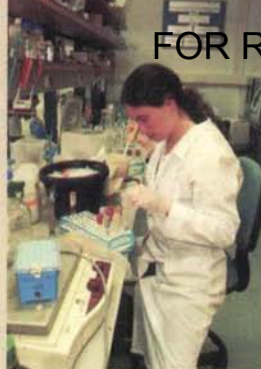


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SEWAGE POLLUTION AND MICROBIOLOGY

B. D. TIWARI



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**Sewage Pollution
and
Microbiology**

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B.D. Tiwari



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SEWAGE POLLUTION AND MICROBIOLOGY

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PREFACE

This is an introduction to sewage pollution and microbiology for students of science, medicine, and environmental science which is designed to hold the reader's attention and to stimulate his interest. Author has kept the book short so as to encourage the student to feel that he can grasp and understand the whole subject; a point especially important at a time when other subjects are making large demands on his time. This book is concerned more with current ideas in sewage pollution than with a list of the currently known facts of the subject; for author feels that this approach is more likely to interest students who are starting the subject and, in consequence, is more likely to lead to their remembering the subject. The techniques for the detection of pollutants have been described in very lucid style so that an average student may understand them. The methods for water treatment processes, designing and treatment of industrial effluents and methods for prevention or control of pollution have been described.

The author expresses his thanks to all those friends, colleagues, and research scholars whose continuous inspirations have initiated him to bring this title.

The author wishes to thank the publisher, printer and staff members for bringing out this book.

Constructive criticisms and suggestions for improvement of the book will be thankfully acknowledged.

Author

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Introduction

The human population is facing a great challenge to produce more food at a faster rate but in a sustainable way. In our discussion here, 'sustainable' means to have methods of production that protect the environment and the biodiversity and also keep natural resources for future generations. In this context, it is important to analyse the international situation: the world population keeps growing and it is estimated that the mark of 8000 million people will be reached by the year 2005. Most of the increase, 2500 million, will occur in developing countries, and nearly 85% of the total will be living there. In order to cover the food needs, agricultural production will have to be doubled around year 2025; however hunger will still leave about one billion malnourished people.

During 1996 in Mexico the situation of food production was as follows: we imported 10 million tonnes of basic grains, 7 million of which were corn and 250 000 beans, which represents an expenditure of \$3 billion. This is a worrying prospect, not only due to the lack of rain in many agricultural sectors of the country but also because it indicates structural problems in the Mexican agriculture. In 1994 the FAO constructed a simulation model to estimate domestic food demand to the year 2010; the model takes into account all uses of agricultural products (direct human food, industrial uses, animal feed, etc.) and considers that the NGP will grow at 4.8% while population will increase at 1.9% between 1989 and 2000 and 1.6% in the period 2000-2010. In these conditions the total internal demand for agricultural products will grow annually 2.6% in the period 1989-2010, which represents an annual increase of 0.4% of calories available for the population. We can anticipate today that these estimations will never occur, because the growth of the NGP will be smaller than estimated.

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Even though the FAO's model indicates that future growth of demand will be smaller than in the last three decades, the demand will grow faster than the domestic production.

For these reasons the only way to cover the food demand will be through food imports. The yearly rates of imports will be high: 6.7% for wheat, 5.2% for rice and 3.9 for corn. The agricultural commercial deficit will grow 57 and 49% for the periods 1989-2000 and 2000-2010, respectively.

TABLE 1.1 Future demand for food products (thousand t/year)

Product	1988-1990	2010	Increase
Rice	786	1 349	563
Corn	16 395	23 675	7 280
Wheat	4 731	7 613	2 882
Beans	1 384	1 523	139
Barley	597	1 451	854
Sorghum	7 851	12 582	4 731
Vegetable oils	1 248	2 414	1 166
Milk	8 988	13 759	4 771
Bovine meat	1 931	3 129	1 198
Eggs	1 058	1 727	669
Pork meat	835	1 750	915
Bird meat	768	1 793	1 025

For several years domestic production has been increased by using new agricultural methods developed by adoption of technology of the type known as 'Green Revolution' and also by the opening of large areas for irrigation, which at the same time had important negative environmental impacts. The contamination related to agricultural inputs has been growing mainly due to the extended use of pesticides. These products have grown 5% annually, from 14 000 t in 1960 to 60 000 t in 1986, as a result of the diffusion of this type of technology and also due to the fact that many pathogenic agents have developed resistance to chemical products and new pests have appeared. From 1988 to 1992 the import of plaguicides to Mexico increased from 30 000 t to 60 000 t, but how much of this is applied domestically, and the amount of pesticides produced and applied in Mexico, is not known.

It is thus necessary to increase food production but how could it be done keeping the environment and the biodiversity safe and

TABLE 1.2 Trends, future demand and importation of foods products: annual growth rate

<i>Product</i>	<i>Domestic demand</i>		<i>Projection</i>	<i>Importation</i>
	<i>Historical trends</i>			
	<i>1988-2010</i>	<i>1988-201</i>		
Wheat	5.1	2.1	2.3	6.7
Corn	3.4	2.7	1.8	3.9
Rice	3.6	3.0	2.6	5.2
Sorghum	11.2	0.0	2.3	2.3
Cereals	4.8	1.8	2.1	4.0
Meats	5.3	3.5	3.1	0.9
Total foods	4.8	2.6	2.7	3.0
Total (foods/other products)	4.6	2.6	2.6	3.0

conserving the natural resources for the future? A satisfactory answer faces problems of large technological complexity: agricultural productivity must increase but at the same time consumption of agrochemicals should decrease. This goal can be reached at least partially through

- replacing traditional agrochemicals (insecticides, fungicides, herbicides, etc.) by novel and less toxic products, susceptible to short-term biodegradation in the environment;
- national policies to reduce the input of agrochemicals;
- support of research and development directed towards integrated pest management.

In this chapter, we will describe some of the most interesting aspects of the most widely commercial used bioinsecticide, *Bacillus thuringiensis* (*BT*), because it is a good model to understand the problems of replacing toxic agrochemicals with biodegradable products. The areas that will be covered are:

- the search for novel *BT* activities against different kind of insects;
- study of the mechanism of action of the *BT* δ -endotoxins;
- generation of transgenic plants containing the δ -endotoxins *BT* genes (*cry* genes)
- development of new systems for use of *BT* toxins.

1.1 BIOINSECTICIDES BASED ON BT

Bacillus thuringiensis is a Gram-positive soil bacterium that produces insecticidal crystal proteins (ICP), also known as δ -endotoxins, during the sporulation process; these are toxic to the larvae of a number of destructive insect pests in forestry and agriculture. The spores and the crystals produced by this bacterium were discovered at the beginning of the twentieth century, and commercial products based on it were used until 1956 in France. However, the δ -endotoxins (also called cry proteins), the main constituent of the crystals, have recently attracted the attention of several research groups and also of some international companies for two reasons: δ -endotoxins with novel activities have been discovered, and the arrival of molecular biology to agriculture has generated transgenic plants containing the cry genes.

In 1990 it was reported that *BT* represents around 95% of the total biopesticides market, its sales volume being \$260 million. It has been estimated that the demand for these products will grow at least 10% annually until the end of the twentieth century. The principal producers of *BT* biopesticides are Abbott, Sandoz, Du Pont and Novo Nordisk. The market distribution by regions is: USA and Canada 50%, Orient 18%, China 10%, 8% Central and South America, rest of the world 14%.

If we follow the historical development of the discovery of δ -endotoxins with novel activities we can see that its growth rate has been practically exponential since the 1980s and today we can foresee that with new biological methodologies available more toxins will be found with novel activities. Particularly it must be pointed out that *BT* toxins are not toxic to humans and other mammals after 40 years of use and this characteristic has made them very attractive because it is not necessary to carry out long and costly clinical trials to probe its lack of toxicity and, at the same time, the past experience of commercial use makes easier and faster the introduction of similar products.

At the present time several *BT* strains have been identified with different insecticidal activities; most of them are toxic to lepidopteran larvae (caterpillars), but there are also strains with toxic activity against dipterans (flies and mosquitoes), coleopterans (beetles), mites and ants (hymenopterans), platyhelminthes (flat worms), protozoans (amoebae) and nematodes (roundworms). It is estimated that the list of susceptible organisms will keep growing and this area, the search of toxins with novel activities, is the principal goal of many research groups around the world, in academia as well as in private companies.

1.2 MODE OF ACTION OF BT Δ -ENDOTOXINS

The elucidation of the mechanism of action of the *BT* toxins is, besides the search for novel activities, the big area of interest in the field of the *BT* bioinsecticides. This knowledge will allow the design of more potent molecules or manufacture of molecules with a novel and/or higher spectrum of action. The current knowledge about the mode of action of the *BT* S-endotoxins.

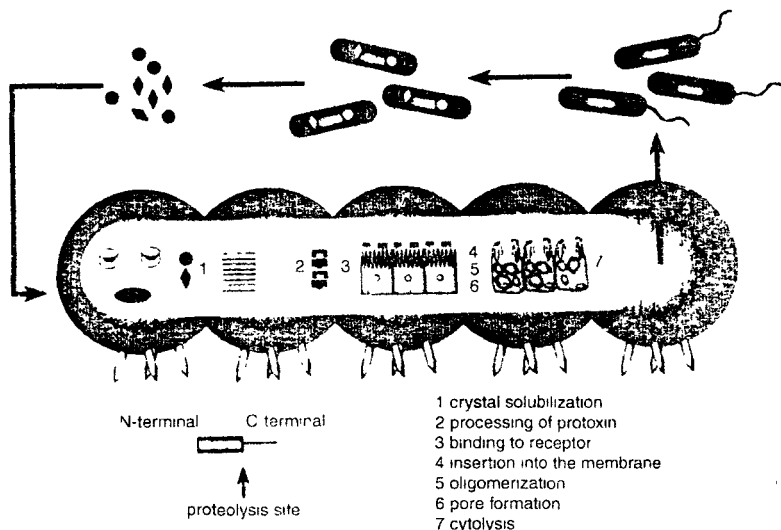


Figure 1.1 Mechanism of action of *Bacillus thuringiensis* S-endotoxins.

It is known that cry proteins accumulate within the cell, forming crystals during the sporulation process. In the crystals, the proteins are immature products (protoxins); in order to be active the crystal must be solubilized in the extreme pH conditions of the insect midgut and in the same place also must be activated by proteolytic processing. The mature products or toxins bind to specific receptors in the brush border membrane of the midgut columnar cells where, after a substantial conformational change caused by interaction with the receptor, it is inserted into the membrane and, presumably by oligomerization, forms pores. These pores disturb the endogenous permeability properties of the target membrane. After that, there is a massive entry of water into the cells, causing the destruction of the epithelial tissue and finally the death of the larvae.

The putative receptor has recently been isolated and characterized. It has been reported that the Cry IAc receptor in *Manduca sexta*, *Heliothis virescens* and *Lymantria dispar* is an aminopeptidase N

that is linked to the cell membrane by a glycosyl-phosphatidylinositol anchor.

Using several methods *in vitro* to know with more detail the effect of the δ -endotoxins in the membrane that contains the receptor, several authors have suggested that the primary action of the cry proteins in the membrane is to induce some endogenous proteins to allow the flux of ions through the membrane - in other words, to open an ionic channel selective to cations, or forms (alone or with the receptor) a cationic channel. Some δ -endotoxins have been studied incorporating them into planar bilayers formed by synthetic lipids, where it has been shown that these toxins could form ionic channels *per se*. The ionic channels formed by these proteins allow the preferential flux of monovalent cations; other important characteristic of these channels is that apparently they are formed by one or more molecules, because its conductance (the property that describes how much resistance must an ion overcome to pass through the channel) is very variable (500-4000 pS). It is important to point out that in order to observe these channels experimentally micromolar concentration of toxins have to be used, while the effect of them in the apical membrane of the epithelial cells occur at nanomolar or picomolar concentration. This fact suggests that the receptor reduces the toxin concentration required for the protein or the protein-receptor complex to insert and form pores into the membrane. There is still an open question: do the channels observed in the black lipid bilayers in the absence of the receptor correspond to the channels formed by the cry proteins *in vivo*?

At least 60 different cry genes have been described and classified considering the similarity of the amino acid sequence in 19 different groups (1, 2, ... , 19) and subgroups (A, B, ... , etc.) (Crickmore *et al.*). Doing alignments of the primary structure of the cry protein toxic regions it has been possible to identify five very conserved regions (blocks 1, 2, 3, 4 and 5) that are separated from others of low similarity and variable length.

1.3 STRUCTURE AND FUNCTION OF δ -ENDOTOXINS

The tridimensional structure determined by X-ray diffraction studies of the toxic portion of one of these proteins, Cry3A, has shown that it is organized in three domains, to each of which a specific function has been assigned. Domain I (residues 1 to 290) is constituted of six amphipathic helices surrounding another hydrophobic one, α -helix 5;

these are possible constituents of a structure that forms pores. Domain II (residues 290-500) is formed by three β -antiparallel layers that end in loops in the vertex of the molecule; this conformation is called β -prisms. The region that forms the loops is the less conserved (hypervariable) region; the interchange of fragments of the hypervariable region between three closely related proteins allows interchange of specificities, and for this reason the domain is associated with the interaction with the receptor. Domain III is composed of β -sheet arrays in the form of a β -sandwich (this topology is called double helix β); toxins with mutations in this domain are very unstable to protease treatment, and the role of this domain is to be responsible for the structural stability of the whole molecule. It is important to note that the five conserved regions are in the central part of the molecule, and it is proposed that the cry proteins have a common conformation and similar mechanism of action. This proposal was corroborated recently when the structure of another member of the cry family CryIAa, was published. There is only one significant difference between Cry3A and Cry IAa: the loops of domain II of the latter toxin are longer. This structural information supports the idea that the hypervariable region is a determinant of the different specificity of each one of these toxins.

There is evidence that Domain I, specifically block 1 (this region corresponds to the α -helix 5), is involved in pore formation. Mutants of CryIAc in α -helix 5 were equally effective as the wild type when binding to the receptor in brush border membrane vesicles (BBMV) obtained from the midgut of three susceptible insects (*Manduca sexta*, *Heliothis virescens* and *Trichoplusia ni*); however, the activity in bioassays of some of these mutants decreased dramatically (between 10 and 1000 times) when proline or other charged residues were introduced. It was proved that these mutants lose the capacity to inhibit the leucine transport dependent on the K^+ gradient in the BBMV; this is an indirect measure that indicates that these proteins are capable of altering the vesicle membrane permeability. Other results that support the hypothesis that the central helix is a structural component of the pores were obtained when peptides corresponding to the α -helix 5 of Cry3A and CryIAc were synthesized; both peptides maintain the capacity of the whole molecule to form ionic channels selective to cations in black lipid bilayers. In the literature it is proposed that at least one α -helix of Domain I (α -helix 7) can also participate as a structural component of the pore. This possibility has not been eliminated, although the evidence available suggests that α -helix 7 could participate in addition to the B-1 of Domain II as a bridge of communication

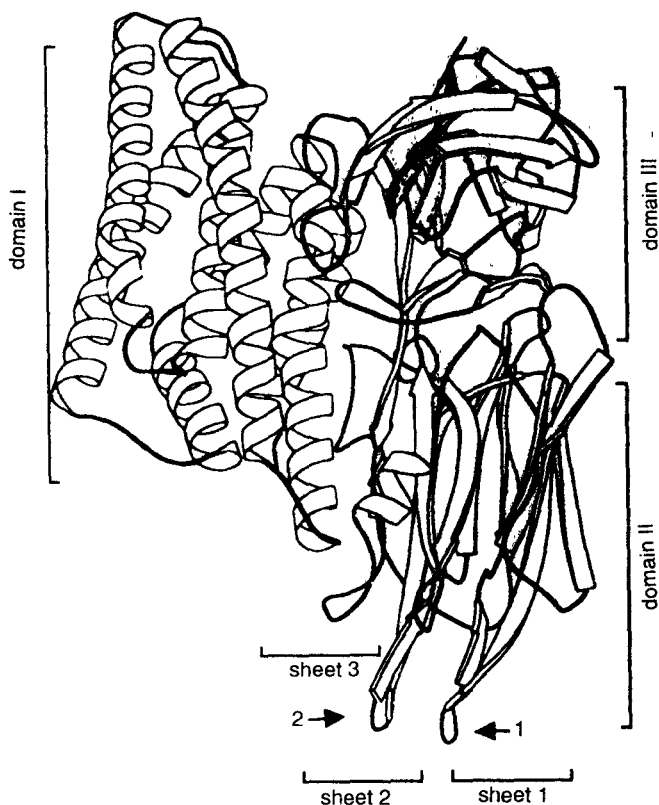


Figure 1.2 The three-dimensional structure of *Bacillus thuringiensis* Cry3a toxin. Domain I contains seven α -helices, six of them arranged the seventh around. α -helix 5. Domain II is formed by three β -sheets (loops 1 and 2 are denoted by arrows). Domain III has a β -sandwich structure with a topology typical of double β -helix

between these two domains to allow the molecule to make the conformational change necessary for the toxin to pass from a soluble conformation to a state that will insert into a hydrophobic environment, which is present in biological membranes.

During experiments of site-directed mutagenesis, where one or more amino acid residues present in the protein structure are substituted by others, it has been confirmed that the regions determinant of the high specificity of each one of the toxins are those corresponding to the loops in the apex of the molecule (Domain II). When this region in proteins CryIAa, CryIAb, CryIC and Cry3A was mutated, it was observed that the affinity of the mutant proteins to the receptor are substantially changed.

Three of the five regions of conserved amino acids in the cry family are localized in Domain III (blocks 3, 4 and 5). The evidence obtained from several authors suggests that preservation of the molecule's integrity depends on the maintenance of the globular structure of this domain. This structure depends on the salt bridges between the positively charged residues of block 4 and its negative counterparts in block 5.

The results of experiments in which fragments of CryIAa and CryIAc were interchanged and of the interactions between these chimeric proteins and the receptor obtained from *Lymantria dispar* suggest that some residues of Domain III also participate in the interaction with the receptor. It is not known if this is a general function of the domain in the *BT* toxins.

It is proposed that when δ -endotoxins form pores in the membrane they cause the death of the epithelial cells because they disturb the system that maintains the pH gradient. This inactivation is the result of the alteration in membrane permeability because the target membrane is less permeable to cations. When the motive force that maintains the gradient of 1000 times more protons in the cytoplasm than in the lumen is disturbed, the cytoplasm becomes alkalized and this disturbs the cellular metabolism, causing the destruction of the intestinal tissue. Once this physical barrier is destroyed, the spores obtain access to the haemolymph, where they proliferate because there is plenty of nutrients. The larva dies due to inanition and septicemia.

1.4 TRANSGENIC PLANTS RESISTANT TO INSECTS

Since the beginning of agrobiotechnology in 1983, *BT* cry genes have been identified as key elements in order to increase the agricultural productivity, decreasing the use of agrochemicals. Many important crops and different types of plants have been genetically transformed, and in many cases *BT* genes have been introduced. The main results are reported below.

During the period 1987-1995, 2261 field trials were done with transgenic plants in the USA, in 7095 different locations. From that total, 23.1% correspond to insect-resistant plants; 27.8% to herbicide resistance; 26.8% to improved characteristics; 11.5% to virus resistance and 2.9% fungus resistance.

In the USA from 1993 to 1996, 17 transgenic plants were approved for widespread use and production, six of which were resistant to insects (lepidoptera and coleoptera) due to the introduction of the δ -endotoxin *BT* genes. Now the methodology for plant transformation of

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dicotyledons is available and there have been important improvements in the transformation of monocotyledons.

TABLE 1.3 Global status of applications for the commercialization of transgenic crops with BT insect resistance

<i>Country</i>	<i>Crop</i>	<i>Year of approval (company)</i>
Argentina	Corn/maize	P (Ciba)
	Corn/maize	P (Monsanto)
	Corn/maize	P (Northrup King)
Australia	Cotton	P (Monsanto)
Canada	Corn/maize	1996 (Mycogen-Ciba)
	Potato	1996 (Monsanto)
European Union	Corn/maize	P (Ciba)
	Corn/maize	P (Monsanto)
Japan	Corn/maize	P (Ciba)
	Corn/maize	P (Northrup King)
	Potato	P (Monsanto)
	Cotton	1996 (Monsanto)
Mexico	Corn/maize	P (CIMMYT)
	Potato	1996 (Monsanto)
	Cotton	1995 (Monsanto)
USA	Corn/maize	1995 (Ciba)
	Corn/maize	1996 (Northrup King)
	Corn/maize	1996 (Monsanto)
	Potato	1995 (Monsanto)
	Potato	1996 (Monsanto)

In Mexico the International Center for Improvement of Wheat and Corn has' been developing, with financial support from the United Nations, a project to oBTain corn varieties with resistance to insects. In February 1996, the first transgenic corn was fieldtested, containing the cryIAb gene of *BT*. This field trial was very important because Mexico is the centre of origin of corn, and the possibility exists of genetic flux between transgenic plants and its ancestor, the teocintle; in this case it was necessary to plan and design a very careful trial and to oBTain the approval of the National Agricultural Biosafety Committee of the Secretary of Agriculture. Also it was necessary to assess the possible implications of introducing transgenic corn in productive areas where teocintle could exchange genetic material with transgenic corn.

1.5 NOVEL SYSTEMS USING *BT*

Recently, the use of *BT* genes and its proteins has increased, because several research groups have developed novel systems to use and produce them. For example:

- introduction of *BT* genes in *Pseudomonas fluorescens* (CellCap technology);
- introduction of *cry* genes into algae to control *Anopheles* mosquitoes (malaria);
- introduction of *BT* genes into endophytic bacteria in order to control sucking insects (aphids);
- production of *BT* toxins in baculoviruses as an alternative expression system.

All these efforts are based on the fact that *cry* proteins are recognized as non-toxic to humans, mammals and other commercially important species. It also means that *BT* will be used against a wide variety of pests that affect not only agriculture but also other areas such as human health (e.g. in destruction of mosquitoes that transmit malaria).

1.6 CONCLUSION

We believe that the discovery of novel *BT* activities and strains will continue because there are many insects, mostly in the developing world, that are not effectively controlled with the known *cry* toxins. Our knowledge about the structure and mode of action of *BT* toxins will help us to increase the speed of discovery of new *cry* proteins.

The great challenge of this research field is to avoid or at least delay insect resistance, and that is why it is necessary to improve the understanding of the mechanism of action of *cry* toxins in order to develop new strategies to face this problem. The increasing use of transgenic plants makes this challenge bigger.

2

Water Pollution

Any physical, biological, or chemical change in water quality that adversely affects living organisms or makes water unsuitable for desired uses can be considered pollution. Often, however, a change that adversely affects one organism may be advantageous to another. Nutrients that stimulate oxygen consumption by bacteria and other decomposers in a river or lake, for instance, may reduce some fish populations, but will stimulate a flourishing community of decomposers. Whether the quality of the water has suffered depends on your perspective. There are natural sources of water contamination, such as poison springs, oil seeps, and sedimentation from erosion, but in this chapter we will focus primarily on humancaused changes that affect water quality or usability.

Pollution control standards and regulations usually distinguish between point and nonpoint pollution sources. Factories, power plants, sewage treatment plants, underground coal mines, and oil wells are classified as *point sources* because they discharge pollution from specific locations, such as drain pipes, ditches, or sewer outfalls. These sources are discrete and identifiable, so they are relatively easy to monitor and regulate. It is generally possible to divert effluent from the waste streams of these sources and treat it before it enters the environment.

In contrast, *nonpoint sources* of water pollution are scattered or diffuse, having no specific location where they discharge into a particular body of water. Nonpoint sources include runoff from farm fields and feedlots, golf courses, lawns and gardens, construction sites, logging areas, roads, streets, and parking lots. Whereas point sources may be fairly uniform and predictable throughout the year, nonpoint sources are often highly episodic. The first heavy rainfall after a dry

period may flush high concentrations of gasoline, lead, oil, and rubber residues off city streets, for instance, while subsequent runoff may have lower levels of these pollutants. Spring snowmelt carries high levels of atmospheric acid deposition into streams and lakes in some areas. The irregular timing of these events, as well as their multiple sources and scattered location, makes them much more difficult to monitor, regulate, and treat than point sources.

Perhaps the ultimate in diffuse, nonpoint pollution is *atmospheric deposition* of contaminants carried by air currents and precipitated into watersheds or directly onto surface waters as rain, snow, or dry particles. The Great Lakes, for example, have been found to be accumulating industrial chemicals such as PCBs and dioxins, as well as agricultural toxins such as the insecticide toxaphene that cannot be accounted for by local sources alone. The nearest sources for many of these chemicals are sometimes thousands of kilometers away.

Amounts of these pollutants can be quite large. It is estimated that there are 600,000 kg of the herbicide atrazine in the Great Lakes, most of which is thought to have been deposited from the atmosphere. Concentration of persistent chemicals up the food chain can produce high levels in top predators. Several studies have indicated health problems among people who regularly eat fish from the Great Lakes.

Ironically, lakes also can be pollution sources as well. In the past twelve years, about 26,000 metric tons of PCBs have "disappeared" from Lake Superior. Apparently, these compounds are released from the lake surface and moved to other areas where they are re-deposited.

2.1 TYPES AND EFFECTS OF WATER POLLUTION

Although the types, sources, and effects of water pollutants are often interrelated, it is convenient to divide them into major categories for discussion. Let's look more closely at some of the important sources and effects of each type of pollutant.

2.1.1 Infectious Agents

The most serious water pollutants in terms of human health worldwide are pathogenic organisms. Among the most important waterborne diseases are typhoid, cholera, bacterial and amoebic dysentery, enteritis, polio, infectious hepatitis, and schistosomiasis. Malaria, yellow fever, and filariasis are transmitted by insects that have aquatic larvae. Altogether, at least 25 million deaths each year are blamed on these water-related diseases.

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Nearly two-thirds of the mortalities of children under 5 years old are associated with waterborne diseases.

The main source of these pathogens is from untreated or improperly treated human wastes. Animal wastes from feedlots or fields near waterways and food processing factories with inadequate waste treatment facilities also are sources of disease-causing organisms.

TABLE 2.1 Major categories of water pollutants.

Category	Examples	Sources
A. Causes health problems		
1. Infectious agents	Bacteria, viruses, parasites	Human and animal excreta
2. Organic chemicals	Pesticides, plastics, detergents, oil,	Industrial, household, and farm use and gasoline
3. Inorganic chemicals	Acids, caustics, salts, metals	Industrial effluents, household cleansers, surface' runoff
4. Radioactive materials	Uranium, thorium, cesium, iodine, radon	Mining and processing of ores, power plants, weapons production, natural sources
B. Causes ecosystem disruption		
1. Sediment	Soil, silt	land erosion
2. Plant nutrients	Nitrates, phosphates, ammonium	Agricultural and urban fertilizers, sewage, manure
3. Oxygen-demanding wastes	Animal manure and plant residues food processing	Sewage, agricultural runoff, paper mills,
4. Thermal	Heat	Power plants, industrial cooling

In developed countries, sewage treatment plants and other pollution-control techniques have reduced or eliminated most of the worst sources of pathogens in inland surface waters. Furthermore, drinking water is generally disinfected by chlorination so

epidemics of waterborne diseases are rare in these countries. The United Nations estimates that 90 percent of the people in developed countries have adequate (safe) sewage disposal, and 95 percent have clean drinking water.

The situation is quite different in less-developed countries. The

United Nations estimates that at least 2.5 billion people in these countries lack adequate sanitation, and that about half these people also lack access to clean drinking water. Conditions are especially bad in remote, rural areas where sewage treatment is usually primitive or nonexistent, and purified water is either unavailable or too expensive to obtain. The World Health Organization estimates that 80 percent of all sickness and disease in lessdeveloped countries can be attributed to waterborne infectious agents.

If everyone had pure water and satisfactory sanitation, the World Bank estimates that 200 million fewer episodes of diarrheal illness would occur each year, and 2 million childhood deaths would be avoided. Furthermore, 450 million people would be spared debilitating roundworm or fluke infections. Surely these are goals worth pursuing.

Detecting specific pathogens in water is difficult, timeconsuming, and costly; thus, water quality control personnel usually analyze water for the presence of *coliform bacteria*, any of the many types that live in the colon or intestines of humans and other animals. If large numbers of these organisms are found in a water sample, recent contamination by untreated feces is indicated. Exposure to an alien strain of coliform bacteria is usually the cause of upset stomach and diarrhea that often strike tourists. It is usually assumed that if coliform bacteria are present in a water sample, infectious pathogens are present also.

To test for coliform bacteria, a 100 ml (4 oz) sample of water is passed through a filter that removes bacterial cells. The filter is placed in a dish containing a liquid nutrient medium that supports bacterial growth. After twenty-four hours at the appropriate temperature, each living cell will have produced a small colony of cells on the filter. If any colonies are found in drinking water samples, the U.S. Environmental Protection Agency considers the water unsafe and requiring disinfection. The EPA recommended maximum coliform count for swimming water is 200 colonies per 100 ml, but some cities and states allow higher levels. If the limit is exceeded, the contaminated pool, river, or lake usually is closed to swimming.

2.1.2 Oxygen-Demanding Wastes

The amount of oxygen dissolved in water is a good indicator of water quality and of the kinds of life it will support. Water with an oxygen content above 6 parts per million (ppm) will support game fish and other desirable forms of aquatic life. Water with less than 2 ppm oxygen will support mainly worms, bacteria, fungi, and other detritus feeders and decomposers. Oxygen is added to water by

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diffusion from the air, especially when turbulence and mixing rates are high, and by photosynthesis of green plants, algae, and cyanobacteria. Oxygen is removed from water by respiration and chemical processes that consume oxygen.

The addition of certain organic materials, such as sewage, paper pulp, or food-processing wastes, to water stimulates oxygen consumption by decomposers. The impact of these materials on water quality can be expressed in terms of *biochemical oxygen demand* (BOD): a standard test of the amount of dissolved oxy-

gen consumed by aquatic microorganisms over a five-day period. An alternative method, called the chemical oxygen demand (COD), uses a strong oxidizing agent (dichromate ion in 50 percent sulfuric acid) to completely break down all organic matter in a water sample. This method is much faster than the BOD test, but normally gives much higher results because it oxidizes compounds not ordinarily metabolized by bacteria. A third method of assaying pollution levels is to measure *dissolved oxygen* (DO) content directly, using an oxygen electrode. The DO content of water depends on factors other than pollution (for example, temperature and aeration), but it is usually more directly related to whether aquatic organisms survive than is BOD.

The effects of oxygen-demanding wastes on rivers depends to a great extent on the volume, flow, and temperature of the river water. Aeration occurs readily in a turbulent, rapidly flowing river, which is, therefore, often able to recover quickly from oxygendepleting processes. Downstream from a point source, such as a municipal sewage plant discharge, a characteristic decline and restoration of water quality can be detected either by measuring dissolved oxygen content or by observing the flora and fauna that live in successive sections of the river.

The oxygen decline downstream is called the *oxygen sag*. Upstream from the pollution source, oxygen levels support normal populations of clean-water organisms. Immediately below the source of pollution, oxygen levels begin to fall as decomposers metabolize waste materials. Rough fish, such as carp, bullheads, and gar, are able to survive in this oxygen-poor environment where they eat both decomposer organisms and the waste itself. Further downstream, the water may become anaerobic (without oxygen) so that only the most resistant microorganisms and invertebrates can survive. Eventually, most of the nutrients are used up, decomposer populations are smaller, and the

water becomes oxygenated once again. Depending on the volumes and flow rates of the effluent plume and the river receiving it, normal communities may not appear for several miles downstream.

2.1.3 Plant Nutrients and Cultural Eutrophication

Water clarity (transparency) is affected by sediments, chemicals, and the abundance of plankton organisms and is a useful measure of water quality and water pollution. Rivers and lakes that have clear water and low biological productivity are said to be *oligotrophic* (oligo = little + trophic = nutrition). By contrast, *eutrophic* (eu + trophic = truly nourished) waters are rich in organisms and organic materials. Eutrophication, an increase in nutrient levels and biological productivity, is a normal part of successional changes in most lakes. Tributary streams bring in sediments and nutrients that stimulate plant growth. Over time, the pond or lake tends to fill in, eventually becoming a marsh and then a terrestrial biome. The rate of eutrophication and succession depends on water chemistry and depth, volume of inflow, mineral content of the surrounding watershed, and the biota of the lake itself.

Human activities can greatly accelerate eutrophication. An increase in biological productivity and ecosystem succession caused by human activities is called *cultural eutrophication*. Cultural eutrophication can be brought about by increased nutrient flows, higher temperatures, more sunlight reaching the water surface, or a number of other changes. Increased productivity in an aquatic system sometimes can be beneficial. Fish and other desirable species may grow faster, providing a welcome food source. Often, however, eutrophication has undesirable results. An oligotrophic lake or river usually has aesthetic qualities and species of organisms that we value.

The high biological productivity of eutrophic systems is often seen in "blooms" of algae or thick growths of aquatic plants stimulated by elevated phosphorus or nitrogen levels. Bacterial populations also increase, fed by larger amounts of organic matter. The water often becomes opaque and has unpleasant tastes and odors. The deposition of silt and organic sediment caused by cultural eutrophication can accelerate the "aging" of a water body enormously over natural rates. Lakes and reservoirs that normally might exist for hundreds or thousands of years can be filled in a matter of decades.

Eutrophication also occurs in marine ecosystems, especially in near-shore waters and partially enclosed bays or estuaries. Blooms of minute organisms called dinoflagellates produce toxic *red tides* that

kill fish. Partially enclosed seas such as the Black Sea, the Baltic, and the Mediterranean tend to be in especially critical condition. During the tourist season, the coastal population of the Mediterranean, for example, swells to 200 million people. Eighty-five percent of the effluents from large cities go untreated into the sea. Beach pollution, fish kills, and contaminated shellfish result. Extensive "dead zones" often form where rivers dump nutrients into estuaries and shallow seas. The largest in the world occurs during summer months in the Gulf of Mexico at the mouth of the Mississippi River. This hypoxic zone (less than 2 mg oxygen per liter) can cover 18,000 km² (7,000 mi²).

2.1.4 Toxic Inorganic Materials

Some toxic inorganic chemicals are released from rocks by weathering, are carried by runoff into lakes or rivers, or percolate into groundwater aquifers. This pattern is part of natural mineral cycles. Humans often accelerate the transfer rates in these cycles thousands of times above natural background levels through the mining, processing, using, and discarding of minerals.

In many areas, toxic, inorganic chemicals introduced into water as a result of human activities have become the most serious form of water pollution. Among the chemicals of greatest concern are heavy metals, such as mercury, lead, tin, and cadmium. Supertoxic elements, such as selenium and arsenic, also have reached hazardous levels in some waters. Other inorganic materials, such as acids, salts, nitrates, and chlorine, that normally are not toxic at low concentrations may become concentrated enough to lower water quality or adversely affect biological communities.

2.1.4.1 Heavy metals

Many metals such as mercury, lead, cadmium, and nickel are highly toxic. Levels in the parts per million range—so little that you cannot see or taste them—can be fatal. Because metals are highly persistent, they accumulate in food chains and have a cumulative effect in humans. A famous case of mercury poisoning in Japan in the 1950s was one of our first warnings of this danger.

Another mercury-poisoning disaster appears to be in process in South America. Since the mid-1980s, a gold rush has been under way in Brazil, Ecuador, and Bolivia. Forty thousand *garimpeiros* or prospectors have invaded the jungles along the Amazon River and its tributaries to pan for gold. They use mercury to trap the gold and separate it from sediments. Then, the mercury is boiled off with a

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blow torch. Miners and their families suffer nerve damage from breathing the toxic fumes. Estimates are that 130 tons of mercury per year are deposited in the Amazon, which will be impossible to clean up.

We have come to realize that other heavy metals released as a result of human activities also are concentrated by hydrological and biological processes so that they become hazardous to both natural ecosystems and human health. A condition known as Itaitai (literally ouch-ouch) disease that developed in Japanese living near the Jintsu River was traced to cadmium poisoning. Bacteria forming methylated tin have been found in sediments in Chesapeake Bay, leading to worries that this toxic metal also may be causing unsuspected health effects. The use of tin compounds as antifouling agents on ship bottoms has been banned because of its toxic effects.

Lead poisoning has been known since Roman times to be dangerous to human health. Lead pipes are a serious source of drinking water pollution, especially in older homes or in areas where water is acidic and, therefore, leaches more lead from pipes. Even lead solder in pipe joints and metal containers can be hazardous. In 1990, the EPA lowered the maximum limit for lead in public drinking water from 50 parts per billion (ppb) to 20 ppb. Some public health officials argue that lead is neurotoxic at any level, and the limits should be less than 10 ppb.

Mine drainage and leaching of mining wastes are serious sources of metal pollution in water. A survey of water quality in eastern Tennessee found that 43 percent of all surface streams and lakes and more than half of all groundwater used for drinking supplies was contaminated by acids and metals from mine drainage. In some cases, metal levels were two hundred times higher than what is considered safe for drinking water.

2.1.4.2 Nonmetallic salts

Desert soils often contain high concentrations of soluble salts, including toxic selenium and arsenic. You have probably heard of poison springs and seeps in the desert where these compounds are brought to the surface by percolating groundwater. Irrigation and drainage of desert soils mobilize these materials on a larger scale and can result in serious pollution problems, as in Kesterson Marsh in California where selenium poisoning killed thousands of migratory birds in the 1980s.

Such salts as sodium chloride (table salt) that are nontoxic at

low concentrations also can be mobilized by irrigation and concentrated by evaporation, reaching levels that are toxic for plants and animals. Salt levels in the San Joaquin River in central California rose from 0.28 gm/l in 1930 to 0.45 gm/l in 1970 as a result of agricultural runoff. Salinity levels in the Colorado River and surrounding farm fields have become so high in recent years that millions of hectares of valuable croplands have had to be abandoned. The United States has built a huge desalinization plant at Yuma, Arizona, to reduce salinity in the river. In northern states, millions of tons of sodium chloride and calcium chloride are used to melt road ice in the winter. The corrosive damage to highways and automobiles and the toxic effects on vegetation are enormous. Leaching of road salts into surface waters may have a similarly devastating effect on aquatic ecosystems.

2.1.4.3 Acids and Bases

Acids are released as by-products of industrial processes, such as leather tanning, metal smelting and plating, petroleum distillation, and organic chemical synthesis. Coal mining is an especially important source of acid water pollution. Sulfur compounds in coal are solubilized to make sulfuric acid. Thousands of kilometers of streams in the United States have been acidified by acid mine drainage, some so severely that they are essentially lifeless.

Coal and oil combustion also leads to formation of atmospheric sulfuric and nitric acids, which are disseminated by long-range transport processes and deposited via precipitation (acidic rain, snow, fog, or dry deposition) in surface waters. Where soils are rich in such alkaline material as limestone, these atmospheric acids have little effect because they are neutralized. In high mountain areas or recently glaciated regions where crystalline bedrock is close to the surface and lakes are oligotrophic, however, there is little buffering capacity (ability to neutralize acids) and aquatic ecosystems can be severely disrupted. These effects were first recognized in the mountains of northern England and Scandinavia about thirty years ago. In recent years, aquatic damage due to acid precipitation has been reported in about two hundred lakes in the Adirondack Mountains of New York State and in several thousand lakes in eastern Quebec, Canada. Game fish, amphibians, and sensitive aquatic insects are generally the first to be killed by increased acid levels in the water. If acidification is severe enough, aquatic life is limited to a few resistant species of mosses and fungi. Increased acidity may result in leaching of toxic metals, especially aluminum, from soil and rocks, making water unfit for drinking or irrigation, as well.

2.1.5 Organic Chemicals

Thousands of different natural and synthetic organic chemicals are used in the chemical industry to make pesticides, plastics, pharmaceuticals, pigments, and other products that we use in everyday life.

Many of these chemicals are highly toxic. Exposure to very low concentrations (perhaps even parts per quadrillion in the case of dioxins) can cause birth defects, genetic disorders, and cancer. Some can persist in the environment because they are resistant to degradation and toxic to organisms that ingest them. Contamination of surface waters and groundwater by these chemicals is a serious threat to human health.

The two most important sources of toxic organic chemicals in water are improper disposal of industrial and household wastes and runoff of pesticides from farm fields, forests, roadsides, golf courses, and other places where they are used in large quantities. The U.S. EPA estimates that about 500,000 metric tons of pesticides are used in the United States each year. Much of this material washes into the nearest waterway, where it passes through ecosystems and may accumulate in high levels in certain nontarget organisms. The bioaccumulation of DDT in aquatic ecosystems was one of the first of these pathways to be understood. Dioxins, and other chlorinated hydrocarbons (hydrocarbon molecules that contain chlorine atoms) have been shown to accumulate to dangerous levels in the fat of salmon, fish-eating birds, and humans and to cause health problems similar to those resulting from toxic metal compounds.

Hundreds of millions of tons of hazardous organic wastes are thought to be stored in dumps, landfills, lagoons, and underground tanks in the United States. Many, perhaps most, of these sites have leaked toxic chemicals into surface waters or groundwater or both. The EPA estimates that about 26,000 hazardous waste sites will require cleanup because they pose an imminent threat to public health, mostly through water pollution.

2.1.6 Sediment

Sediment and suspended solids make up the largest volume of water pollution in the United States and most other parts of the world. Rivers have always carried sediment to the oceans, but erosion rates in many areas have been greatly accelerated by human activities. As chapter 11 describes, some rivers carry astounding loads of sediment. Erosion and runoff from croplands contribute about 25 billion metric tons of soil, sediment, and suspended solids to world surface waters

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each year. Forests, grazing lands, urban construction sites, and other sources of erosion and runoff add at least 50 billion additional tons. This sediment fills lakes and reservoirs, obstructs shipping channels, clogs hydroelectric turbines, and makes purification of drinking water more costly. Sediments smother gravel beds in which insects take refuge and fish lay their eggs. Sunlight is blocked so that plants cannot carry out photosynthesis and oxygen levels decline. Murky, cloudy water also is less attractive for swimming, boating, fishing, and other recreational uses.

Sediment also can be beneficial. Mud carried by rivers nourishes floodplain farm fields. Sediment deposited in the ocean at river mouths creates valuable deltas and islands. The Ganges River, for instance, builds up islands in the Bay of Bengal that are eagerly colonized by land-hungry people of Bangladesh. In Louisiana, lack of sediment in the Mississippi River (it is being trapped by dams upstream) is causing biologically rich coastal wetlands to waste away. Sediment also can be harmful. Excess sediment deposits can fill estuaries and smother aquatic life on coral reefs and shoals near shore. As with many natural environmental processes, acceleration as a result of human intervention generally diminishes the benefits and accentuates the disadvantages of the process.

2.1.7 Thermal Pollution and Thermal Shocks

Raising or lowering water temperatures from normal levels can adversely affect water quality and aquatic life. Water temperatures are usually much more stable than air temperatures, so aquatic organisms tend to be poorly adapted to rapid temperature changes. Lowering the temperature of tropical oceans by even one degree can be lethal to some corals and other reef species. Raising water temperatures can have similar devastating effects on sensitive organisms. Oxygen solubility in water decreases as temperatures increase, so species requiring high oxygen levels are adversely affected by warming water.

Humans cause thermal pollution by altering vegetation cover and runoff patterns, as well as by discharging heated water directly into rivers and lakes. As chapter 19 shows, nearly half the water we withdraw is used for industrial cooling. Electric power plants, metal smelters, petroleum refineries, paper mills, food-processing factories, and chemical manufacturing plants all use and release large amounts of cooling water.

The cheapest way to remove heat from an industrial facility is to draw cool water from an ocean, river, lake, or aquifer, run it through

a heat-exchanger to extract excess heat, and then dump the heated water back into the original source. A *thermal plume* of heated water is often discharged into rivers and lakes, where raised temperatures can disrupt many processes in natural ecosystems and drive out sensitive organisms. To minimize these effects, power plants frequently are required to construct artificial cooling ponds or wet- or dry-cooling towers in which heat is released into the atmosphere and water is cooled before being released into natural water bodies. Wet cooling towers are cheaper to build and operate than dry systems, but lose large quantities of water to evaporation.

In some circumstances, introducing heated water into a water body is beneficial. Warming catfish-rearing ponds, for instance, can increase yields significantly. Warm water plumes from power plants often attract fish, birds, and marine mammals that find food and refuge there, especially in cold weather. This artificial environment can be a fatal trap, however. Organisms dependent on the warmth may die if they leave the plume or if the flow of warm water is interrupted by a plant shutdown. The manatee, for example, is an endangered marine mammal species that lives in Florida. Manatees are attracted to the abundant food supply and warm water in power plant thermal plumes and are enticed into spending the winter much farther north than they normally would. On several occasions, a midwinter power plant breakdown has exposed a dozen or more of these rare animals to a sudden thermal shock that they could not survive.

2.2 WATER QUALITY TODAY

In 1996, the U.S. EPA announced that about 16,000 segments of surface water in the United States and its territories were contaminated by toxic chemicals, sewage, or other pollutants. This contamination affects about 10 percent of the river, stream, coastal water, lake, and estuary mileage in the country. In addition, between 1 to 2 percent of the groundwater near the surface is also polluted. How does this situation compare to past pollution levels? How do the United States and Canada compare to other countries? In the next section, we will look at areas of progress and remaining problems in water pollution control.

2.2.1 Surface Waters in the United States and Canada

Water pollution problems in surface waters are often both highly visible and a direct threat to environmental quality. Consequently, more has been done to eliminate surface water pollution than any other type. This is probably the greatest success story in our antipollution efforts. Much remains to be done, however.

2.2.1.1 Areas of progress

Like most developed countries, the United States and Canada have made encouraging progress in protecting and restoring water quality in rivers and lakes over the past forty years. In 1948, only about one-third of Americans were served by municipal sewage systems, and most of those systems discharged sewage without any treatment or with only primary treatment (the bigger lumps of waste are removed). Most people depended on cesspools and septic systems to dispose of domestic wastes.

The 1972 Clean Water Act established a National Pollution Discharge Elimination System (NPDES), which requires an easily revoked permit for any industry, municipality or other entity dumping wastes in surface waters. The permit requires disclosure of what is being dumped and gives regulators valuable data and evidence for litigation. As a consequence, only about 10 percent of our water pollution now comes from industrial or municipal point sources. One of the biggest improvements has been in sewage treatment.

Since the Clean Water Act was passed in 1972, the United States has spent more than \$180 billion in public funds and perhaps ten times as much in private investments on water pollution control. Most of that effort has been aimed at point sources, especially to build or upgrade thousands of municipal sewage treatment plants. As a result, nearly everyone in urban areas is now served by municipal sewage systems and no major city discharges raw sewage into a river or lake except as overflow during heavy rainstorms.

This campaign has led to significant improvements in surface water quality in many places. The U.S. EPA reports that gross pollution of rivers, lakes, and coastal waters by sewage and industrial wastes is largely a thing of the past. Fish and aquatic insects have returned to waters that formerly were depleted of life-giving oxygen. Swimming and other water-contact sports are again permitted in rivers, lakes, and at ocean beaches that once were closed by health officials.

The national goal of making all U.S. surface waters "fishable and swimmable" has not been fully met, but nearly 90 percent of the river miles and lake acres that are assessed for water quality fully or partly support their designated uses.

An encouraging example of improved water quality is seen in Lake Erie. Although widely regarded as "dead" in the 1960s, the lake today is promoted as the "walleye capital of the world." Bacteria counts and algae blooms have decreased more than 90 percent since

1962. Water that once was murky brown is now clear. Interestingly, part of the improved water quality is due to immense numbers of exotic zebra mussels, which filter the lake water very efficiently. Swimming is now officially safe along 96 percent of the lake's shoreline. Nearly 40,000 nesting pairs of double-crested cormorants nest in the Great Lakes region, up from only about 100 in the 1970s. These improvements bring economic benefits as well as a sense of civic pride. Lake-based tourism on the U.S. side of the Great Lakes brings in more than \$10 billion each year.

Passage of the 1970 Water Act in Canada has produced comparable results. Seventy percent of all Canadians in towns over 1,000 population are now served by some form of municipal sewage treatment. In Ontario, the vast majority of those systems include tertiary treatment. After ten years of controls, phosphorus levels in the Bay of Quinte in the northeast corner of Lake Ontario have dropped nearly by half, and algal blooms that once turned waters green are less frequent and less intense than they once were. Elimination of mercury discharges from a pulp and paper mill on the Wabigoon-English River system in western Ontario has resulted in a dramatic decrease in mercury contamination that produced Minamata-like symptoms in local native people twenty years ago. Extensive flooding associated with hydropower projects has raised mercury levels in fish to dangerous levels elsewhere, however.

2.2.1.2 Remaining problems

The greatest impediments to achieving national goals in water quality in both the United States and Canada are nonpoint discharges of pollutants. These sources are harder to identify and to reduce or treat than are specific point sources. About three-fourths of the water pollution in the United States comes from soil erosion, fallout of air pollutants, and surface runoff from urban areas, farm fields, and feedlots. In the United States, as much as 25 percent of the 46,800,000 metric tons (52 million tons) of fertilizer spread on farmland each year is carried away by runoff.

Cattle in feedlots produce some 129,600,000 metric tons (144 million tons) of manure each year, and the runoff from these sites is rich in viruses, bacteria, nitrates, phosphates, and other contaminants. A single cow produces about 30 kg (14 lb) of manure per day, or about as much as that produced by ten people. Some feedlots have 100,000 animals with no provision for capturing or treating runoff water. Imagine drawing your drinking water downstream from such

a facility. Pets also can be a problem. It is estimated that the wastes from about a half million dogs in New York City are disposed of primarily through storm sewers, and therefore do not go through sewage treatment.

Loading of both nitrates and phosphates in surface water have decreased from point sources but have increased about fourfold since 1972 from nonpoint sources. Fossil fuel combustion has become a major source of nitrates, sulfates, arsenic, cadmium, mercury, and other toxic pollutants that find their way into water. Carried to remote areas by atmospheric transport, these combustion products now are found nearly everywhere in the world. Toxic organic compounds, such as DDT, PCBs, and dioxins, also are transported long distances by wind currents.

2.2.2 Surface Waters in Other Countries

Japan, Australia, and most of Western Europe also have improved surface water quality in recent years. Sewage treatment in the wealthier countries of Europe generally equals or surpasses that in the United States. Sweden, for instance, serves 98 percent of its population with at least secondary sewage treatment (compared with 70 percent in the United States), and the other 2 percent have primary treatment. Poorer countries have much less to spend on sanitation. Spain serves only 18 percent of its population with even primary sewage treatment. In Ireland, it is only 11 percent, and in Greece, less than 1 percent of the people have even primary treatment. Most of the sewage, both domestic and industrial, is dumped directly into the ocean.

This lack of pollution control is reflected in inland water quality as well. In Poland, 95 percent of all surface water is unfit to drink. The Vistula River, which winds through the country's most heavily industrialized region, was so badly polluted in 1978 that only 432 of its 1068 km were suitable even for industrial use. It was reported to be "utterly devoid of life." Recently, however, the Polish government instituted an ambitious program to build domestic and industrial waste treatment plants and to clean up the river.

There are also some encouraging pollution control stories. In 1997, Minamata Bay in Japan, long synonymous with mercury poisoning, was declared officially clean again. Another important success is found in Europe, where one of its most important rivers has been cleaned up significantly through international cooperation. The Rhine, which starts in the rugged Swiss Alps and winds 1,320 km through five countries before emptying through a Dutch delta into the North Sea,

has long been a major commercial artery into the heart of Europe. More than 50 million people live in its catchment basin and nearly 20 million get their drinking water from the river or its tributaries. By the 1970s, the Rhine had become so polluted that dozens of fish species disappeared and swimming was discouraged along most of its length.

Efforts to clean up this historic and economically important waterway began in the 1950s, but a disastrous fire at a chemical warehouse near Basel, Switzerland, in 1986 provided the impetus for major changes. Through a long and sometimes painful series of international conventions and compromises, land-use practices, waste disposal, urban runoff, and industrial dumping have been changed and water quality has significantly improved. Oxygen concentrations have gone up fivefold since 1970 (from less than 2 mg/l to nearly 10 mg/l or about 90 percent of saturation) in long stretches of the river. Chemical oxygen demand has fallen fivefold during this same period, and organochlorine levels have decreased as much as tenfold. Many species of fish and aquatic invertebrates have returned to the river. In 1992, for the first time in decades, sexually mature salmon were caught in the Rhine.

The less-developed countries of South America, Africa, and Asia have even worse water quality than do the poorer countries of Europe. Sewage treatment is usually either totally lacking or woefully inadequate. In urban areas, 95 percent of all sewage is discharged untreated into rivers, lakes, or the ocean. Low technological capabilities and little money for pollution control are made even worse by burgeoning populations, rapid urbanization, and the shift of much heavy industry (especially the dirtier ones) from developed countries where pollution laws are strict to lessdeveloped countries where regulations are more lenient.

Appalling environmental conditions often result from these combined factors. Two-thirds of India's surface waters are contaminated sufficiently to be considered dangerous to human health. The Yamuna River in New Delhi has 7,500 coliform bacteria per 100 ml (thirty-seven times the level considered safe for swimming in the United States) *before* entering the city. The coliform count increases to an incredible 24 million cells per 100 ml as the river leaves the city! At the same time, the river picks up some 20 million l of industrial effluents every day from New Delhi. It's no wonder that disease rates are high and life expectancy is low in this area. Only 1 percent of

India's towns and cities have any sewage treatment, and only eight cities have anything beyond primary treatment.

In Malaysia, forty-two of fifty major rivers are reported to be "ecological disasters." Residues from palm oil and rubber manufacturing, along with heavy erosion from logging of tropical rainforests, have destroyed all higher forms of life in most of these rivers. In the Philippines, domestic sewage makes up 60 to 70 percent of the total volume of Manila's Pasig River. Thousands of people use the river not only for bathing and washing clothes but also as their source of drinking and cooking water. China treats only 2 percent of its sewage. Of seventy-eight monitored rivers in China, fifty-four are reported to be seriously polluted. Of fortyfour major cities in China, forty-one use "contaminated" water supplies, and few do more than rudimentary treatment before it is delivered to the public.

2.2.3 Groundwater and Drinking Water Supplies

About half the people in the United States, including 95 percent of those in rural areas, depend on underground aquifers for their drinking water. This vital resource is threatened in many areas by overuse and pollution and by a wide variety of industrial, agricultural, and domestic contaminants. For decades it was widely assumed that groundwater was impervious to pollution because soil would bind chemicals and cleanse water as it percolated through. Springwater or artesian well water was considered to be the definitive standard of water purity, but that is no longer true in many areas.

The U.S. EPA estimates that every day some 4.5 trillion l (1.2 trillion gal) of contaminated water seep into the ground in the United States from septic tanks, cesspools, municipal and industrial landfills and waste disposal sites, surface impoundments, agricultural fields, forests, and wells. The most important of these in terms of toxicity are probably waste disposal sites. Agricultural chemicals and wastes are responsible for the largest total volume of pollutants and area affected. It usually takes hundreds to thousands of years for most deep aquifers to turn over their water content, and many contaminants are extremely stable once underground. It is possible, but expensive, to pump water out of aquifers, clean it, and then pump it back. For very large aquifers, pollution may be essentially irreversible.

We don't know exactly how contaminated our aquifers already are because access is difficult, and testing is expensive. Results of recent groundwater studies have been alarming, however. In Iowa, pesticides and other synthetic chemicals were detected in half of all

wells tested. One-fifth of these wells had nitrate levels from fertilizer infiltration that exceeded federal standards.

In farm country, especially in the Midwest's Corn Belt, fertilizers and pesticides commonly contaminate aquifers and wells. Herbicides such as atrazine and alachlor are widely used on corn and soybeans and show up in about half of all wells tested in Iowa, for example. Nitrates from fertilizers often exceed safety standards in rural drinking water. These high nitrate levels are dangerous to infants (nitrate combines with hemoglobin in the blood and results in "blue-baby" syndrome). They also are transformed into cancer-causing nitrosamines in the human gut. In Florida, one thousand drinking water wells were shut down by state authorities because of excessive levels of toxic chemicals, mostly ethylene dibromide (EDB), a pesticide used to kill nematodes (roundworms) that damage plant roots.

The United States has at least 2.5 million underground chemical storage tanks. Many of these tanks were left behind at abandoned gasoline stations or industrial sites, their exact whereabouts and contents unknown. The EPA estimates that about 42 million l (11 million gal) of gasoline are lost each year from leaking underground storage tanks (LUST). Considering that a single gallon of gasoline can make an aquifer unsuitable for drinking, these old, rusting, forgotten tanks represent a problem of tremendous proportions. The EPA now requires that all new tanks have double walls or be placed in concrete vaults to help prevent leaks into groundwater.

Aquifers in the United States also are threatened by direct injection of wastes. Every year, 38 billion l (10 billion gal) of liquid wastes, such as oil field brine, effluents from chemical plants, and treated sewage, are pumped down deep wells as an alternative to incineration or other treatment. The EPA estimates that 58 percent of all hazardous wastes generated are injected into deep wells. No permits are required, nor are there any limits on where or how wastes are pumped. Opponents of this disposal method argue that we don't know exactly how underground aquifers are connected or how toxic substances might flow through them, but it seems likely that some of those wastes have made, or will make, their way into aquifers used for domestic and municipal water supplies.

Abandoned wells represent another major source of groundwater contamination. Most domestic wells have no casings to prevent surface contaminants from leaking directly into aquifers that they penetrate. When these wells are no longer in use, they often are not capped

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adequately, and people forget where they are. They become direct routes for drainage of surface contaminants into aquifers. Oil wells and municipal water wells have casings to prevent leakage into aquifers, but these casings corrode and crack as they age.

In addition to groundwater pollution problems, contaminated surface waters and inadequate treatment make drinking water unsafe in many areas. A 1996 survey concluded that nearly 20,000 public drinking water systems in the United States expose consumers to contaminants such as lead, pesticides, and pathogens at levels that violate EPA rules. A vast majority of these systems are small, serving fewer than 3,000 people, but altogether some 50 million people are sometimes at risk. Problems often occur because small systems can't afford modern purification and distribution equipment, regular testing, and trained operators to bring water quality up to acceptable standards. Some aging central cities find themselves in a similar situation. Boston, for instance, still uses some wooden pipelines originally installed more than a century ago for water distribution.

Every year epidemiologists estimate that around 1.5 million Americans fall ill from infections caused by fecal contamination. In 1993, for instance, a pathogen called cryptosporidium got into the Milwaukee public water system, making 400,000 people sick and killing at least 100 people. The total costs of these diseases amount to billions of dollars per year. Preventive measures such as protecting water sources and aquifer recharge zones, providing basic treatment for all systems, installing modern technology and distribution networks, consolidating small systems, and strength-packaging material, and other litter are tossed from ships every year into the ocean where they ensnare and choke seabirds, mammals, and even fish. Sixteen states now require that sixpack yokes be made of biodegradable or photodegradable plastic, limiting their longevity as potential killers. The amount of municipal and industrial plastic that finds its way to the ocean is unknown but immense. In one day, volunteers in Texas gathered more than three hundred tons of plastic refuse from Gulf Coast beaches.

Few coastlines in the world remain uncontaminated by oil or oil products. Tar granules and sticky crude oil droplets stick to feet on beaches everywhere. Because oil floats on water, it is easily detected on the open ocean. Where ening the Clean Water Act and the Safe Drinking Water Act would cost far less. Unfortunately, in the present climate of budget-cutting and anti-regulation, these steps seem unlikely.

Relatively little information is available about groundwater quality

in most countries, especially in less-developed countries, because (1) it is expensive to drill test wells and to monitor pollutants and (2) this is not yet a major priority. In Europe, where fertilizer use is even more intensive than in the United States, nitrate levels in groundwater are reported to be alarmingly high in many areas. Britain, for instance, calculates that half of its underground reservoirs have been contaminated by fertilizer nitrates. China reports that forty-one of forty-four large cities suffer from polluted groundwater. As agricultural modernization increases the use of fertilizer and pesticides in less-developed countries, we may see more groundwater pollution there as well.

2.2.4 Ocean Pollution

During the summer of 1988, bathers from New Jersey to Massachusetts experienced unwelcome firsthand evidence of increasing levels of ocean pollution. They found floating garbage, ranging from untreated sewage to used drug paraphernalia and medical wastes, washing up on their favorite beaches. One author reported that the experience was as safe and appealing as bathing in an unflushed toilet.

This distressing situation is only one aspect of a global problem. Near-shore zones around the world, especially bays, estuaries, shoals, and reefs near large cities or the mouths of major rivers, are being overwhelmed by human-caused contamination. Suffocating and sometimes poisonous blooms of algae regularly deplete ocean waters of oxygen and kill enormous numbers of fish and other marine life. High levels of toxic chemicals, heavy metals, disease-causing organisms, oil, sediment, and plastic refuse are adversely affecting some of the most attractive and productive ocean regions. The potential losses caused by this pollution amount to billions of dollars each year. In terms of quality of life, the costs are incalculable. Oceanographer Jacques Cousteau warned that the oceans are dying and that our own survival is threatened.

One of the most massive and least understood sources of this pollution is agricultural and urban runoff. Fertilizers, manure, pesticides, and crop residues from farm fields combine with oil, rubber, metals, salts, and other urban contaminants and are carried by rivers to the ocean. Industrial wastes and municipal sewage effluents are also chronic pollution sources of near-shore ocean zones. In the United States, 1,300 major industrial and 600 municipal facilities dump untreated wastewater directly into estuaries and coastal regions. Thousands of other facilities discharge a variety of toxic wastes into rivers that run into the oceans.

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Discarded plastic flotsam and jetsam are becoming an ubiquitous mark of human impact on the oceans. Since plastic is lightweight and nonbiodegradable, it is carried thousands of miles on ocean currents and lasts for years. Even the most remote beaches of distant islands are likely to have bits of polystyrene foam containers or polyethylene packing material that were discarded half a world away by some careless person. It has been estimated that some 6 million metric tons of plastic bottles, visible oil slicks have been reported. Oceanographers estimate that somewhere between 3 million and 6 million metric tons of oil are discharged into the world's oceans each year from both land- and sea-based operations. About half of this amount is due to maritime transport. Most of the 40 million liters of discharge (nearly 11 million gal) is not from dramatic, headline-making accidents, such as the 1989 oil spill from the *Exxon Valdez* in Prince William Sound, Alaska, but from routine open-sea bilge pumping and tank cleaning, which are illegal but, nonetheless, are carried out once ships are beyond sight of land. Much of the rest comes from land-based municipal and industrial runoff or from atmospheric deposition of residues from refining and combustion of fuels.

The transport of huge quantities of oil creates opportunities for major pollution episodes through a combination of human and natural hazards. Military conflict in the Middle East and increasing amounts of oil being pumped and shipped from off-shore drilling areas in inhospitable places, such as the notoriously rough North Sea and the Arctic Ocean, make it likely that more oil spills will occur. Plans to drill for oil along the seismically active California and Alaska coasts have been controversial because of the damage that oil spills could cause to these biologically rich coastal ecosystems.

The toxic chemicals of all sorts that we dump into the ocean are having deadly effects on marine life. Around the world tragic die-offs of marine mammals have been reported with increasing frequency. Often snouts, flippers, and tails are pocked with blisters and craters. Others have huge patches of skin sloughed off. The most likely cause of these distressing conditions is viral infections.

It is thought that exposure to pesticides and other water pollutants may have weakened the mammals' immune systems and made them susceptible to infections. Harbor seals in the Gulf of Maine have the highest pesticide levels of any U.S. mammals on land or sea. Fishers bring up lobsters and crabs with gaping holes in their shells, and fish have rotted fins and ulcerous lesions. In Louisiana, 35 percent of the

oyster beds have been closed because of sewage contamination. Japan's heavily used inland sea has more than two hundred toxic red tides each year. One of these episodes killed more than 1 million yellowtail that would have been worth \$15 million in the market.

We often don't know exactly which pollutant is causing these distressing biological effects. In many cases, the cause may not be a single pollutant but a complex series of interactions in marine ecosystems that have many manifestations. Sometimes symptoms of shifting balances may not be noticeable until a disastrous population crash occurs. We have always assumed that the oceans are so vast that their capacity to absorb and neutralize contaminants would be inexhaustible. Marine ecosystems do have an enormous ability to recover from pollution episodes and to regenerate biological communities. Some enormous oil spills, such as the wreck of the *Amoco Cadiz* on the coast of France in 1978 or the blowout of a Mexican oil well in the Gulf of Mexico in 1979, had far less disastrous consequences than had been feared. Many scientists feel that localized, short-term pollution episodes in warm tropical waters may not cause serious long-term damage. Spills in cold arctic waters, like that of the *Exxon Valdez*, may take much longer to dissipate and may cause much more damage. Attention is now turning more to the chronic, land-based pollution from industrial, municipal, and agricultural wastes that slowly build up until they overwhelm natural systems and destroy the ocean's regenerative capacity.

2.3 WATER POLLUTION CONTROL

Appropriate land-use practices and careful disposal of industrial, domestic, and agricultural wastes are essential for control of water pollution.

2.3.1 Source Reduction

In many cases, the cheapest and most effective way to reduce pollution is to avoid producing it or releasing it to the environment in the first place. Elimination of lead from gasoline has resulted in a widespread and significant decrease in the amount of lead in surface waters in the United States. Studies have shown that as much as 90 percent less road deicing salt can be used in many areas without significantly affecting the safety of winter roads. Careful handling of oil and petroleum products can greatly reduce the amount of water pollution caused by these materials. Although we still have problems with persistent chlorinated hydrocarbons spread widely in the environment, the banning of DDT and PCBs in the 1970s has resulted in significant reductions in levels in wildlife.

Industry can modify manufacturing processes so fewer wastes are created. Recycling or reclaiming materials that otherwise might be discarded in the waste stream also reduces pollution. Both of these approaches usually have economic as well as environmental benefits. "Sewering" of heavy metals by industry has been outlawed. Producers are required instead to separate their wastes. It turns out that a variety of valuable metals can be recovered from wastes and reused or sold for other purposes. The company benefits by having a product to sell, and the municipal sewage treatment plant benefits by not having to deal with highly toxic materials mixed in with millions of gallons of other types of wastes.

2.3.2 Nonpoint Sources and Land Management

Among the greatest remaining challenges in water pollution control are diffuse, nonpoint pollution sources. Unlike point sources, such as sewer outfalls or industrial discharge pipes, which represent both specific locations and relatively continuous emissions, nonpoint sources have many origins and numerous routes by which contaminants enter ground and surface waters. It is difficult to identify-let alone monitor and control-all these sources and routes. Some main causes of nonpoint pollution are:

- 1 *Agriculture:* The EPA estimates that 60 percent of all impaired or threatened surface waters are affected by sediment from eroded fields and overgrazed pastures; fertilizers, pesticides, and nutrients from croplands; and animal wastes from feedlots.
- 1 *Urban runoff.* Pollutants carried by runoff from streets, parking lots, and industrial sites contain salts, oily residues, rubber, metals, and many industrial toxins. Yards, golf courses, parklands, and urban gardens often are treated with far more fertilizers and pesticides per unit area than farmlands. Excess chemicals are carried by storm runoff into waterways.
- 1 *Construction sites:* New buildings and land development projects such as highway construction affect relatively small areas but produce vast amounts of sediment, typically ten to twenty times as much per unit area as farming.
- 1 *Land disposal:* When done carefully, land disposal of certain kinds of industrial waste, sewage sludge, and biodegradable garbage can be a good way to dispose of unwanted materials. Some poorly run land disposal sites, abandoned dumps, and leaking septic systems, however, contaminate local waters.

Often the best way to control nonpoint pollution is through improved land-use practices. Measures can be taken to decrease runoff and erosion by maximizing cover on croplands, terracing slopes, increasing soil water retention, prohibiting logging or cultivation on steep slopes, protecting vegetation in and along watercourses, and banning clear-cutting of forests. Generally these methods are the same ones that conserve soil. Applying precisely determined amounts of fertilizer, irrigation water, and pesticides saves money and reduces contaminants entering the water. Preserving wetlands that act as natural processing facilities for removing sediment and contaminants helps protect surface and groundwaters.

In urban areas, reducing materials carried away by storm runoff is helpful. Citizens can be encouraged to recycle waste oil and to minimize use of fertilizers and pesticides. Regular street sweeping greatly reduces contaminants. Runoff can be diverted away from streams and lakes. Many cities are separating storm sewers and municipal sewage lines to avoid overflow during storms. Contractors generally are required to place erosion barriers around construction sites to contain sediments.

One of the best examples of watershed management now in place in the United States is that for the Chesapeake Bay, America's largest estuary. Once famed for its abundant oysters, crabs, shad, striped bass, and other valuable fisheries, the Bay had deteriorated seriously by the early 1970s. Citizens' groups, local communities, state legislatures, and the federal government together established an innovative pollution control program that made the Bay the first estuary in America targeted for protection and restoration.

Among the principal objectives of this plan is reducing nutrient loading through land-use regulations in the six watershed states to control agricultural and urban runoff. Pollution prevention measures such as banning phosphate detergents also are important, as are upgrading wastewater treatment plants and improving compliance with discharge and filling permits. Efforts are underway to replant thousands of hectares of seagrasses and to restore wetlands that filter out pollutants. In the 1980s, annual phosphorous discharges into the Bay dropped 40 percent. Nitrogen levels, however, have remained constant or have even risen in some tributaries. Although progress has been made, the goals of reducing both nitrogen and phosphate levels by 40 percent and restoring viable fish and shellfish populations are still decades away. Still, as EPA Administrator Carol Browner says, it demonstrates the "power of cooperation" in environmental protection.

2.3.3 Human Waste Disposal

As we have already seen, human and animal wastes usually create the most serious health-related water pollution problems. More than 500 types of disease-causing (pathogenic) bacteria, viruses, and parasites can travel from human or animal excrement through water. In this section, we will look at how to prevent the spread of these diseases.

2.3.3.1 Natural Processes

In the poorer countries of the world, most rural people simply go out into the fields and forests to relieve themselves as they have always done. Where population densities are low, natural processes eliminate wastes quickly, making this an effective method of sanitation. The high population densities of cities make this practice unworkable, however. Even major cities of many less-developed countries are often littered with human waste which has been left for rains to wash away or for pigs, dogs, flies, beetles, or other scavengers to consume. This is a major cause of disease, as well as being extremely unpleasant. Studies have shown that a significant portion of the airborne dust in Mexico City is actually dried, pulverized human feces.

Where intensive agriculture is practiced-especially in wet rice paddy farming in Asia-it has long been customary to collect "night soil" (human and animal waste) to be spread on the fields as fertilizer. This waste is a valuable source of plant nutrients, but it is also a source of disease-causing pathogens in the food supply. It is the main reason that travelers in less-developed countries must be careful to surface sterilize or cook any fruits and vegetables they eat. Collecting night soil for use on farm fields was common in Europe and America until about one hundred years ago when the association between pathogens and disease was recognized.

Until about fifty years ago, most rural American families and quite a few residents of towns and small cities depended on a pit toilet or "outhouse" for waste disposal. Untreated wastes tended to seep into the ground, however, and pathogens sometimes contaminated drinking water supplies. The development of septic tanks and properly constructed drain fields represented a considerable improvement in public health. In a typical septic system, wastewater is first drained into a septic tank. Grease and oils rise to the top and solids settle to the bottom, where they are subject to bacterial decomposition. The clarified effluent from the septic tank is channeled out through a drainfield of small perforated pipes embedded in gravel just below

the surface of the soil. The rate of aeration is high in this drainfield so that pathogens (most of which are anaerobic) will be killed, and soil microorganisms can metabolize any nutrients carried by the water. Excess water percolates up through the gravel and evaporates. Periodically, the solids in the septic tank are pumped out into a tank truck and taken to a treatment plant for disposal.

Where land is available and population densities are not too high, this can be an effective method of waste disposal. It is widely used in suburban areas, but as suburban densities grow, groundwater pollution often becomes a problem, indicating the need to shift to a municipal sewer system. It doesn't work well in cold, rainy climates where the drainfield may be too wet for proper evaporation, or where the water table is close to the surface.

2.3.3.2 Municipal Sewage Treatment

Over the past one hundred years, sanitary engineers have developed ingenious and effective municipal wastewater treatment systems to protect human health, ecosystem stability, and water quality. This topic is an important part of pollution control, and is a central focus of every municipal government; therefore, let's look more closely at how a typical municipal sewage treatment facility works.

Primary treatment is the first step in municipal waste treatment. It physically separates large solids from the waste stream. As raw sewage enters the treatment plant, it passes through a metal grating that removes large debris. A moving screen then filters out smaller items. Brief residence in a grit tank allows sand and gravel to settle. The waste stream then moves to the primary sedimentation tank where about half the suspended, organic solids settle to the bottom as sludge. Many pathogens remain in the effluent and it is not yet safe to discharge into waterways or onto the ground.

Secondary treatment consists of biological degradation of the dissolved organic compounds. The effluent from primary treatment flows into a trickling filter bed, an aeration tank, or a sewage lagoon. The trickling filter is simply a bed of stones or corrugated plastic sheets through which water drips from a system of perforated pipes or a sweeping overhead sprayer. Bacteria and other microorganisms in the bed catch organic material as it trickles past and aerobically decompose it.

Aeration tank digestion is also called the activated sludge process. Effluent from primary treatment is pumped into the tank and mixed with a bacteria-rich slurry. Air pumped through the mixture encourages

bacterial growth and decomposition of the organic material. Water flows from the top of the tank and sludge is removed from the bottom. Some of the sludge is used as an inoculum for incoming primary effluent. The remainder would be valuable fertilizer if it were not contaminated by metals, toxic chemicals, and pathogenic organisms. The toxic content of most sewer sludge necessitates disposal by burial in a landfill or incineration. Sludge disposal is a major cost in most municipal sewer budgets.

Where space is available for sewage lagoons, the exposure to sunlight, algae, aquatic organisms, and air does the same job more slowly but with less energy costs. Effluent from secondary treatment processes is usually disinfected with chlorine, UV light, or ozone to kill harmful bacteria before it is released to a nearby waterway.

Tertiary treatment removes plant nutrients, especially nitrates and phosphates, from the secondary effluent. Although wastewater is usually free of pathogens and organic material after secondary treatment, it still contains high levels of inorganic nutrients, such as nitrates and phosphates. When discharged into surface waters, these nutrients stimulate algal blooms and eutrophication. To preserve water quality, these nutrients also must be removed. Passage through a wetland or lagoon can accomplish this or chemicals often are used to bind and precipitate nutrients.

In many American cities, sanitary sewers are connected to storm sewers, which carry runoff from streets and parking lots. A large line called an interceptor delivers the combined stream of storm runoff and domestic and industrial waste to the municipal treatment plant. Storm sewers are routed to the treatment plant rather than discharged into surface waters because runoff from streets, yards, and industrial sites generally contains a variety of refuse, fertilizers, pesticides, oils, rubber, tars, lead (from gasoline), and other undesirable chemicals. During dry weather, this plan works well. Heavy storms often overload the system, however, causing bypass dumping of large volumes of raw sewage and toxic surface runoff directly into receiving waters. To prevent this overflow, cities are spending hundreds of millions of dollars to separate storm and sanitary sewers. These are huge, disruptive projects. When they are finished, surface runoff will be diverted into a river or lake and cause another pollution problem.

2.3.3.3 Low-Cost Waste Treatment

The municipal sewage systems used in developed countries are often too expensive to build and operate in the developing world where

low-cost, low-tech alternatives for treating wastes are needed. One option is *effluent sewerage*, a hybrid between a traditional septic tank and a full sewer system. A tank near each dwelling collects and digests solid waste just like a septic system. Rather than using a drainfield, however, to dispose of liquids-an impossibility in crowded urban areas-effluents are pumped to a central treatment plant. The tank must be emptied once a year or so, but because only liquids are treated by the central facility, pipes, pumps, and treatment beds can be downsized and the whole system is much cheaper to build and run than a conventional operation.

Another alternative is to use natural or artificial wetlands to dispose of wastes. Arcata, California, for instance, needed an expensive sewer plant upgrade. By transforming a 65-hectare (160acre) garbage dump into a series of ponds and marshes that serve as a simple, low-cost waste treatment facility, the city saved millions of dollars and improved the environment simultaneously. Sewage is piped to holding ponds where solids settle out and are digested by bacteria and fungi. Effluent flows through marshes where it is filtered and cleansed by aquatic plants and microorganisms. The marsh is a haven for wildlife and has become a prized recreation area for the city. Eventually, the purified water flows into the bay where marine life flourishes.

Similar wetland waste treatment systems are now operating in many developing countries. Effluent from these operations can be used to irrigate crops or raise fish for human consumption if care is taken to first destroy pathogens. Usually 20 to 30 days of exposure to sun, air, and aquatic plants is enough to make the water safe. These systems make an important contribution to human food supplies. A 2,500-hectare (6,000-acre) waste-fed aquaculture facility in Calcutta, for example, supplies about 7,000 metric tons of fish annually to local markets. The World Bank estimates that some 3 billion people will be without sanitation services by the middle of the next century under a business-as-usual scenario. With investments in innovative programs, however, sanitation could be provided to about half those people and a great deal of misery and suffering could be avoided.

2.4 WATER LEGISLATION

Water pollution control has been among the most broadly popular and effective of all environmental legislation in the United States. It has not been without controversy, however. In this section, we will look at some of the major issues concerning water quality laws and their provisions.

2.4.1 The Clean Water Act

Passage of the Clean Water Act of 1972 was a bold, bipartisan step determined to “restore and maintain the chemical, physical, and biological integrity of the Nation’s waters” that made clean water a national priority. Along with the Endangered Species Act and the Clean Air Act, this is one of the most significant and effective pieces of environmental legislation ever passed by the U.S. Congress. It also is an immense and complex law, with more than 500 sections regulating everything from urban runoff, industrial discharges, and municipal sewage treatment to land-use practices and wetland drainage.

The ambitious goal of the Clean Water Act was to return all U.S. surface waters to “fishable and swimmable” conditions. For specific “point” sources of pollution such as industrial discharge pipes or sewage outfalls, the act requires discharge permits and *best practicable control technology* (BPT). It sets national goals of *best available, economically achievable technology* (BAT), for toxic substances and zero discharge for 126 priority toxic pollutants. As we discussed earlier in this chapter, these regulations have had a positive effect on water quality. While not yet swimmable or fishable everywhere, surface water quality in the United States has significantly improved on average over the past quarter century. Perhaps the most important result of the act has been investment of \$54 billion in federal funds and more than \$128 billion in state and local funds for municipal sewage treatment facilities.

Not everyone, however, is completely happy with the Clean Water Act. Industries, state and local governments, farmers, land developers, and others who have been forced to change their operations or spend money on water protection often feel imposed upon. One of the most controversial provisions of the act has been Section 404, which regulates draining or filling of wetlands. Although the original bill only mentions wetlands briefly, this section has evolved through judicial interpretation and regulatory policy to become one of the principal federal tools for wetland protection. Environmentalists applaud the protection granted to these ecologically important areas that were being filled in or drained at a rate of about half a million hectares per year before the passage of the Clean Water Act. Farmers, land developers, and others who are prevented from converting wetlands to other uses often are outraged by what they consider “taking” of private lands.

Another sore point for opponents of the Clean Water Act are what are called “unfunded mandates,” or requirements for state or local governments to spend money that is not repaid by Congress.

You will notice that the \$128 billion already spent by cities to install sewage treatment and stormwater diversion to meet federal standards far exceeds the \$54 billion in congressional assistance for these projects. Estimates are that local units of government could be required to spend another \$130 billion to finish the job without any further federal funding. Small cities that couldn't afford or chose not to

Table 2.2 Some important U.S. water quality legislation.

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1. *Federal Water Pollution Control Act* (1972). Established uniform nationwide controls for each category of major polluting industries.
 2. *Marine Protection Research and Sanctuaries Act* (1972). Regulates ocean dumping and established sanctuaries for protection of endangered marine species.
 3. *Ports and Waterways Safety Act* (1972). Regulates oil transport and the operation of oil handling' facilities.
 4. *Safe Drinking Water Act* (1974). Requires minimum safety standards for every community water supply. Among the contaminants regulated are bacteria, nitrates, arsenic, barium, cadmium, chromium, fluoride, lead, mercury, silver, pesticides; radioactivity and turbidity also regulated. This act also contains provisions to protect groundwater aquifers.
 5. *Resource Conservation and Recovery Act* (RCRA) (1976). Regulates the storage, shipping, processing, and disposal of hazardous wastes and sets limits on the sewerage of toxic chemicals.
 6. *Toxic Substances Control Act* (TOSCA) (1976). Categorizes toxic and hazardous substances, establishes a research program, and regulates the use and disposal of poisonous chemicals.
 7. *Comprehensive Environmental Response, Compensation, and Liability Act* (CERCLA) (1980) and *Superfund Amendments and Reauthorization Act* (SARA) (1984). Provide for sealing, excavation, or remediation of toxic and hazardous waste dumps.
 8. *Clean Water Act* (1985) (amending the 1972 Water Pollution Control Act). Sets as a national goal the attainment of "fishable and swimmable" quality for all surface waters in the United States.
 9. *London Dumping Convention 1990*. Calls for an end to all ocean dumping of industrial wastes, tank washing effluents, and plastic trash. The United States is a signatory to this international convention.
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participate in earlier programs in which the federal government paid up to 90 percent of water quality programs are especially hard hit by requirements that they upgrade municipal sewer and water systems. They now are faced with carrying out those same projects entirely on their own funds.

2.4.2 Clean Water Act Reauthorization

The Clean Water Act has been amended many times, with the most substantial changes occurring in 1977 and 1987. In 1995, as a part of the conservative Republican "Contract with America," the U.S. House of Representatives passed a bill that would have significantly weakened water quality protection. Only a flood of public opposition and a veto threatened by President Clinton prevented this bill from becoming law. Although the crusade to revise the Clean Water Act seems to have diminished for now, it is important to understand what industry groups, local governments, land developers, and others hoped to accomplish.

Among the provisions of the 1995 "dirty water act," as it was called by environmental groups, were:

- "Regulatory relief" from permitting and reporting provisions that would allow much greater releases of toxic chemicals and wastes into waterways.
- Elimination of requirements for pre-treating industrial wastes before discharge into municipal sewer systems.
- Weakening of federal regulations for agricultural and urban runoff.
- Redefinition of wetlands to remove protection for about half of all remaining wetlands.
- Easing of drinking water standards to allow less frequent testing and higher levels of toxins.
- Permission for cities to dump sewage into coastal waters without secondary treatment.
- Cost/benefit analysis that gives greater weight to economic interests in all environmental planning.
- Payments to landowners whose property values are diminished by federal regulations.

These proposals not only failed to pass Congress but many incumbents who voted for them in 1995 lost their seats two years later in election races based mainly on their environmental records. But you can be sure that these issues will come up again. Issues of

private property rights and questions about how much environmental protection is necessary and who will pay for it remain foremost in many areas of environmental politics.

Even those who support the Clean Water Act in principle would like to see it changed and strengthened. Among these proposals are a shift from "end-of-the-pipe" focus on removing specific pollutants from effluents to more attention to changing industrial processes so toxic substances won't be produced in the first place. Another important issue is nonpoint pollution from agricultural runoff and urban areas, which has become the largest source of surface water degradation in the United States. Regulating these sources remains a difficult problem.

Environmentalists also would like to see stricter enforcement of existing regulations, mandatory minimum penalties for violations, more effective community right-to-know provisions, and increased powers for citizen lawsuits against polluters. Under the current law, using data that polluters themselves are required to submit, groups such as the Natural Resources Defense Council and the Citizens for a Better Environment have won million-dollar settlements in civil law suits (the proceeds generally are applied to clean-up projects) and some transgressors have even been sent to jail. Not surprisingly, environmentalists want these powers expanded while polluters find them very disagreeable.

2.4.3 Other Important Water Legislation

In addition to the Clean Water Act, several other laws help to regulate water quality in the United States and abroad. Among these is the Safe Drinking Water Act, which regulates water quality in commercial and municipal systems. Critics complain that standards and enforcement policies are too lax, especially for rural water districts and small towns. Some researchers report pesticides, herbicides, and lead in drinking water at levels they say should be of concern. Atrazine, for instance, a widely used herbicide, was detected in 96 percent of all surface water samples in one study of 374 communities across twelve states. Remember, however, that simply detecting a toxic compound is not the same as showing dangerous levels.

The Superfund program for remediation of toxic waste sites was created in 1980 by the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) and was amended by the Superfund Amendments and Reauthorization Act (SARA) of 1984. This program is designed to provide immediate response to emergency situations and to provide permanent remedies for abandoned or inactive

sites. These programs provide many jobs for environmental science majors in monitoring and removal of toxic wastes and landscape restoration. A variety of methods have been developed for remediation of problem sites.

Among the most important international agreements on water quality is the 1972 Great Lakes Water Quality Agreement between Canada and the United States. The agreement has produced encouraging progress in cleaning up the world's largest freshwater system. Another important international agreement is the 1990 London Dumping Convention, which calls for phasing out all ocean dumping of industrial waste, tank-washing effluent, and plastic trash by 1995. The sixty-four nations that have signed the Law of the Sea Treaty are bound by this agreement. The United States has already passed legislation to support its provisions. Whether this can be enforced remains to be seen, however.

Is it safe to assume that we are well on our way to solving our water pollution problems? We are better off in terms of legislation, policy, and practice than we were in 1960. Laws, however, are only as good as (1) the degree to which they are not weakened by subsequent amendments and exceptions and (2) the degree to which they are funded for research and enforcement. Economic interests cause continued pressure on both of these points, so that the importance of an overriding national attitude to maintain the intent of protective legislation must continually be stressed and retaught.

3

Residential Waste

As we carry on our daily lives, each of us produces a wide variety of waste products, ranging from our body excrements through wastepaper, glass, and other trash from the products we use. These “personal” wastes are called *domestic wastes*, since they originate primarily in homes, offices, schools, and stores. Thus they are distinguished from *industrial wastes*, that come primarily from factories, and from agricultural wastes from farm operations.

Domestic wastes are divided into two categories: *sewage wastes* and *solid wastes*, based on the method of handling. Sewage wastes are all materials that are washed or flushed down drains into the sanitary sewer system. Personal excrementsurine and fecal matter are of primary importance but other materials that go down the drain also enter into the problem of treatment and disposal. Solid wastes are all materials that go out in the trash and are handled as solids. The handling and disposal of both sewage and solid wastes pose many environmental problems. The first part of this chapter will focus on sewage wastes; the second, on solid wastes.

3.1 TREATMENT AND DISPOSAL OF SEWAGE WASTES

3.1.1 Historical Perspective

To gain a better understanding of the status of sewage treatment in the United States, some background information will be helpful. Urine and fecal wastes contain the mineral nutrients that were originally absorbed by plants. In natural ecosystems these wastes are recycled back through the soil, assuring a steady supply of nutrients for continuing plant growth. The fundamental importance of this cycle has been recognized by some human societies: For many centuries

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the Chinese have returned their excrements to their agricultural fields. However, Western cultures still largely fail to appreciate the nutrient value of wastes and generally treat them with disinterest if not disdain. As long as populations were relatively sparse, outdoor privies sufficed. When the stench became too obnoxious, the latrine was moved to a new hole. But, with the intense urbanization resulting from the Industrial Revolution, wastes accumulated in the back-to-back privies until conditions became almost unbearable.

Sewer systems had been constructed in cities for the purpose of draining off storm water, not for the removal of human wastes. In fact, many cities had ordinances prohibiting the dumping of wastes into the storm sewers, which persisted until the turn of the century.

However, in the latter half of the 1800's, it was discovered that certain dread diseases such as typhoid fever and cholera were caused by bacteria which were spread by the contamination of water supplies with human wastes. Intensive efforts therefore began to clean up and protect water supplies by funneling human wastes out and away from cities. The flush toilet was developed and, expediently, pipes for wastes were tapped into the storm water sewers which led into streams and rivers. This was certainly the fastest way to clean up cities, and it was not considered imprudent. After all, people believed then, and the saying still persists, that a natural stream or river purifies itself every 10 miles.

Streams do self-purify, but to only a limited extent. Organic detritus dumped into a stream serves as food for bacteria, the first step of a food chain which leads through protozoans and insect larvae to fish. Thus, the stream ecosystem will purify itself of organic detritus and produce fish in return. However, this works only as long as the system is not overloaded. If too much organic matter is put in, the growth of bacteria is so prolific that protozoans, and the rest of the food chain, cannot keep the balance. The population of bacteria becomes so large that they use all the available oxygen in their respiration, suffocating the other organisms. Thus, little remains but dead organic matter, bacteria, and the foul-smelling waste products of the bacterial metabolism.

Consequently, waterways became hopelessly polluted as human wastes from cities were funneled through storm sewers into streams and rivers. In many cases, streams became so bad that they were simply roofed over and incorporated as part of the sewer system. Eventually, to alleviate the pollution of important waterways, cities

began to construct sewage treatment plants at the principal sewage outfalls.

In spite of these efforts, we have not caught up with the situation. First, there are still rural communities and even pockets in urban areas that continue to use outdoor privies. In some areas that do have indoor plumbing, toilets and other drains still empty directly into the nearest stream. Not only do old urban areas lack appropriate disposal systems, but many new suburban developments are constructed without central sewer systems. Instead, each home has its own septic system (to be discussed later). In theory, these individual systems are adequate for waste disposal, but in practice they tend to clog up after some years of operation and the raw sewage then forces its way to the surface, producing a situation that is little improved from conditions in the nineteenth century.

Second, as new areas are hooked into a central sewer system, it is often found that pipes in older parts of the system are simply not large enough to handle the additional flow, especially at times when additional storm water enters the system. To prevent sewage from backing up into homes, it has been routine practice to install overflow valves in the sewer lines. When pressures become too great, these valves allow the excess sewage to overflow directly into any convenient stream or river. Even so, many people have experienced raw sewage bubbling out of their drains during storms.

Third, the legacy of interconnected storm and sanitary sewers creates a tremendous problem at the sewage treatment plant. In times of heavy rains, the storm water added to the sewage water creates a volume so great that treatment plants cannot handle the total flow. Again, the excess flow is simply allowed to bypass the treatment plant, and raw sewage is discharged into the *receiving* body—the lake, river, stream or ocean which receives the wastes. Until recently, many American cities have been able to treat no more than half of the sewage water during heavy rains, the other half going directly into the receiving body. In yet other areas, populations have grown too large for the sewage treatment plant to handle even the normal flow of sewage.

Finally, the treatment processes provided in sewage treatment plants are seldom adequate to remove many of the pollutants now found in sewage water. Consequently, environmental problems continue to arise from the discharge of even treated wastes. Lack of adequate treatment is also a tremendous human health problem in that many

of the lakes that receive human sewage also serve as sources of drinking water. Such water is generally treated with chlorine to kill organisms that may cause disease, but is seldom treated in a way that will remove all the chemical pollutants. In short, Americans may laugh at the Chinese for putting sewage on their agricultural soils, but we often put sewage into our drinking water.

To overcome these problems, laws in most areas now require that separate storm and sanitary sewerage systems be installed in new developments. However, law is only as good as its enforcement, and it is not unusual to find cases in current developments where builders have tapped roof and patio drains into the sanitary sewers as a matter of expediency. Also, sanitary sewers are still occasionally tapped into storm drains, in spite of building codes.

Local governments have ongoing programs in expanding and upgrading sewage collection systems and more ambitious jurisdictions are gradually undertaking to dig up the old interconnected systems and install separate storm and sewage systems. Similarly, sewage treatment plants are gradually being expanded and upgraded. However, a general lack of public interest, demonstrated by the failure to vote for bond issues, higher sewer assessments, or other means of financing for such projects, is a tremendous deterrent to such improvements.

Do people really prefer polluted rivers and beaches rather than the payment of a few extra tax dollars a year for adequate sewage treatment? Probably not. The explanation may be in a lack of understanding of how the drains of our spotless kitchens and bathrooms are connected to our polluted beaches. In the following discussion we will attempt to make this connection clearer.

3.1.2 Sewage Water-Its Treatment and Disposal

3.1.2.1 Materials Contained in Sewage Water

Sewage or wastewater is divided into water itself and three categories of pollutants: *suspended solids*, *colloidal materials*, and *dissolved material*.

3.1.2.1.1 Water

By far the largest constituent of wastewater is water itself. Even without dilution with storm water, wastewater is about 99.9 percent water. All the contaminating or polluting material makes up only about one-tenth of one percent of the total mass, or one part in 1000 parts of water. The reason for this is that we use very large amounts of water to flush away very little bits of waste. In taking a bath or

shower, we may use 40-160 liters of water to remove a few grams of dirt from our bodies. Flushing a toilet requires three to five gallons of water. The amount of water used in washing dishes and clothes also is enormous when compared to the amount of dirt removed: 100-200 liters per load of laundry. Overall, an average of between 400 and 600 liters (100 and 150 gallons) of water per person per day goes down sewer drains.

This profligate use of water to flush small amounts of waste is beginning to strain freshwater resources in many areas, in addition to being a burden on treatment facilities. Some effort is being made to substitute the term *wastewater* for sewage to emphasize the fact that sewage is mostly just wasted water. As described below, the undesirable qualities result from relatively small amounts of polluting substances carried in the water. If polluting material is removed, the water may be used and reused in many ways.

3.1.2.1.2 *Suspended solids*

These materials are mostly inorganic sand and silt particles that originate primarily from soil and street runoff entering storm drains connected to the sanitary sewer system. Lesser amounts come from washing hands, clothing, and so forth. This material is carried along by the agitated water as it flows through pipes but it settles rapidly when the water becomes quiet. Material that is carried by water only through its constant mixing action is said to *be in suspension*; hence the name *suspended solids*. These solids do not constitute any great pollution hazard, but they must be removed early to prevent their interfering with later stages in the treatment process.

3.1.2.1.3 *Colloidal material*

This is mostly organic material, bits of undigested cellulose from feces, paper fibers (also cellulose) from toilet tissue, and fibers from clothing. A rapidly increasing proportion of colloidal material is ground garbage, because of the installation of garbage disposal units in sink drains. Bacteria, largely from fecal matter, are also placed in the category of colloidal material. Such material settles very slowly even in still water, and some may not settle at all. The presence of colloidal material may cause severe pollution problems in terms of both endangering human health and degrading the quality of natural waters.

Regarding human health, the bacterium that causes typhoid fever continues to grow and multiply to some degree in the intestinal tract of a person for many years after physical recovery from the disease. Also, some people may harbor low populations of typhoid bacteria

without ever showing symptoms of the disease. Such bacteria continue to be excreted in the feces. Consequently, untreated wastewater has a fair probability of carrying typhoid bacteria. Likewise bacteria, viruses, and parasites responsible for many other diseases may also be present. Obviously effective sewage treatment demands that such organisms be killed or at least that human contact and reinfection be prevented.

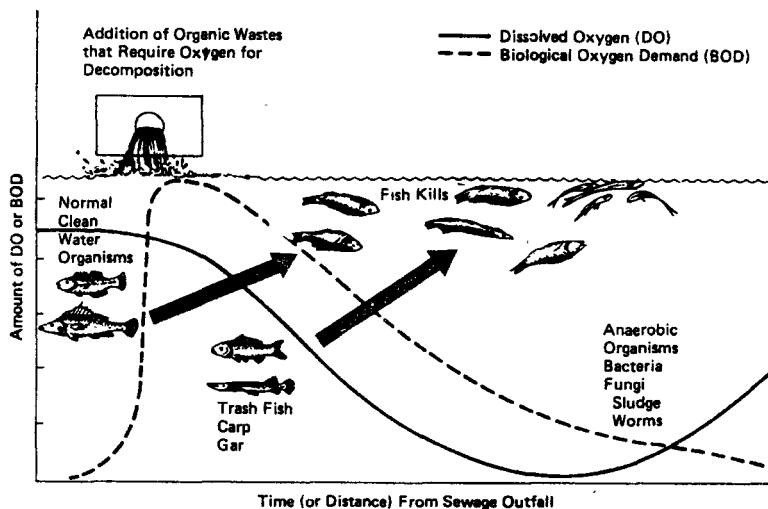


Figure 3.1 Dissolved oxygen (DO) and biological oxygen demand (BOD). Dissolved oxygen is crucial in supporting fish and other aquatic organisms. Natural organic wastes may cause death of fish because their decomposition uses oxygen (BOD). The dissolved oxygen concentration may drop to fatally low levels during decomposition. After decomposition is complete, recovery of the ecosystem may occur.

The *fecal coliform test* is commonly used as an indicator for the presence of these disease-causing organisms. The fecal coliform test is for the presence of *Escherichia coli*, the most common bacteria present in the intestinal tract of humans and other animals. *E. coli*, itself, does not cause disease; in fact our intestines would not function normally without it. However, *E. coli* is much easier to test for than specific pathogens, and since *E. coli* is invariably present in feces and does not exist elsewhere in the environment, its presence indicates contamination with raw sewage and the probable presence of disease-causing organisms.

Even the non-bacterial portion of the colloidal material in wastewater may be highly polluting, because as microorganisms in the water attack and oxidize the organic matter they may utilize most

or even all the available dissolved oxygen. Consequently, fish and other aquatic organisms which are dependent upon oxygen will suffocate. Thus, the concentration of *dissolved oxygen* is frequently taken as a measure of pollution-the lower the dissolved oxygen the greater the pollution by organic matter. A more informative indication of organic matter pollution is provided by the *biological oxygen demand* or BOD test. The BOD test is a measurement of the total amount of oxygen that will be consumed by microorganisms in the process of decomposing all the organic matter present in a sample of water. If the biological oxygen demand is too high, fish and other aquatic life are obviously endangered.

Another pollution hazard from colloidal materials concerns pathogenic organisms present in soils, which are frequently carried into bodies of water by runoff. If the water is clean, these organisms die very quickly, but if abundant organic matter is present to serve as a food source, their survival time is greatly increased.

3.1.2.1.4 Dissolved Material

These are materials and compounds that are completely dissolved-that is, individual ions and molecules of various substances in solution. It is important to note that material in solution will not settle out regardless of how long the water may remain still. In domestic sewage, the dissolved materials consist mainly of the various nutrient ions, such as nitrate, ammonium, phosphate, and potassium, released from the human body through the urine and from the metabolism of microorganisms as they carry on the decomposition of organic material. In recent years the use of phosphate-based detergents has resulted in greatly increased levels of phosphate in wastewater. All these ions are required in plant growth and in the proper place and in proper amounts they are beneficial. However, when added to bodies of water they upset the natural balance and lead to a very severe type of pollution.

What about industrial wastes? Local industries may use the domestic sewage system for disposal of any number of manufacturing and/or processing wastes. Industrial wastes are divided into the same three categories as domestic wastes; and insofar as these wastes consist of natural sediments, natural organic material, and nutrient ions they cause no particular problem other than requiring increased treatment capacity.

However, three classes of industrial wastes, all in the category of dissolved materials, do cause particular problems. These are

(1) heavy metals, (2) nonbiodegradable organic compounds, and (3) acidic and caustic compounds. All these compounds are extremely toxic and cause numerous problems in the treatment and disposal of domestic sewage wastes, as will be discussed later. These industrial-type wastes may also originate from domestic sources as various cleaning compounds, solvents, pesticides, and other materials are disposed of down drains.

3.1.2.2 Sewage Treatment

Sewage treatment plants also called wastewater treatment plants vary in details of design but their basic principles of operation have been standardized at least through the first three steps: pretreatment, primary treatment, and secondary treatment. Pretreatment removes debris and suspended solids; primary and secondary treatment both remove colloidal material. Dissolved materials are, for the most part, not removed by these processes.

3.1.2.2.1 Pretreatment

As wastewater enters a treatment plant, it first flows through a *bar screen*, a row of iron bars mounted about one inch apart. Large pieces of debris such as rags, pieces of wood, and plastic are removed by the bar screen. The amount of such material is usually not large and hence it is not often listed among the contaminating materials. However, if it were not removed, it could clog or damage pipes or pumps further along in the process. The material that gets caught on the barscreen may be removed by hand or by mechanical rakes and taken to an incinerator. In more modern plants a mechanical apparatus associated with the screen grinds this material finely enough to pass through the screen.

Suspended solids are removed in a second phase of pretreatment. After the bar screen the wastewater flows through *grit settling tanks* where its velocity and agitation is slowed sufficiently to allow most of the material in suspension (sand and silt) to settle out. The settled material is removed mechanically or by hand, depending on the size of the operation, and taken to a landfill.

Note that pretreatment does not remove any of the obnoxious components from the wastewater. As the name implies, these steps must be carried out before treatment itself begins to protect plant equipment and to prevent buildup of this inert material in the succeeding treatment units where its presence would prevent efficient operation.

3.1.2.2.2 Primary Treatment

After leaving the grit settling tanks, the wastewater enters larger

sludge settling tanks or *primary clarifiers*. Here the velocity of the water is slowed almost to a standstill, allowing denser particles of colloidal material to settle to the bottom and grease and oil to float to the top. Chemicals may be added which cause colloidal particles to clump together and thus hasten their settling. The settled colloidal material is mechanically removed from the bottom, while fatty or oily material is skimmed from the top; together these comprise *raw sludge*. The treatment and/or disposal of this raw sludge is discussed later. Primary treatment removes only about 30-50 percent of the colloidal material. Stated the other way around, wastewater after primary treatment still contains 50-70 percent of the colloidal material and nearly all the dissolved material, although some dissolved material goes with the sludge.

3.1.2.2.3 *Secondary treatment*

Secondary treatment is also called *biological treatment* because it employs organisms to break down the organic colloidal material. Through several trophic levels of detritus feeders, most of the remaining colloidal material is oxidized to carbon dioxide, water, and mineral nutrients as in other food chains. The principle of secondary treatment, then, is to create an environment optimal for the growth of organisms that will feed on the colloidal matter in the wastewater. *Trickling filter systems* and *activated sludge systems* are two widely used methods of doing this.

In trickling filter systems, the wastewater from primary treatment is applied to and allowed to trickle through a bed of fist-sized rocks, creating an environment similar to a natural stream. The water is well aerated as it trickles over the rocks. Well-aerated water rich in organic matter supports several trophic levels of organisms attached to the rocks. Bacteria are the first organisms to attack and digest organic particles but the food chain continues through protozoans, rotifers, and various small worms. Through the respiration of the organisms at the various trophic levels about 85-90 percent of the organic matter entering the system is broken down to carbon dioxide, water, and inorganic mineral nutrients. Carbon dioxide is dissipated into the atmosphere; the mineral nutrients (NH_4^{+} , NO_3^{-} , PO_4 , K^{+} , and so forth) remain in the water. Organisms and clumps of detritus that do occasionally wash from the trickling filters are mostly removed by passing the wastewater from trickling filters into *secondary clarifiers*. These tanks, similar to primary clarifiers, accomplish removal by permitting the material to settle, after which it is treated as raw sludge.

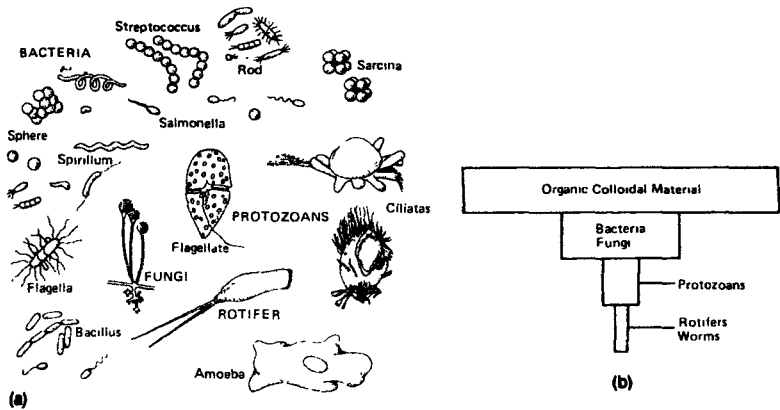


Figure 3.2 (a) Some of the organisms adhering to rocks in the trickling filter. (b) They form a series of trophic levels so that overall biomass is reduced by 85-90 percent.

In the activated sludge system, the water from the primary treatment flows into a long, relatively narrow "aeration tank" in which air or pure oxygen is continuously bubbled up from the bottom. This is another method of creating a food and oxygen-rich environment which is ideal for the growth of organisms. A mixture of organisms, *activated sludge*, is added to the wastewater as it enters and these organisms feed and grow on the organic matter as the water passes through the tank. As the wastewater leaves the aeration tank, it still contains a rich mixture of the feeding organisms. Therefore, following the aeration tank, the wastewater is passed into a secondary settling tank. Since the feeding organisms are mostly in clumps feeding on bits of detritus, settling is relatively efficient. The settled organisms are the "activated sludge" which is continuously pumped from the bottom of the settling tank back to the entrance of the aeration tank, assuring a continued high population of active organisms. Excess amounts of the activated sludge are mixed and handled with the raw sludge.

About 95 percent of the colloidal material entering the activated sludge system is removed through the trophic levels in the aeration tank and the final settling. However, most of the dissolved material and the mineral nutrients resulting from the breakdown of organic matter remain in the water. As before, some goes with the sludge that is removed.

In construction of new wastewater treatment plants or in upgrading old ones, the activated sludge system is generally being installed

because it offers several advantages. First, it removes a somewhat higher proportion of the colloidal material, 90-95 percent vs. 85-90 percent for the trickling filter. Second, it is a much more compact system, saving space which is often limited, especially on sites of old plants. Third, it is less subject to clogging, adding even more to its greater efficiency of removing organic material. It has one important disadvantage, however: Activated sludge systems require large amounts of energy for the aeration pumps and also for producing pure oxygen if it is used. With rising energy costs, activated sludge systems are becoming increasingly expensive to operate. Trickling filter systems, on the other hand, are generally gravity feed systems and their operation requires little if any additional energy.

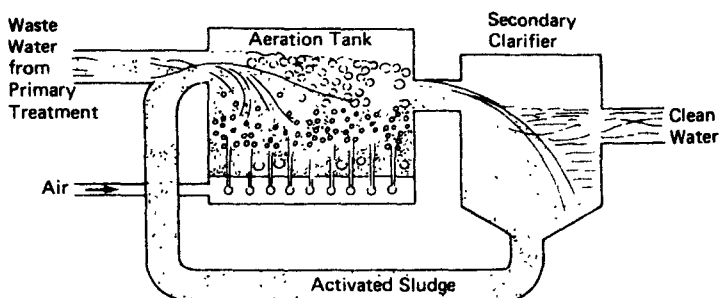


Figure 3.3 Activated sludge treatment In the oxygen-rich environment of the aeration tank, microorganisms consume the organic matter. Organisms (activated sludge) settle out in the secondary clarifier and are returned to the aeration tank while the clarified water flows on.

It is important to note that neither system of secondary treatment provides systematic removal of dissolved materials. Fertilizer nutrients from urine and the breakdown of natural organic compounds, and additional phosphate from detergents, mostly remain with the water, as do most of the heavy metals, nonbiodegradable synthetic organic compounds, and caustic or acidic compounds from industrial wastes. Not only are they not removed, but toxic industrial wastes may reduce the efficiency of secondary treatment or destroy it altogether by killing the organisms. Significantly the dissolved toxic material that doesn't go with the water goes with the various sludges.

After secondary treatment, *advanced treatment* may be applied to remove one or more of the mineral nutrients or other materials dissolved in the water. Numerous methods of advanced treatment are possible, but at present very few are in operation mainly because the need for advanced treatment has not been clearly appreciated until recently.

Various methods of advanced treatment will be described after such needs have been discussed.

3.1.2.2.4 Degree of wastewater treatment

It may be noted here that there are alternatives as to how much treatment to provide. Sewage water may be discharged with no treatment; it may be discharged after primary treatment; after secondary treatment; or it may be carried on through some form of advanced treatment. A primary sewage treatment plant is one which carries the water only through primary treatment. A secondary sewage treatment plant carries it through secondary treatment, and an advanced sewage treatment plant carries it on through some form of advanced treatment. In addition, at whatever stage the wastewater is discharged, it is generally disinfected as will be described later.

It is interesting to note that as recently as 1972, 5 percent of the wastewater in the United States was discharged with no treatment; another 24 percent received only primary treatment. Less than 2 percent received advanced treatment. It is small wonder that practically all the nation's rivers, lakes, and beaches near cities were becoming or had become intolerably polluted with sewage water effluents.

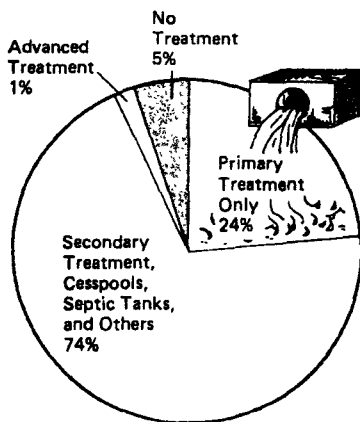


Figure 3.4 There is still a long way to go in providing adequate sewage water treatment

Given this deplorable situation, Congress, with the backing of environmentally conscious citizens, passed the Clean Water Act of 1972. Among other things, this act mandated and provided Federal funding for upgrading all sewage treatment to include the secondary stage by 1983 at the latest. In recent years, tremendous strides have been made toward this goal and sewage pollution problems in many

areas have declined accordingly. However, even secondary treatment of wastewater does not resolve all pollution problems.

3.1.2.2.5 Disinfection of wastewater

As noted previously, untreated wastewater has a very high probability of carrying disease-causing organisms. After primary treatment at least 50-70 percent of these organisms remain. Even after secondary treatment, a significant number of human fecal bacteria are still present, indicating some potential for the continued presence of disease-causing organisms, even though most have unquestionably been removed. To reduce the public health threat from these organisms, it is common practice to disinfect (kill the organisms in) wastewater as it is discharged whether it is untreated or has received primary or secondary treatment. Especially since 1972 efforts have been intensified to disinfect wastewater effluents.

Frequently, disinfection is referred to as "purification." It is important to make a distinction here between *biological purification* and *chemical purification*. Biological purification involves only the killing of disease-causing organisms. It does not remove anything from the water. Chemical purification, on the other hand, involves the actual removal of contaminating materials. Disinfection does the former, not the latter. Indeed, the most common method of disinfection is *chlorination*, the addition of chlorine usually in the form of chlorine gas (Cl_2). Chlorination is effective in killing microorganisms, but far from removing anything, it involves the *addition* of one more toxic chemical.

In fact, in cases where the wastewater has received secondary treatment, there is debate as to whether the addition of chlorine itself poses a greater threat than would the remaining microorganisms. Several studies indicate that the chlorine contained in the wastewater discharge causes more damage to the ecosystem of the receiving body of water than would the discharge of unchlorinated wastewater. It has also been found that chlorine reacts with some organic waste materials to form *chlorinated hydrocarbons*, organic molecules with chlorine atoms attached. Many of the chlorinated hydrocarbons are extremely toxic, long-lived chemicals, and some have been identified as carcinogens (cancer-causing chemicals). Finally, there is a potent accident hazard in transporting tank-car loads of highly poisonous chlorine gas from points of manufacture to sewage treatment plants. Several such accidents have already claimed human lives, and more can be expected.

Fortunately, there is an alternative to chlorine, namely, ozone (O_3). Ozone is extremely effective in killing microorganisms and in the process it breaks down to oxygen gas, which improves water quality. Because of its explosive nature, ozone would have to be manufactured at the point of use, a step which demands considerable capital investment in the short term. However, in the longer term and as technology improves, costs might be comparable to using chlorine and safety hazards might be less.

3.1.2.2.6 Sludge treatment and potential use

The raw sludge that is collected from primary and secondary settling tanks is a black, foul smelling, syrupy liquid of about 98 percent water and 2 percent organic matter which includes many pathogenic organisms. However, raw sludge can be treated and made into highly valuable resources.

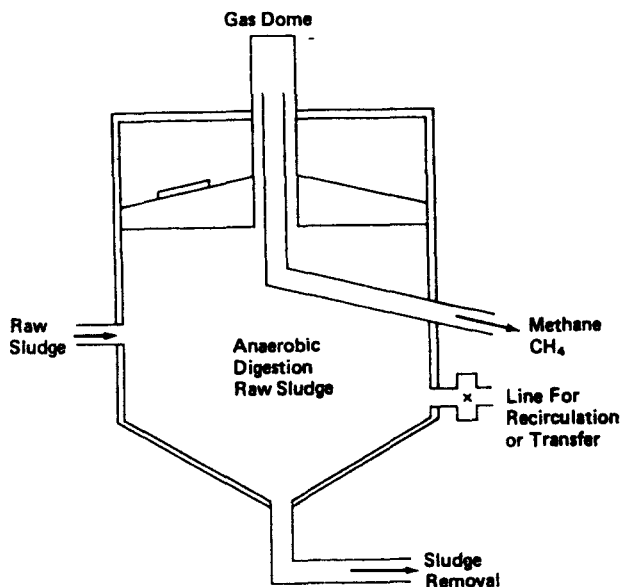


Figure 3.5 Sludge treatment. Sludge from clarifiers may be treated by digestion for several weeks in an airtight tank. Byproducts of treatment are methane gas from the anaerobic digestion and a humus-rich, nutrient-rich sludge

The most common technique is to put the sludge into large airtight tanks called *sludge digesters*. In the absence of oxygen, certain bacteria carry on *anaerobic respiration*, a process in which organic molecules are broken down to methane gas (CH_4). Methane is the same as natural

gas and it can be used for the same purposes. It is common practice to tap the methane from the tanks during the digestion period and use it to heat the buildings of the treatment plant and the digesters themselves, which operate best at 35-38°C (90-95°F). Excess methane may be sold to the local gas company, but the amounts involved are not great. The quantity of methane that may be obtained from a sewage treatment plant is about one cubic foot per day per person served. By comparison, through a gas heater, an average individual uses 25-30 cubic feet per day just for personal hot water needs. Nevertheless, the value of the methane produced may significantly offset the cost of treatment.

After a digestion period of 30-50 days, the process is virtually complete. All that remains of the sludge is the portion of the organic matter which is relatively stable, odorless, and resistant to further digestion, and water rich in nutrients released from decomposing organic matter. Pathogenic organisms have been eliminated in the competition with the natural decay organisms and no longer present any great hazard. Thus, the digested sludge is essentially the same as the humus resulting from natural decay in a natural ecosystem. Like natural humus, it has great value in improving soil quality. It may be applied directly to lawns and agricultural fields in the highly liquid state as it comes from the digester, thus obtaining benefit from both the humus and the nutrient-rich water. Alternatively, the liquid, digested sludge may be filtered, leaving a semisolid humus "sludge cake". Such a cake is easier to handle, but most of the nutrients are lost with the water, which is generally put back into the wastewater stream.

Where sewage sludge is treated as described above, the semidry "humus" is usually available to the public free of charge. Denver, Colorado, and many smaller communities, especially in the Southwest, apply all their sludge production to agricultural fields. Chicago is presently using much of its sludge to reclaim soils ravaged by strip mining. Milwaukee, which has a particularly rich sludge resulting from the brewing industry, pasturizes, bags, and sells it under the trade name "Milorganite".

Raw sludge may also be treated by *composting*. In this process, raw sludge is filtered to make it semisolid, mixed with wood chips or other material to improve aeration, and piled in windrows. As shown, additional air may be drawn through the piles to further increase aeration. As long as the system is kept well aerated, obnoxious odors that come from waste products of anaerobic respiration are negligible. Aerobic respiration of organisms breaks

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down organic material, pathogenic organisms lose out in the competition, and a nutrient-rich humus-like material is produced. Washington, D.C., is using this approach.

Unfortunately, most people have the impression that treated sewage sludge differs little, if at all, from the raw "stuff" straight out of the toilet. Consequently, not only do they refuse to use it themselves, they often go so far as to bring court action against individuals or municipalities who do use it. Also, many people in government, and especially those in the sewage treatment branch, have a strong prejudice that they should not involve themselves in the "fertilizer business."

It is interesting that while we in America generally reject putting treated sludge on lawns or agricultural fields, we tolerate untreated sewage going into lakes and rivers that serve as water supplies. This attitude is extremely unfortunate because it leads to our throwing away tremendous quantities of sludge that might be used as valuable organic fertilizer. Many soils have been sadly degraded by loss of organic matter. Applications of treated sewage sludge could go a long way toward correcting this condition.

Not only is value lost in not using the sludge; alternative methods of disposal are expensive and plagued with problems. Most of the treated sludge now goes into landfills but these are becoming critically limited as will be described later. Some sludge is incinerated, but its high moisture content makes burning difficult and its high ash content (mineral nutrients) makes control of resulting air pollution difficult. New York, Philadelphia, and other large coastal cities barge *untreated* sludge out to sea and dump it. In recent years beaches on the East Coast have been contaminated by dumped sludge which has migrated back to shore. Cities which barge sludge to sea are now under orders from the Environmental Protection Agency to find alternative methods of disposal.

One note of caution is necessary regarding use of sewage-derived humus, namely, possible contamination by industrial wastes, particularly the heavy metals. Some sludges have such high concentrations of heavy-metal ions such as copper, zinc, lead, and cadmium that they are poisonous to agricultural crops. Grasses, however, seem to be more resistant to the toxic effects of these metals, so the sludge may still be used with benefit on lawns.

3.1.2.2.7 *Other techniques of handling sewage wastewater*

Sewage Lagoons. In some cases sewage from small communities or large housing developments is piped into shallow open ponds or

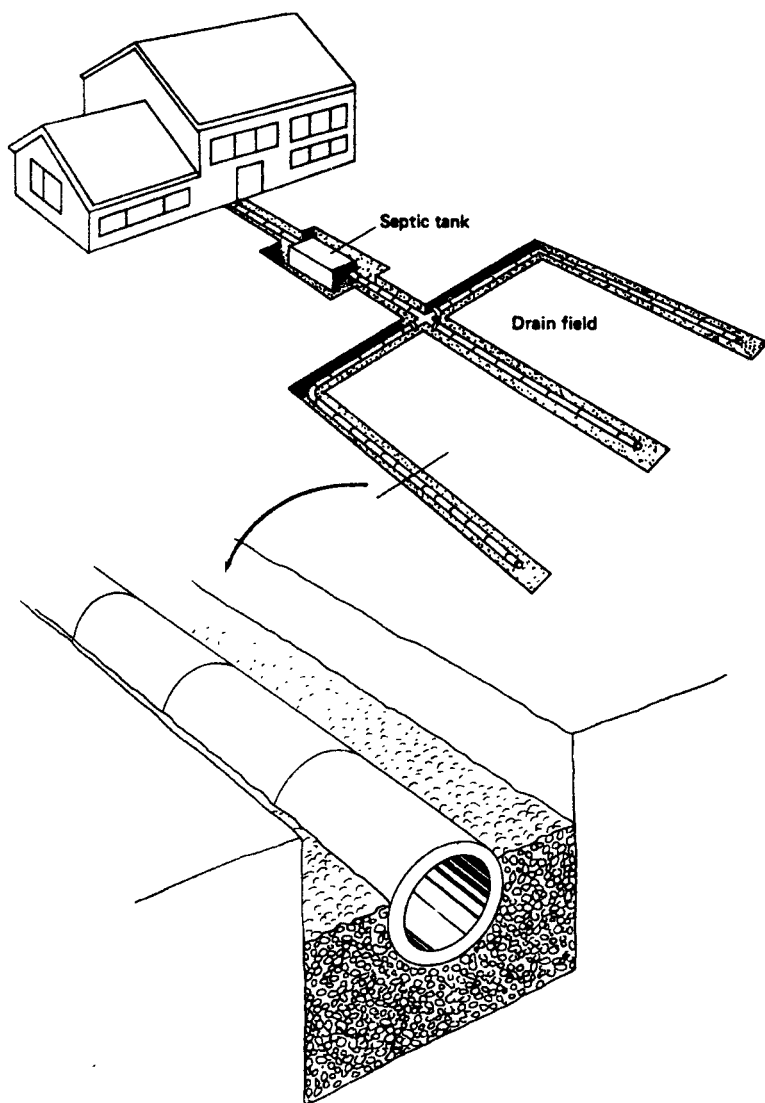


Figure 3.6 Individual sewage treatment. Septic tank and drain field.

catch basins where the material is allowed to decompose. Occasionally a mechanical device is added to increase aeration and hence decomposition. The water gradually percolates into the soil or it may be permitted to overflow into natural waterways in times of high

rainfall, when dilution is possibly sufficient to reduce hazards of contamination.

Although sanitary engineers refer to this technique of handling wastes as *lagooning* and the ponds as *sewage lagoons*, these terms are but euphemisms for what are actually open cesspools. Although sewage lagoons offer natural waters a little more protection than straight dumping of wastes, the open pools are a source of objectionable odors and may constitute a public health hazard.

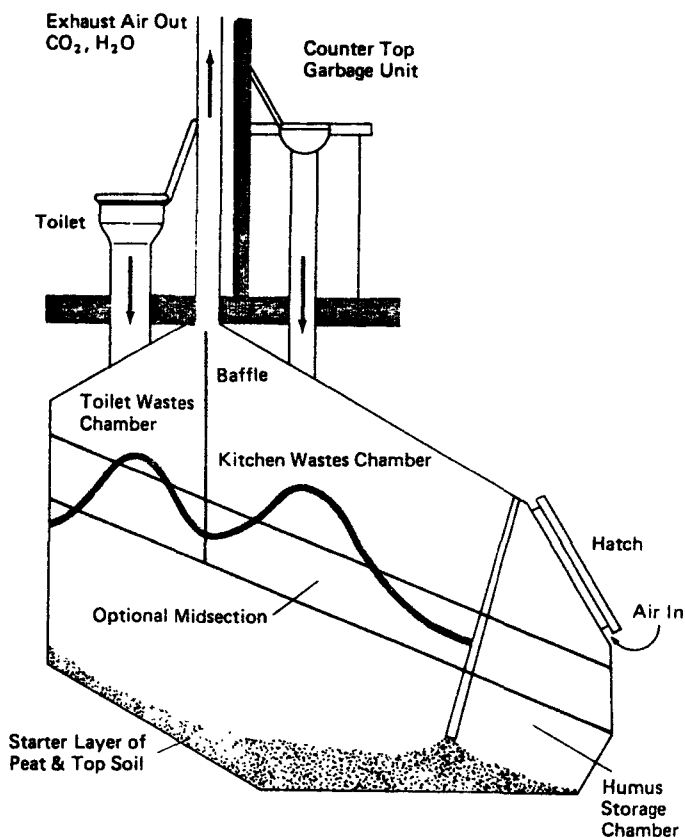


Figure 3.7 Clivus Multrum—a dry waste treatment system. Sewage and garbage wastes deposited in the Clivus Multrum decompose aerobically. A dry humus- and nutrient-rich compost is removed from the Clivus Multrum as a byproduct of treatment.

Individual Septic Systems. In areas that do not have a centralized sewerage system, a common practice is to install a septic tank and drain field for each individual home or commercial building. The

effluent from the home flows into a large tank where the heavier particles settle to the bottom. The water, still carrying much of the colloidal and nearly all the dissolved material, overflows into a system of drain tiles buried in the ground, and gradually percolates into the soil. Bacteria gradually digest the organic material that settles in the tank, reducing it to a stable humus. Likewise soil bacteria decompose the organic colloidal material that comes through the drain tiles.

Septic systems are more sound ecologically than centralized sewer systems in that they do return nutrients to the soil. In fact, some people establish successful vegetable gardens over septic drain fields, thus using and recycling the nutrients from wastes rather than using commercial fertilizer from nonrenewable resources.

If conditions are ideal, a septic tank from which the humus material is periodically removed may function indefinitely. However, it is frequently found that colloidal material enters the soil at a rate faster than it is decomposed, gradually clogging up the soil pores and preventing percolation of the water. With nowhere else to go, effluent with the objectionable colloidal material forces its way to the surface where it is unsightly, causes objectionable odors, contaminates surface waters, and is a general health hazard. If the lot is not large enough to relocate the drain field, there is little to be done about this problem except to try to get centralized sewerage as soon as possible. In many areas of the country, expensive suburban developments using individual septic systems have become extremely obnoxious as the septic systems have failed and lot sizes have not permitted relocation of systems. How long individual septic systems function depends to a large extent on how intensively they are loaded. Disposal of paper diapers, sanitary products, and kitchen garbage into such systems will clog them in relatively short order. The average life expectancy of a system is 7-14 years.

Some new septic tanks incorporate air pumps and effectively provide secondary activated sludge treatment as well as the primary settling. Decomposing much of the colloidal organic matter in this way should permit such septic systems to function at least twice as long as nonaerated systems. Also, public health laws in many regions are being made more strict so that individual septic systems are not permitted in poor drainage areas or on lots too small to permit relocation.

An interesting alternative for the handling of personal wastes in individual homes is the Clivus Multrum developed in Sweden. The

“multrum” receives only personal excrements and food wastes-no water other than urine. These wastes pass through a series of chambers as they decompose and after two to four years they arrive at the final chamber as a stable, nutrient-rich humus that is suitable for application on gardens. Bath, dish, and other “gray water” must still be disposed of by some other pathway but, without contamination by human excrement, this offers relatively little problem.

3.1.3 Eutrophication: A Problem of Nutrient-Rich Water

3.1.3.1 Eutrophication: The process

After secondary treatment, waste water still contains most of the nutrient ions from urine, from the breakdown of organic material and from detergents. In the past 20 years or so, we have learned that even these “beneficial” nutrient ions may have a profound polluting effect on aquatic ecosystems, an effect known as *eutrophication*. The term *eutrophic* comes from the Greek: *eu* = well; *trophic* = to feed. Hence, eutrophication is the process of feeding well-too well!

In order to understand the problems of a eutrophic or nutrient-loaded lake, it is helpful to start with consideration of a lake or other body that has clear water aesthetically pleasing for swimming, boating, and fishing. The water in a crystal-clear lake is clear because growth of phytoplankton (floating forms of algae which give water a cloudy green appearance) is limited by *lack* of nutrients. In natural situations the water of lakes tends to be low in nutrients because leaching from undisturbed ecosystems is generally slight. Nutrients are largely held by and continue to be recycled by land ecosystems. Nutrients that do enter the lake with soil particles tend to settle near the mouth of the entering stream or river. These areas support an abundant growth of algae and other water plants, and the productivity of these areas, in turn, supports food chains leading to desirable game fish such as bass, trout, and pike. The addition of nutrients from wastewater or other sources changes this situation.

The nutrients discharged from a sewage treatment plant are mostly free in water solution. Therefore they mix freely with the water of the entire lake rather than settling with soil particles at the mouth of an entering stream or river. The nutrient loading of the water permits growth of phytoplankton which was held in check by the relative lack of nutrients. Given nutrients, the growth and division of phytoplankton may be extremely rapid, turning clear water to cloudy green in a matter of a few days. This sudden appearance of large quantities of phytoplankton is called an *algal bloom*.

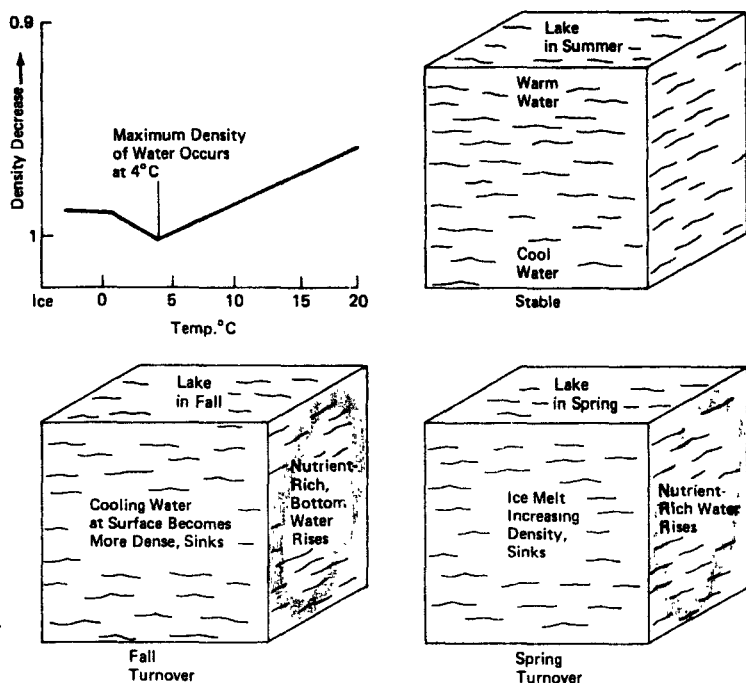


Figure 3.8 Temperature changes in the spring and fall result in a turnover of the water in a lake as shown. The nutrient-rich water brought to the top may stimulate a natural algal bloom at these times

Algal blooms are not a new phenomenon. In many lakes, even lakes that are generally clear, blooms may occur in the spring when cold water from melting ice sinks to the bottom and bottom water rises to the top, bringing along nutrients from the sediments. A similar turnover occurs in the fall as surface water cools, sinks, and forces nutrient-rich bottom water to the surface, frequently resulting in a fall algal bloom. In lakes that are relatively short of nutrients, however, the algal bloom is a short-lived phenomenon. All the available nutrients are soon absorbed by the growing phytoplankton. Thus limited, most of the cells soon die and settle to the bottom, taking the nutrients which they absorbed with them. With the addition of extra nutrients from wastewater discharge or other sources, however, the duration and intensity of the algal bloom is vastly increased. In severe cases, the water may be covered with a green scum of algae from as soon as water temperature permits growth in the spring to when temperature again limits growth in the fall.

The bloom itself is an aesthetic problem, detracting from the pleasure of swimming and boating. Also, if the lake is used as a source of drinking water, there is added expense in filtering out the algae and an unpleasant taste may remain. Further, the algal bloom is only the beginning of a set of ecological problems. The phytoplankton population soon reaches its maximum size, at which time the continued high reproductive rate is matched by an equally high death rate. The dead cells sink to the bottom, taking nutrients with them, but with the still-abundant supply of nutrients, the growth and death processes continue. A vastly increased amount of organic matter therefore accumulates on the bottom.

As in every ecosystem, the dead organic matter serves as food for bacteria and other decomposers. Consequently, there is a tremendous increase in the growth and reproduction of these organisms. The oxygen needed by the bacteria in their respiration is obtained from the supply of dissolved oxygen in the water. Oxygen is only sparingly soluble in water and it diffuses very slowly through still water; therefore the bacteria soon deplete the oxygen supply of the lower levels of water. However, the lack of oxygen does not stop the growth of all the bacteria. Some are able to continue growth and reproduction by using anaerobic respiration. As additional oxygen becomes available, aerobic respiration is resumed. Thus decay organisms can effectively keep the water depleted of oxygen until the organic matter is largely decomposed.

The effects of depleting the water of dissolved oxygen should be obvious: All fish and other organisms which depend upon oxygen for respiration die of asphyxiation. Game fish are especially vulnerable because they both require large amounts of oxygen and tend to inhabit the deeper water that is the first to be depleted of oxygen. Some fish, like carp, tend to survive or even thrive because they can tolerate much lower concentrations of oxygen and they inhabit shallow areas where adequate oxygen diffuses into the water from the atmosphere.

This, then, is the eutrophic condition: a body of water rich in nutrients supporting an abundance of phytoplankton and having a low or zero level of dissolved oxygen in the lower layers. Such lakes have been termed "dead"-Lake Erie is often cited as a classic example of a dead lake. Biologically, the term *dead* is a definite misnomer, since the total biomass and productivity are many times greater in a eutrophic lake than in a clear, nutrient-poor lake. However, the lake is dead in terms of providing the aesthetic pleasures of swimming, boating, and sport and commercial fishing. Also, where the lake is a

source of drinking water, its value may be greatly impaired because of offensive odors and toxic byproducts produced by the algae. Algal cells also rapidly clog water purification filters. Baltimore, Maryland, for example, had to change filters every 14 hours during an algal bloom in one of its reservoirs compared to every 70 hours during normal times.

Unless it is a small lake or pond, an entire body of water does not become eutrophic at one time because complete and uniform mixing of a large body of water is very slow. Evidence of eutrophication—the algal scum and low dissolved oxygen *first observed around the area where nutrients enter. As nutrient loading continues*, eutrophication gradually spreads in area, intensity, and duration. Eutrophication of Lake Erie developed over a period of more than 20 years and parts of the lake never have become eutrophic.

In a river, the steps of eutrophication are spread out in a linear manner, depending upon the rate of river flow. Nutrients discharged into the river are carried downstream and development of an algal bloom occurs some distance from the nutrient source. Decomposing algae continue to be carried downstream and cause the decrease in dissolved oxygen further from the nutrient source. Even further downstream, the river may recover if there are not more additions of nutrients and/or organic wastes. However, even relatively small areas of eutrophication in a body of water can have profound effects on fish populations. A school of fish may swim into a eutrophic area and be suddenly killed by lack of oxygen, or a eutrophic segment drifting in a river or bay may entrap schools of fish. Many massive, “mysterious” fishkills of recent years probably stem from this source. The decaying bodies of fish then create an additional oxygen demand which compounds the cycle of eutrophication.

It is ironic that the sewage treatment process is directed to the removal of organic matter largely because decomposition of this material in natural waters depletes the dissolved oxygen. However, the failure to remove nutrients creates the same problem in the form of decomposing algae. Obviously the treatment process must go further and remove the nutrients; or the wastewater carrying the nutrients must be diverted to other uses rather than being discharged into natural rivers and lakes. Even if this is done it may be many years, if ever, before a eutrophic lake returns to a more normal condition. The reason for slow recovery is that decaying organic matter releases nutrients back into the water, perpetuating the nutrient-rich, eutrophic condition.

However, there is much evidence that the nutrients gradually become stabilized in the bottom sediments, permitting gradual recovery. Lake Washington in Seattle, Washington, is an example of a lake that made a recovery over a period of nearly 15 years following reduction of nutrients flowing into the lake. Nutrient additions were reduced by both upgrading existing sewage treatment plants and diverting new sewage water flows to other locations. Lake Erie is also recovering slowly as nutrient additions are increasingly controlled.

3.1.3.1 *Point and non point sources of nutrients*

It should be evident that eutrophication may occur whenever a body of water receives too many nutrients. Although we have stressed nutrient loading from waste water discharge, nutrients may come from a variety of other sources. A major source of nutrients in many areas is the leaching of fertilizer applied to agricultural fields or residential lawns. Also drainage from barnyards and/or cattle feeding operations may carry tremendous nutrient loads from animal urine and fecal wastes. The same is true of drainage from housing developments that use individual septic systems.

The origins of nutrients (or other pollutants) are generally divided into *point* and *nonpoint* sources. Point sources are specific, identifiable discharges such as outlets from sewage treatment plants or factory drains. Nonpoint sources involve generalized drainage such as that from agricultural fields, runoff from city streets, or drainage from many individual septic systems. Which of these categories contributes the most pollutants will vary with the particular situation. Therefore, each case of pollution must be studied individually to determine the major sources of pollutants.

3.1.4 Controlling Eutrophication

Eutrophication should be looked at, not by itself, but in a total ecological context. Eutrophication results from the failure to return nutrients to the soil from which they came. Thus, eutrophication is only one side of the nutrient coin; the other is that the soil is being depleted of nutrients. To replenish soils, necessary nutrients are mined from deposits elsewhere in the Earth's crust. These deposits of phosphate rock, potash (potassium) and other nutrients are neither unlimited nor renewable. Some authorities predict, for example, that reserves of phosphate available to the United States may be depleted within the next 50 years. Consequently, the flow of nutrients from soils to bodies of water may ultimately be a greater disaster in terms of soil depletion and lack of fertilizer than in terms of eutrophication.

But it is hardly worthwhile to dwell on which aspect of the problem is of greater consequence. Conspicuously, both problems may be solved by prudent steps toward returning the nutrients to the soil. Recycling nutrients in a highly urbanized society is, however, easier said than done, and cost will always be a moderating factor in striking a compromise between what should be done and what can be done. The following methods of dealing with eutrophication should be considered in light of these factors.

3.1.4.1 Chemical treatment

Algae may be poisoned by the addition of chemical herbicides to the water. This is the most expedient solution and is frequently used on small lakes in resort or residential communities, but it is ecologically the worst. In attempting to control the undesirable phytoplankton by this means, other desirable forms of algae may also be killed and in turn all the fish and other organisms that depend on these producers are affected. Even if fish, ducks, and other forms of wildlife continue to thrive in the short *term*, many questions concerning long-term health effects remain unresolved, for these chemicals and/or their breakdown products may be passed up the food chain and may accumulate in fish, ducks, and humans who eat them. Chemical treatment may similarly affect the stream or river draining the lake as the herbicide washes downstream. Importantly, the chemical treatment does *not* alleviate the basic problem of too many nutrients. As soon as the chemical breaks down or is diluted enough to become nontoxic, the algal bloom may recur, demanding continued treatment.

Paradoxically, some toxic industrial wastes are probably playing this role of chemically preventing algal pollution. When industrial pollutants were eliminated from the Thames River in Lōndon, for example, Londoners were confronted by algal blooms and eutrophication.

3.1.4.2 Aeration

Installing a mechanical aeration system in a eutrophic lake or pond will keep the dissolved oxygen high and at least will prevent the fishkills due to suffocation. But aeration has another remarkable effect: When dissolved oxygen is low, phosphate is quite soluble; when dissolved oxygen is high, phosphate tends to crystallize in insoluble phosphate compounds. Since phosphate is often the limiting nutrient in growth of algae, decreasing phosphate solubility by means of aeration may significantly reduce algal blooms.

Obviously, aeration does not solve the initial problem of nutrient

loading, nor does it address the long-range problem of nutrient recycling. However, aeration produces none of the undesirable ecological side effects or long-term human health hazards of herbicides.

3.1.4.3 Harvesting algae

Various mechanical harvesters have been designed to physically strain the algae from the water. Sometimes people, in "labors of love," have removed algae by hand. Since the algae have absorbed the nutrients from the water, removal of the algae removes the nutrients as well. If the quantity of nutrients flowing or leaching into the lake is not too great, an effective harvesting program during one season may be sufficient to last for several years before enough nutrients again accumulate to stimulate eutrophication.

Algae are rich in nutrients and decompose readily; therefore harvested algae make an excellent organic fertilizer. However, the cost of harvesting algae on a commercial basis is too high to be offset by its fertilizer value.

3.1.4.4 Alternative disposal of nutrient-rich water

3.1.4.4.1 Diversion

When pollution comes mainly from a point source such as discharge from a sewage treatment plant, it may be expedient to divert the effluent to another location. For example, Seattle, Washington, and surrounding cities used to discharge wastewater from sewage treatment into Lake Washington, a favorite recreational lake. When faced with the problem of eutrophication, citizens undertook a costly project to pipe the wastewater to Puget Sound. This step presupposes that Puget Sound has enough tidal flushing action to dilute the nutrients sufficiently with ocean water so that eutrophication of the Sound does not occur. Other communities facing similar problems are considering or have completed such diversion projects. It should be obvious that "diversion" is simply a euphemism for throwing garbage out the back door instead of the side door. It may solve the immediate problem for the short term, but with respect to the long-term global ecology, it solves nothing.

3.1.4.4.2 Use of wastewater for irrigation

A form of diversion which does incorporate the ecological concept of nutrient recycling is the use of the nutrient-rich wastewater as irrigation water. St. Petersburg, Florida, recently installed a system for sprinkling treated wastewater on city parks and golf courses. Pennsylvania State University is experimentally spraying wastewater

on a forest. Muskegon County, Michigan, combines sewage treatment and land application of effluents. Raw sewage is pumped to large lagoons where settling and decomposition take place, and the overflow from the lagoon is sprayed on surrounding croplands. Other communities have similar projects.

Land irrigation with wastewater, however, does have several restrictions. First, the wastewater must be relatively free of toxic compounds, particularly the heavy metals that can accumulate in soils to toxic levels. Second, an abundance of land must be available in or near the city to receive the wastewater. It has been estimated that one hectare will absorb the wastewater effluent of only about 30 people. Thus, for a relatively small community surrounded by agricultural land, such a project would be feasible. But in most large cities, particularly in the eastern United States, there is not sufficient land close enough to absorb the enormous quantities of wastewater involved. Transporting and distributing such large volumes of water over long distances is presently economically impractical. Ten thousand tank trucks, each with a capacity of 10,000 gallons, making one round trip per day would be required to transport wastewater effluent from a city of one million persons, assuming an average water use of 100 gallons per person per day.

Topography is also a consideration. The land to receive the water should be lower than or at least level with the community; otherwise huge pumping costs will be incurred. Finally, storage capacity must be built into the system to hold the wastewater during periods when the ground cannot absorb it, such as during winter months when the ground is frozen. Even with these limitations, however, there are many regions, particularly warm dry regions, that could profitably irrigate land with wastewater.

3.1.4.5 Advanced treatment

If the problem-causing source of nutrients is a discharge of wastewater after secondary treatment, further treatment may be initiated to remove the nutrients. Such treatment is called *advanced* treatment, or *tertiary* (third-order) treatment. Advanced treatment may be designed to remove all or part of the nutrients. A wide variety of both technological and biological methods are either in use or under consideration, some of which are briefly described below.

3.1.4.5.1 Partial removal of nutrients

Partial advanced treatment is based on the principle of limiting factors—that is, only one nutrient needs to be absent to limit growth.

It has been determined that in most natural, clear water lakes phosphate (PO_4^{3-}) is the limiting nutrient. Therefore, if only phosphate is removed from discharges, algal growth will be limited despite the presence of other nutrients.

Fortunately, it is relatively easy to remove phosphate. Passing the wastewater through a "filter" of lime (calcium carbonate) results in the formation of insoluble calcium phosphate. Thus the phosphate ions are pulled out of the water. Although the filter must be changed frequently to replace the lime and dispose of the calcium phosphate, this method of treatment may still be less expensive than others.

Nitrogen compounds may also be specifically removed by passing the water through filter columns of denitrifying bacteria, which convert the soluble mineral nitrogen compounds, namely, nitrate (NO_3^-) and ammonium (NH_4^+), to nitrogen gas which then goes off in the air. The denitrifying system may be used when nitrogen rather than phosphate is the limiting factor.

3.1.4.5.2 Complete removal of nutrients: Technological methods

There are four technological systems that will remove all the ions from wastewater: (1) distillation, (2) electrodialysis, (3) microfiltration or reverse osmosis, and (4) ion exchange.

In distillation, water is heated, evaporated, and recondensed. Mineral ions stay behind in the boiler and the recondensed water is

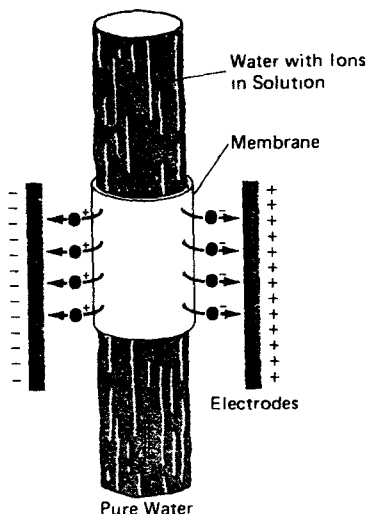


Figure 3.9 Electrodialysis. As water flows between electrically charged plates, polluting ions are attracted and held.

pure water. In electrodialysis, the wastewater flows between positively and negatively charged electric plates. The positively charged ions are attracted to the negative electrode and the negatively charged ions to the positive electrode. Thus, the ions are captured and pure water flows on through. In microfiltration, or reverse osmosis, the water is forced under very high pressure through a membrane filter that is fine enough to prevent the passage of ions and contaminating molecules, but allows water molecules to pass through. In ion exchange, the water is passed through a column of claylike particles which absorb the mineral ions and release hydrogen ions (H^+) in place of positive ions and hydroxyl ions (OH^-) in place of negative ions. The H^+ and OH^- ions subsequently combine to form water molecules.

All these methods require sophisticated facilities which are expensive to build and maintain and they require large amounts of energy to operate. However, they produce chemically pure water that

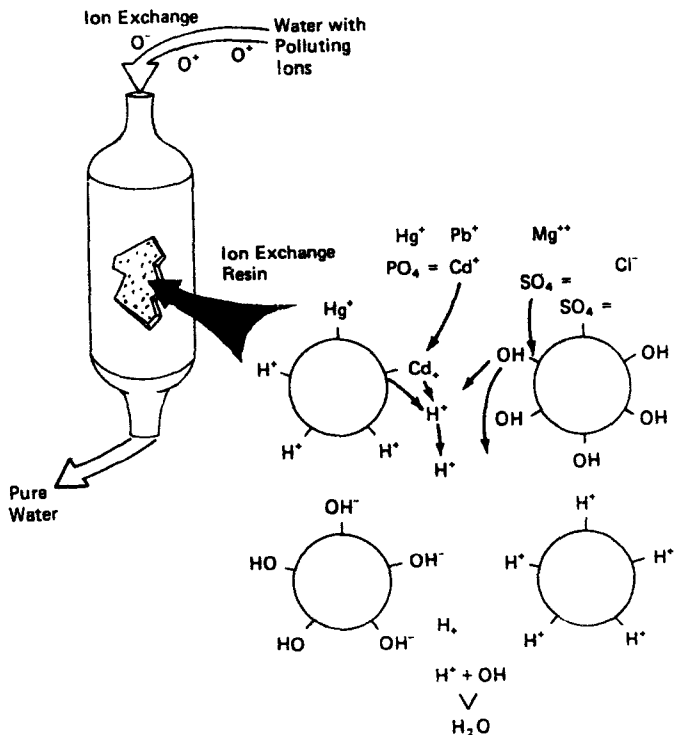


Figure 3.10 Ion exchange As water flows through the ion exchange material, polluting ions are bound and H^+ and OH^- are released in their place. H^+ and OH^- combine to form water molecules.

can be put directly back into the municipal water system and recycled. As water shortages in various parts of the country become more severe, this possibility is becoming increasingly attractive, and may justify the cost of advanced treatment.

Many people still oppose recycling wastewater into drinking water, but this is mostly a problem of misunderstanding. People need to be reminded that *all* water is recycled by nature. There is hardly a molecule of water from the purest mountain spring that has not already passed through organisms, including humans, perhaps countless times. You can think of water molecules as babies. You don't throw a baby away when it gets dirty; you wash it off, and it's as good as ever. Likewise, water is as good as ever when polluting materials are removed.

Many cities take their water from rivers and lakes into which they themselves or other communities dump sewage and industrial wastes. Most communities convert this into drinking water only by filtering out the large visible particles and adding sufficient chlorine to kill the microorganisms. This is recycling at its worst, and indeed, the water quality of many cities in this country is so bad that it is beginning to receive attention as a serious health problem. In short, methods of complete advanced treatment should be looked at as providing a source of high-quality drinking water as well as recovering nutrients and reducing the problem of eutrophication.

3.1.4.5.2 Complete removal of nutrients: Biological methods

The high energy demand of technological methods of advanced treatment and our recognition that energy is becoming a limited resource are arousing increasing interest in biological advanced treatment. The concept is simple. Basically, the problem of eutrophication is that plants—phytoplankton—grow profusely on nutrient-rich water. Why not turn this phenomenon to our advantage and set up systems that will grow *desirable* plants on such water? Nutrient-rich wastewater can be passed into long, shallow ponds where the plants absorb the nutrients from the flowing water. The ponds may be constructed to facilitate the harvesting of plants. The result is more or less pure water flowing from the end of the pond and successive harvests of the desired plants.

A variety of plants might be used in such systems: Plants such as water hyacinths and water lilies can be used for livestock feed. Cattails and other such "reeds" may be used in weaving "straw" mats, baskets, and so on. Or, such plants may be anaerobically decomposed to produce methane, fermented to produce alcohol, or burned directly as a boiler fuel.

Also, a biological advanced treatment system may include a second trophic level. For example, the ponds may contain algae and fish that eat the algae, thus producing relatively clean water plus fish. Shellfish, ducks, or other waterfowl might be grown in place of or along with fish. The possibilities are almost infinite.

If there are doubts about eating fish, or other products that have been raised in this way, recall that the nutrient-rich wastewater being used has been through primary and secondary treatment and may be disinfected with ozone. The chance of pathogenic organisms remaining in the water after such treatment is remote. By contrast, most of our oysters presently come from bays and estuaries which receive large quantities of animal and human wastes that have had no treatment at all. Even the most stringent water regulations permit oysters to be harvested from waters that contain some fecal bacteria. The general conclusion is that use of discharge water after secondary treatment for carefully controlled aquaculture of fish, shellfish, or other organisms is even less likely to lead to disease than our present practices.

However, biological advanced treatment does have some limits. Ponds require considerable land area or water area. In the Philippine Islands and other parts of the world, sections of coastal wetlands have been "fenced off" to create the ponds. A warm sunny climate is an advantage, but some of the climatic limitations may be overcome by judicious choice of species to be grown. Also, waste heat from power plants might be used to maintain desired temperatures. Again, heavy metals and other toxic waste products in wastewater may present a problem, particularly if the food chain involved will lead to humans.

3.1.5 Controlling Inputs Vs. Treatment

In light of the problems caused by certain contaminants in wastewater and the difficulty or expense in removing them, more consideration is being given to controlling what goes down the drain in the first place. This concept of control is particularly applicable in three areas: (1) industrial wastes, (2) phosphates, and (3) water itself.

3.1.5.1 Industrial wastes

As we have discussed, wastewater treatment as it currently exists through primary and secondary steps depends upon settling of solids and on biological oxidation of organic matter. These processes do not cope with toxic industrial wastes such as heavy metal ions, nonbiodegradable organic compounds, or caustic or acidic compounds in solution. In fact, the presence of these pollutants may impede treatment and render potentially valuable byproducts worthless. In

summary, these substances may lessen the efficiency of biological treatment by poisoning the decomposer organisms. Their presence may preclude: the use of treated sludge as a soil conditioner; the use of discharge water as irrigation water; further advanced biological treatment; or food use of plants or animals grown in such systems. Finally, if such toxic wastes are not removed they may have profound ecological effects when they are finally discharged into the environment.

Many people proclaim that domestic wastewater treatment should be upgraded to handle such toxic wastes, and through various processes of technological advanced treatment it would be possible to do so. However, the overall cost to society will be much less if such treatment is exercised by industry *before* their wastewater goes down the drain into the domestic sewage system. For example, suppose that a discharge from a factory is 1 kilogram (2.2 lb) of lead ions in 40,000 liters of water. You can readily see that it will cost less to let us say, distill the 40,000 liters of water at the factory to remove the lead than it will to distill 400 million liters to remove the same kilogram of lead, which may be the situation after the industrial waste is diluted with domestic wastewater. Further, even distillation of the entire volume of wastewater would still not resolve the problem of sludges being contaminated with toxic wastes. In conclusion, every effort needs to be made to promote practices that will prevent toxic chemicals from going down drains and contaminating domestic sewage wastes.

3.1.5.2 *Phosphate*

Since phosphate is generally the limiting factor for the growth of phytoplankton in natural lakes, limiting the amount of phosphate in discharge water, by itself, may be very effective in controlling eutrophication. This may be done by initiating advanced treatment to remove phosphate as described previously, or by limiting the amount of phosphate that goes down the drain in the first place.

Only about 30 percent of the phosphate in wastewater comes from human excrements. Most comes from the phosphate contained in detergents. It should be observed that "biodegradable," with respect to detergents, refers only to the organic (carbon) portion of the molecules. When this is degraded, the phosphate ions remain in solution. By substituting nonphosphate detergents, the phosphate in wastewater may be reduced approximately 70 percent.

Efforts have been made to ban the sale of phosphate-based detergents in areas experiencing problems with eutrophication, for

example, in the states bordering Lake Erie. However, in this case and in many others, the detergent industry has successfully lobbied to defeat such proposed legislation. Greater public understanding and support is needed before such laws can be passed.

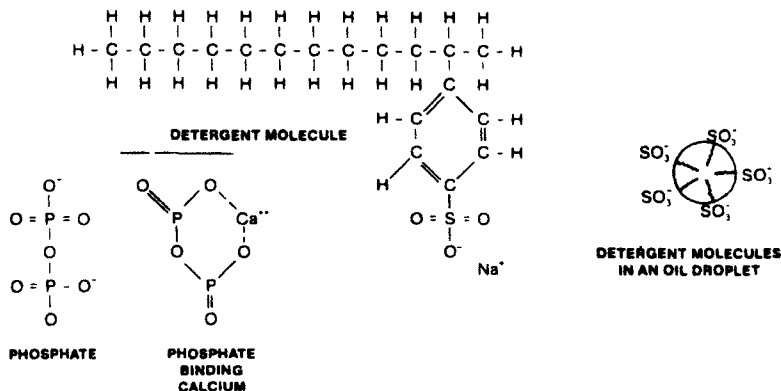


Figure 3.11 Detergent molecule. Its action results from the hydrocarbon end dissolving in fat while the ionic end dissolves in water. Fats are thus brought into solution. The hydrocarbon portion may be biodegradable, but phosphates, added to tie up calcium and thereby aid cleaning action, will remain in solution.

3.1.5.3 Water

Water conservation is generally thought of only in terms of saving water when the supply is limited. However, in many areas water conservation would benefit wastewater treatment. Note that the processes of primary and secondary treatment are time dependent—time is needed for settling and for biological decomposition. The more water used, the more is forced through a given treatment plant, and the faster it must move. Hence, there is less time for settling and decomposition, and the quality of treatment diminishes accordingly. Further, cost is related more to the volume of water to be treated than to the pollutants to be removed. If water conservation is practiced, the same pollutants are carried in less water, the cost of treatment is reduced, and the effectiveness of treatment is increased. There is no danger of clogging sewer lines with solids by practicing water conservation. Cutting water in half would double the pollutant concentration but the doubling is from 0.1 to 0.2 percent. Having 0.2 percent pollutants means that the total volume is still 99.8 percent water.

3.1.6 Cleaning Up

The stage was set for cleaning up our nation's water by the Clean

Water Act of 1972. Under the provisions of this law, states are required to prepare and embark upon programs of upgrading and expanding sewage treatment facilities. The law requires that the best practicable treatment procedures be in operation by July 1, 1983. The law also requires states to prepare plans for controlling nonpoint sources of pollution and provides a large share of federal funding.

However, a law cannot guarantee that the objective will be met. If the public becomes apathetic, we can expect the government to behave likewise. Planning and construction schedules will fall behind, compliance will not be enforced and laws themselves may be changed and made less strict. If we desire clean water, we should become familiar with the Clean Water Act of 1972 and with state and local laws, as well as with plans and progress to conform to these laws. Our public officials may need to be prodded to keep up with the good intentions expressed in the laws.

3.2 DISPOSAL AND RECYCLING OF SOLID WASTES

3.2.1 What is Solid Waste?

Solid waste refers to everything that goes out in trash and is handled as solids in contrast to what is flushed down the drain and handled as liquid. In this section, we shall consider *municipal solid wastes*, that is, wastes from homes, offices, stores, and schools. Solid wastes from agricultural and industrial operations are not considered in this category.

The volume of municipal solid wastes produced each year has grown steadily with increasing population and with increasing affluence. People who are able to buy more ultimately throw away more. In addition, there has been a trend toward use of disposable products such as paper plates and cups and plastic or aluminum food containers and wrappings, and also an increase in the number of inexpensive items that wear out quickly. Therefore, the generation of solid wastes has increased much more rapidly than the population and the trend is still continuing.

On the average, municipal solid wastes consist of the following (by weight):

Paper	41 %
Food wastes	21 %
Glass	12 %
Ferrous metals	10 %

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Plastics	5%
Wood	5%
Rubber and Leather	3%
Textiles	2%
Aluminum	1 %
Other metals	0.3%

However, the proportion may vary greatly from one type of community to another—for example, poor versus affluent. It also varies with the season. At certain times of the year there may be additions of grass clippings, leaves, and other lawn and garden wastes, all of which may nearly equal the listed categories in weight. This extreme variability of municipal solid waste presents many problems in regard to its use or disposal.

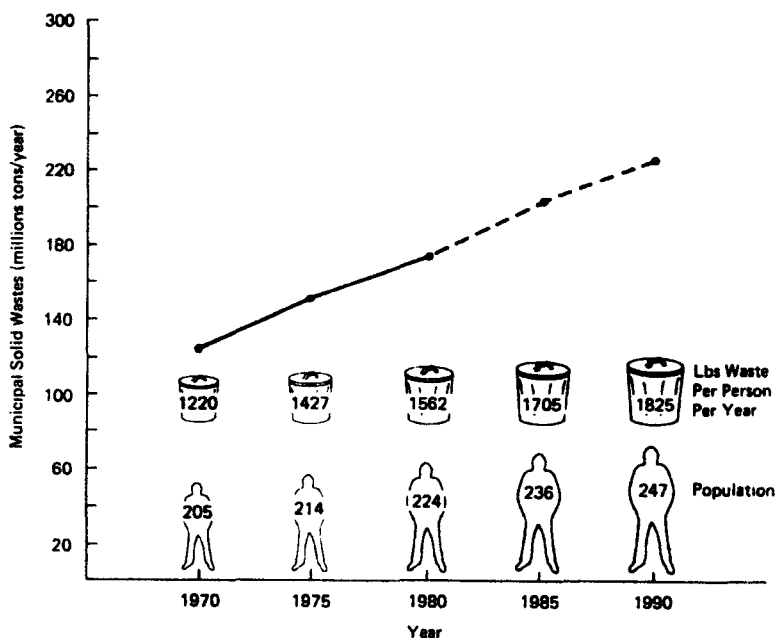


Figure 3.12. Output of solid wastes in the United States has grown much more rapidly than population and the trend is continuing. Can it be reversed?

Traditionally, local governments have assumed the responsibility of collecting and disposing of their municipal wastes. The local jurisdiction itself may own the equipment and employ workers or it may contract with a private firm to provide the service. In any case, the service is paid for by local tax dollars and the type and quality

of the service ultimately depends upon what residents are willing to support and pay for.

3.2.2 Means of Disposal: Past, Present and Future

3.2.2.1 Dumps

Until the 1960's most municipal solid waste was disposed of by open burning in dumps. Burning reduced the volume of waste, but burning was often delayed or incomplete. Consequently, dumps were notorious breeding grounds for rats, flies, and other vermin, and they were always the source of objectionable smoke and odors. Public objection and air pollution laws led to most open-burning dumps being phased out from the 1960's to the early 1970's.

3.2.2.2 Landfills

Sanitary landfills were generally adopted as the substitute for open-burning dumps, and some 90 percent of municipal solid waste is now disposed of in landfills. In a sanitary landfill, the wastes are dumped in a hole in the ground and covered with dirt. A natural valley or ravine may be used as the initial hole, or a trench may be dug. In either case, further excavations are made to expand the hole and to provide a source of dirt to cover each day's dumping.

As long as each day's refuse is well covered with dirt, landfills may be relatively clean and sanitary and air pollution from smoke is eliminated. When filling is completed the top is seeded, and the area may become an attractive park or it may be returned to grassland or forest. Building structures on landfills is not advisable because paper and other organic material in the refuse will gradually decompose, resulting in an inevitable settling of the filled area.

Landfills, however, still have disadvantages. First, the operation of a landfill seldom fulfills the ideal. It is difficult to prevent trash from being spilled or scattered by wind, and hence it is difficult to achieve complete coverage of all the refuse. Further, the covering dirt may be eroded, re-exposing the waste. Consequently, sanitary landfills may still produce objectionable odors and sights and allow breeding of rats and flies, although not as flagrantly as do open-burning dumps. Second, precipitation or ground water may percolate through the fill, leaching various compounds from the decomposing wastes. Countless cases of contaminated wells and pollution leading to fishkills in streams and rivers have been traced to toxic compounds leaching from landfills. In many states, regulations for the establishment of new landfills attempt to minimize the potential leaching problem.

However, the Environmental Protection Agency estimates that better than 80 percent of existing landfills are or potentially may be polluting ground water through leaching.

Finally, and most troublesome from the point of view of government officials responsible for solid waste disposal, is simply the lack of available space. Landfills must be relatively close to cities in order to avoid inordinate hauling expenses. Yet so many suburban and exurban developments have sprung up that there is literally no place near cities that is not close to one or more housing developments. The smell and water pollution from landfills are realistic grounds for objection and if nothing else, the continuous traffic of trash trucks to and from the landfill is objectionable; consequently, proposals for new landfills are frequently defeated by vigorous political counterattacks mounted by local residents.

Because of the lack of acceptable, close-in landfill sites, many cities are turning to remote sites. To minimize the additional hauling expense, this practice requires a transfer station where trash is taken from local collection trucks, compacted, bailed, and put on large trucks or rail cars for final hauling to fills. However, finding locations to build even transfer stations is difficult because they elicit the same objections from nearby residents as do landfills themselves. Furthermore, in large metropolitan regions, even acceptable "remote" sites are becoming increasingly hard to find. Frustrated government officials are led to say, "Everyone wants us to pick the stuff up, but no one wants us to put it down."

Humor aside, the situation is extremely serious. Many cities face the prospect of current landfill sites being filled to capacity within the next five to twenty years with no new sites available. A city of a million persons produces enough solid wastes to fill a large football stadium each year. Even after landfills are full, the trash will continue to come, and it must go somewhere.

3.2.2.3 Reclamation or Recycling

One solution is to look at municipal solid waste not as waste to be disposed of, but as a resource to be recycled back into the same or other useful products. A few of the possibilities include:

1. Paper can be:
 - a. Repulped and made into paper, cardboard, or other paper products.
 - b. Manufactured into cellulose insulation.

- c. Shredded for garden mulch; however, paper is devoid of nutrients.
 - d. Used as a clean-burning fuel since its sulfur content is near zero.
2. Glass can be:
 - a. Returned and refilled.
 - b. Crushed, remelted, and made into new containers.
 - c. Crushed and used as a substitute for gravel in asphalt paving (frequently referred to as *glassphalt*).
 - d. Crushed and used as a substitute for sand on beaches to replace that lost to erosion.
 - e. Crushed and made into "bricks" or "cinder blocks" for construction.
3. Metals can be:
 - a. Remelted and made into new metals. Making new aluminum from scrap aluminum saves more than 95 percent of the energy required to make aluminum from virgin ore.
4. Organic Matter can be:
 - a. Composted to make humus which can be used as a soil conditioner.
 - b. Burned along with paper for energy.
5. Textiles can be:
 - a. Shredded and used in new fiber products.
 - b. Burned along with paper for energy.

Recycling, of course, has been practiced for years by volunteer groups who collect paper, glass, and metals and take them to dealers. Such volunteer efforts generally have not managed to recycle more than 1 percent of any solid waste component. Therefore, as currently practiced, volunteer efforts make an insignificant contribution toward solution of the solid waste problem. Nevertheless, if recycling were to be practiced on a municipal scale, it would offer a substantial solution to the problem. In the bargain, it might produce revenue rather than just consuming it, and would save virgin resources as well.

The appeal of this concept is such that it has been promoted by many environmentalists with an enthusiasm bordering on religious fervor. In spite of the underlying logic of recycling, however, one cannot escape economic reality. In the context of the total municipal solid waste stream, it does not necessarily follow that all recycling is

good, or that lack of recycling certain products is bad. Lack of suitable analysis of recycling efforts can lead to situations where more resources are expended than saved and total wastes are not significantly reduced. In the following section we shall look at some of the problems of recycling on a municipal scale.

3.2.3 Problem of Recycling

Two major problems confront the recycling of municipal solid wastes: (1) separating the waste into its various components; (2) having markets to absorb the material once it is separated.

3.2.3.1 Separation

Present patterns of discard and collection mix all components of solid waste into one great mass (or mess). Recycling requires that the components be separated. This can be done before or after collection, but neither is without problems. In separation before collection, individual residents are asked (or required) to put paper, glass, metals, and garbage into separate containers. Containers are then collected separately, or at least put on different trucks which can deliver the material directly to the dealers. This technique requires exceptional understanding, dedication, and cooperation from residents, to say nothing of the large number of trash cans. Lack of full cooperation can completely defeat this effort.

There are some compromises between no recycling and a multitude of different trash cans and collections. For example, in Greenbelt, Maryland, a suburb of Washington, D.C., residents simply put paper in containers separate from other trash. There is one collection per week for paper and two collections per week for other trash; paper goes to a dealer, other trash still goes to a landfill. In instituting this program, Greenbelt had several factors in its favor. First, its residents generally had a high degree of environmental awareness. Second, as a well-to-do suburban community, the town already afforded three trash collections per week and had the sanitation workers pick up containers from the house, rather than relying on residents to place them by the curbside.

Greenbelt simply purchased one new can marked "Paper Only" for each household and devoted one of the three weekly collections to those cans only. Therefore, beyond the expense of the cans, there was no increased cost to the city. In fact, there was a net return from the sale of the paper, as well as a savings in keeping it out of the landfill. From the point of view of the residents, there was very little additional thought or effort required to put only paper in the

appropriate can, and the remaining collections were still fully adequate to prevent accumulation of other trash.

Other situations would not be so conducive to this system. For example, if there were only one collection per week, the city would have to double its trucks and personnel to provide a weekly collection of both paper and other trash. The value of paper collected would not offset the increased cost. On an every-other-week schedule trash would accumulate, resulting in unsanitary conditions. Likewise, where residents must put their own trash by the curbside, it has been found that not everyone will remember to put out the right can on the right day.

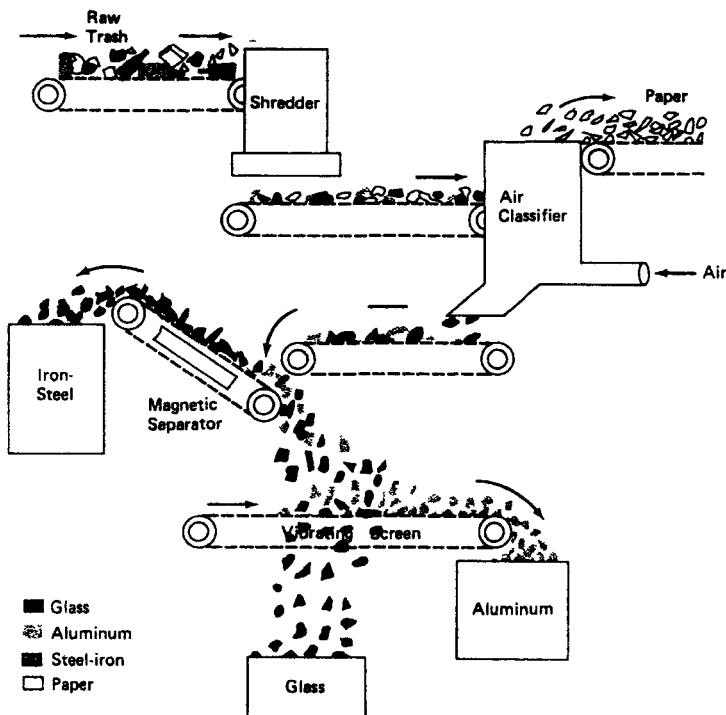


Figure 3.13 Municipal solid wastes can be separated into various components, but only at the cost of expensive equipment and high maintenance. Do the recycled materials justify the economic and resource costs?

Alternatively, various systems exist or are under development to separate municipal solid wastes after they are collected. In this situation, the material is first run through a shredder to reduce size. It then passes through an air stream which blows the lighter paper and organic material away from heavier glass and metals. The metals

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and glass are passed through a magnetic separator which removes ferrous (iron-containing)) metals. A water flotation separator is used to remove other heavy metals from the lighter glass and aluminum, and finally an electrostatic device is used to separate glass from aluminum.

Even with this, or some similar sophisticated technology, complete separation is hardly possible, which detracts from some of the options for use of recovered material. For example, plastic does not separate from other organic material, and this detracts from the use of the organic material as compost. Cans which combine both aluminum and iron lead to inevitable cross-contamination of these metals and to a reduced value as scrap.

Furthermore, it should be clear that however separation is accomplished, it requires a lot of equipment that is expensive to install, operate, and maintain. These costs must be subtracted from the value of the recovered materials and savings in not having to dispose of solid wastes in landfills or by other means. In fact, costs of separation and recovery may exceed the value of recovered materials and savings on disposal costs, leading to a net loss on recycling efforts. That is, in a given city or town, recycling may cost more than simply disposing through of the solid wastes in a landfill. These factors may be better understood by a word equation:

Profit or (Loss) in Recycling	=	Value of Recovered Materials	+	Savings on Alternative Disposal Costs	-	Cost of Separation and Recovery

Sometimes environmentalists are prone to discount the economics and assume that recycling must be good and disposal bad regardless of relative costs. It is well to remember that costs in themselves ultimately reflect the value of resources used. Consequently, a higher cost associated with recycling may be indicative of more precious resources being used than are being recovered. For example, the tungsten used in grinders and shredders is becoming a scarce resource, as is the energy to run them, while the iron and silica recovered from cans and bottles are among the most abundant elements on Earth. From the city officials' point of view, relative costs must be considered; decisions that lead to higher costs, and hence the need to raise taxes, are unpopular indeed.

Further controversy arises, however, because the factors in the recycling equation are not constant. Landfill disposal costs are escalating with increasing land values and increasing costs of fuel for hauling. Likewise, the value of recovered material may increase or decrease with changes in supply and demand, and costs of separation and recovery may change as technology develops.

In conclusion, whether separation and recovery of municipal solid wastes is worthwhile depends on present and projected costs of disposal, and also on present and projected market values of recovered material. This brings us to the second main problem in recycling solid wastes, namely, the problem of finding suitable markets for the recovered material.

3.2.3.2 Finding markets

The absence of sufficient markets that are willing and able to convert the waste materials into new products, and of a consumer market for the recycled products, can readily defeat the best efforts toward recycling. For example, the municipal paper-collecting effort in Greenbelt, Maryland, had to be temporarily abandoned in the early 1970's when the paper buyers had so much paper that they could not accept, even for free, the municipal wastepaper. There were many other instances in the early 1970's where paper and glass, carefully collected and separated by ardent environmentalists, ended up being taken to landfills along with other wastes because markets were insufficient to absorb it. Obviously, collecting and separating efforts in the absence of markets are an additional waste.

Consumer acceptance of recycled products is equally important. No manufacturer can operate without some profit. The virtue of recycling does not alter the fact that income from sales must exceed cost of material (for example, wastepaper) plus the costs of production. Ultimately, therefore, products made from recycled materials must compete in the marketplace with those made from virgin materials; they may or may not be able to compete successfully. For example, in reprocessing wastepaper into paper, there is an inevitable breakage of fibers that provide strength and integrity to the paper. Therefore, recycled paper is of lower quality; yet the cost of producing it is nearly the same as for virgin paper. Consequently, recycled paper has not found a large market. On the other hand, cellulose insulation (made from paper fibers) has proved to be very competitive with other types of insulation, and is now providing a substantial market for wastepaper.

Recycling, then, is as dependent upon supply/demand relationships as any other business. Unfortunately, creating a supply/demand balance with respect to recycled products tends to be impeded by the "chicken or the egg" phenomenon. A city may not invest in a facility to separate its solid wastes because there are too few industries to absorb the reclaimed products (insufficient demand for the supply). On the other hand, a company may not invest in a plant to reprocess waste materials because there is no guarantee of a constant supply of raw materials (insufficient supply for the demand). It is worth noting here that volunteer efforts in collecting materials for recycling tend to be sporadic. Many people become enthusiastic about recycling at one time and produce large amounts of salvagable waste products. Then interest fades and very little is produced. In other words, volunteer efforts generally do not provide the long-term dependability that is necessary for a satisfactory supply/demand balance.

Reprocessing industries and markets for recycled products obviously must develop or expand along with establishment of a municipal solid waste recovery program. This partnership has been promoted in a variety of ways. For instance, large government offices have entered into agreements whereby they promise to collect their wastepaper and, at the same time, buy a certain amount of recycled paper. The office thus simultaneously provides both a constant guaranteed supply of raw material and a guaranteed market for the recycled product. Such a give-and-take operation could be established on a municipal scale for many reclaimable materials in solid wastes.

Even so, the economic advantages of recovery and recycling of many constituents of solid wastes may be dubious. A recent analysis concluded that separation and recovery of municipal solid wastes does not pay for itself on the basis of materials recovered alone; it is only economically viable where alternative disposal costs are very high, thus adding a substantial savings in alternative disposal costs to the value of reclaimed products. However, as present landfills become full, disposal costs will escalate because of higher land costs and/or longer hauling distances to new sites. Also, the value of recovered materials is likely to increase. Therefore, recovery and recycling of municipal solid wastes should be reappraised periodically; if not at present, it may well become more economically justifiable in the future.

3.2.4 Converting Municipal Solid Waste to Energy

The problems of separating municipal solid waste and finding

markets for the recovered materials may be greatly simplified by converting municipal solid wastes to energy. Since 80 to 90 percent of such wastes are burnable, municipal solid waste can be used more or less directly as a boiler fuel without separation. Perhaps most importantly, there is virtually an unlimited market for the energy thus produced. In fact, the municipality itself may be able to use all or most of the energy produced, thus offsetting the cost of buying power or fuel from private utilities. For example, a municipal solid waste energy recovery plant in Nashville, Tennessee, supplies steam heat for state and city office buildings in the winter. In the summer, the steam is used to drive compressors which supply air conditioning. In Akron, Ohio, a solid waste energy recovery plant supplies steam heat for city buildings in winter, and in summer sells the steam to the local tire industry for use in processing rubber.

Glass and metals can still be recovered from the ash if desired, or the remaining ash can be used as fill dirt. Even if all the remaining unburnable material goes to a landfill, at least the total volume is reduced by 80 to 90 percent; therefore, the landfill site will last five to ten times longer. More importantly, since the incinerated material is not subject to further decomposition and settling, the ash may be used as fill dirt in construction sites, road beds, and so forth. In other words, disposal of the incinerated material presents relatively few problems.

Analysis shows that using municipal solid wastes as fuel, with or without recovery of materials from the ash, is more economical than separation and recovery of materials from municipal solid waste as such. Still, alternative disposal costs and the value of energy in the particular area are factors that cannot be neglected in making a decision.

Impeding the progress of energy recovery from solid wastes is the fact that the technology is still largely in the research and development phase. Burning solid wastes under a boiler to generate steam is simple in concept only. There are many practical problems in getting low-quality fuel, which solid wastes are, to burn efficiently so as to produce the most energy with the least air pollution, and in preventing the system from clogging up with ash. The potential risk involved is forcefully brought home by an experience in Baltimore, Maryland. Baltimore spent 22 million dollars for a pyrolysis plant only to find that it failed to operate successfully and required many modifications. However, several plants in the country are operating successfully.

3.2.5 Reducing Waste Volume

It is typical of our approach to living that we jump to add on more technology to overcome a problem whereas a problem might be better solved by cutting its roots. With respect to municipal solid wastes, we are prepared to build separation and recovery or energy conversion facilities, rather than consider whether all the waste is necessary in the first place. In many cases, reducing the volume of waste material that enters the system may be the most practical and economical approach to the problem. For example, recycling an aluminum can saves both disposal costs and 95 percent of the energy required to make a new can from virgin aluminum ore. However, even more would be saved if we were to decide not to make aluminum cans in the first place.

Reducing the volume of material that enters the solid waste stream deserves much more consideration than it has generally received. Approaches to reducing municipal solid waste generation can be divided into four general categories: (1) product reuse; (2) reduced material in products; (3) increased lifetime of products; and (4) decreased consumption.

3.2.5.1 Product reuse

Product reuse is the form of recycling in which the product itself is reused as opposed to the material being reprocessed into new products. The classic example is the returnable bottle vs. the one-way, disposable, or nonreturnable container. Everyone drinks about a liter of liquid per day. For 220 million Americans, this daily consumption amounts to some 1.2 million barrels of liquid. That a significant portion of this fluid should be packaged in single-serving containers that are used once and then thrown away is deplorable. It taxes one's imagination to think of a more costly, resource-squandering way of distributing fluids.

Nonreturnable beer and soft-drink containers comprise about six percent of the total municipal solid waste and about 50 percent of the nonburnable fraction. In addition, such containers make up about 50 percent of the total roadside litter, and nearly 90 percent of the nonbiodegradable fraction of litter. If returnable bottles were substituted for nonreturnable containers, the costs of disposal, the problems inherent in using municipal solid wastes for energy, and aesthetic degradation of the landscape could be greatly alleviated. In addition there would be a considerable savings of resources and energy.

Oregon and other states have laws which require a deposit on all

beverage containers sold within the state. This provides a monetary incentive to return containers and favors a shift toward refillable containers. There have been attempts to pass similar laws in many other states, but most attempts have been defeated by the combined efforts of bottle and can manufacturers, and national distributors of beer and soft drinks. The vested interest of bottle and can manufacturers in one-way containers is obvious, but that of national beer and soft-drink distributors needs a note of explanation.

Although using returnable containers is intrinsically cheaper than using one-way containers, the cost advantage is lost when long transportation distances are involved. To withstand the bangs and knocks of cleaning and reuse, returnable containers must be relatively heavy and bulky compared to one-way bottles or cans. The additional bulk and weight adds to shipping costs and, of course, to return shipping expenses as well. Therefore, returnable containers really have an advantage only when relatively short distribution distances are involved.

Indeed, through the early 1950's most beer and soft drinks were distributed from local bottlers and breweries in returnable bottles. However, by massive advertising campaigns and the use of one-way containers to lower shipping costs, a handful of national distributors were able to gain dominance during the 1950's and 1960's and countless local bottlers and breweries were driven out of business.

Actually, one-way containers appear to be cost competitive on the market shelf only because the distributor does not have to pay disposal costs of the containers. In effect, we, through tax dollars, subsidize national beverage producers and one-way containers by paying disposal and litter cleanup costs. There are also hidden costs in pollution from mining raw material and from producing the containers.

Laws that encourage or demand the use of returnable containers would again shift the advantage back to local bottlers and breweries, to the disadvantage of the huge national distributors. But national distributors are not so forthright in their position. They claim that loss of jobs and economic disruption would follow enactment of "bottle laws." In fact, experience under the Oregon bottle law has shown that the use of returnable bottles has created more jobs than were lost, to say nothing of savings to taxpayers in lower disposal costs and reducing roadside litter by 60 to 95 percent.

Another method of achieving product reuse which is gaining in popularity is the "garage" or "yard" sale. The families of a whole neighborhood get together and pool all their miscellaneous, unwanted,

but still usable items and hold a yard sale. Thus countless unused items are inexpensively traded and put back into use by their new owners. Here there are no blocks from industry or government. Anyone can organize a yard sale in his or her neighborhood.

3.2.5.2 Reduction of material in products

The concept of reducing material in products is most applicable to packaging. Environmentalists have long decried excessive packaging, especially the practice of sealing small items in a plastic cell attached to a large card. This practice does indeed amount to a large use of material which can only add to the volume of solid wastes. The cost of packaging sometimes equals or exceeds that of the product inside. However, it is well to recognize that this "excess" packaging is not completely a frivolous squandering of resources.

First, in today's serve-yourself shopping, the extra-large package provides a final, and perhaps the most important, advertising pitch. Second, and even more importantly, sealing small items on a large card acts as an effective deterrent to shoplifting-the card is difficult to conceal in a pocket, whereas the item itself would not be. For similar reasons, manufacturers quite intentionally make it almost impossible to get the item out of its package. Lastly, what seems to be excessive packaging may be quite necessary to protect items from excessive handling by customers. In short, it will be difficult to change packaging without some fundamental change in today's merchandising practices, but perhaps such changes are in order.

The amount of material in the product itself may be reduced if a smaller or lighter product will do the job equally well. For example, smaller cars provide great material savings as well as increased fuel efficiency. However, in other cases, decreasing material content may be counterproductive in that it frequently results in decreased durability and a shorter product lifetime. Here it may be more effective to increase the material in products in order to achieve a longer product lifetime.

3.2.5.3 Increased product lifetime

Wood, textiles, and leather make up about 10 percent of municipal solid waste. Most of this material consists of various items of clothing and home furnishings. If such items were more durable, they would stay in use longer and there would be less waste. Thus, increased product durability can go a long way toward reducing waste and resource consumption. Even if an individual does not choose to keep a durable item, it can readily be "recycled" through want ads, garage

sales, flea markets, and so forth. Very durable items actually may increase in value as they age and become antiques. Importantly, each individual has an effect in this area as one constantly makes choices between durable and nondurable items in the same product line; between throwing an item in the trash and repairing or reselling it; and between buying an item new or used. As consumers demand more durable products and shun nondurable items, there is no question but that industry will shift its production accordingly.

As we have noted, wastes may be reduced by such actions as using returnable bottles and buying more durable products which can be repaired or resold. However, in the final analysis waste is an inevitable result of consumption. Thus, reducing consumption will reduce waste. There is a real possibility of reducing one's consumption of products and getting along with fewer things. As an alternative to buying things, one may choose to spend more of one's disposable income on human services, such as lessons in art, music, dance, cooking, and so forth. Also, in buying handcrafted items as opposed to mass-produced items, a greater portion of the price is for human service as opposed to materials.

Consideration of our domestic wastes and their disposal emphasizes what a mammoth stream of materials of all kinds flows in one direction from our resource base to disposal sites. Just as natural ecosystems depend upon recycling nutrients, the continuance of technological society will also depend upon our learning to reduce consumption and recycle or reuse not only nutrients but virtually all kinds of other products as well.

4

Commercial Waste

From a broad point of view, industry encompasses all human endeavors to provide a better material life. But every level of industry-obtaining raw material, manufacturing, using products, and ultimately disposing of products-produces wastes which are discharged into the environment. Pollution may be defined as the addition to air, water, or soil of any material (or heat) that is usually not found there, or that is in excess of normal amounts. Thus while the discharge of most wastes is synonymous with pollution, emissions from smokestacks and drains of factories are far from the only sources of industrial pollution. Consider oil, for example. Spillage and pollution may occur in the process of drilling and obtaining crude oil from wells. There may be further spillage and pollution in transporting oil. Refineries pollute as they make oil into gasoline and other fuels. Finally, exhaust pollution results from the burning of gasoline in individual automobiles.

Thus, industrial pollution cannot be blamed solely on manufacturers. The blame must be shared by the whole of industrial society which desires, produces, and uses the products. Conversely, cleaning up pollution and preventing new pollution problems will demand understanding and cooperation by everyone. We cannot all do as we please with material goods and still have a clean environment.

This chapter will examine various pollution problems and discover how many old and still widely held attitudes and assumptions concerning wastes do not apply to modern dimensions of the problems. In turn, this will provide a new basis of understanding necessary to cope with present and future pollution problems.

4.1 ATTITUDES, ASSUMPTIONS, AND POLLUTION PROBLEMS

4.1.1 Why Do Humans Polluted?

Unless we discover the answer to the question "Why do humans

pollute?" and make some basic changes, we are more than likely to clean up one mess only to find ourselves making another. By understanding the causes underlying our pollution habits, we stand some chance of changing basic attitudes and behavior in such a way as to find permanent solutions to pollution problems.

A basic problem is that we tend to concentrate on single goals. As industrialists or consumers, as individuals or groups, humans tend to pursue single narrow objectives and let other things drop where they may. In his book *The Naked Ape*, Desmond Morris describes this tendency as a legacy from our primate ancestors. The ape goes after a banana and drops the peel. We go after a candy bar or soft drink and usually drop the wrapper or container. Similarly, we drive our cars with little concern as to what or how much is coming out of the tailpipe and what damage it does. We wash cleaning fluids, paint thinners, oil, grease, and all manner of other unwanted material down drains and sewers with little thought of where it will come out and the effects that it may have when it does. Factories still are frequently located on rivers or lakes simply to have a convenient way of flushing away wastes. The smokestack is the simplest way of dumping wastes into the air, where wind will presumably carry them away.

Secondly, as individuals we have a generally casual attitude toward accepting pollution. For example, workers could use the power of a strike to demand that a factory or an industry stop polluting, but in fact they don't. Consumers could organize consumer boycotts of goods from a polluting factory, but in fact they don't. Not many consumers are inclined to use pollution by the manufacturer as a criterion in choosing whether or not to buy a particular item. We must seriously ask ourselves, "Is the polluter more guilty than the people who accept the pollution?" Certainly the former could not exist without the latter.

4.1.2 Assumptions Underlying the

Casual Attitude Toward Pollution

The casual attitude toward pollution, in turn, is underlain by one or more of the following assumptions:

(1) *Threshold level:* It is assumed that below a certain level of concentration, pollutants will have no ill effect. This "certain level" is the *threshold level*.

(2) *Dilution:* It is assumed that pollutants will mix freely in air and/or water and will thus be diluted below threshold levels.

(3) *Assimilation:* It is assumed that wastes will re-enter the natural biological or geochemical cycles of the Earth.

(4) *Immobility of solid wastes:* It is assumed that solid wastes will stay where they are put.

(5) *Accidents won't happen:* In all our activities we tend to assume that accidents (oil spills, chemical leaks) won't happen.

These assumptions do have some validity. The first three involve the means which natural ecosystems use to dispose of their own pollutants of both physical and biological origin. For example, volcanoes vent large amounts of sulfur dioxide (SO_2), a gas highly poisonous to both plants and animals. Forest fires produce carbon monoxide (CO), a poisonous gas, as well as the nonpoisonous carbon dioxide (CO_2). Natural vegetation gives off a hydrocarbon gas, ethylene (C_2H_4), which acts as a plant hormone and in higher concentrations is extremely toxic to plants. However, in the amounts produced by nature these gases are readily diluted to threshold levels and then totally removed by assimilatory processes. For example, sulfur dioxide, ethylene, and carbon monoxide are readily absorbed and metabolized by soil microorganisms.

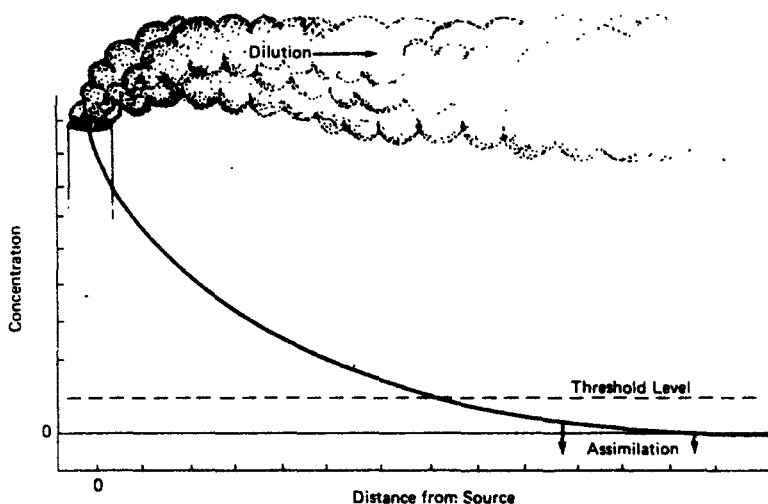


Figure 4.1 By dilution, the concentration of a pollutant diminishes with distance from the source. The *threshold level* is that level below which it is assumed there is no ill effect. After dilution, it is assumed that assimilation will reduce pollution levels to zero. With modern chemical pollutants, both assumptions may be invalid.

Likewise, animals produce urine and fecal wastes which in large concentrated amounts are very serious pollutants. However, in natural ecosystems, populations are small or mobile enough that undesirable amounts of these wastes do not accumulate in one place. Also, these

natural organic and inorganic wastes are readily metabolized by organisms and assimilated into the nutrient cycles.

Sediments may be thought of in a similar way. In runoff from most naturally vegetated land, sediments are dilute enough that they do not harm aquatic life as they wash downstream. Their natural rate of deposition is slow enough that they are assimilated in the natural course of succession without causing disturbance. Further, such natural deposits do not tend to migrate; they tend to stay where they land. These processes work so admirably in natural systems that it is not surprising we should assume that they would work as well for us and our wastes. The question addressed in the next section is why they do not.

4.1.3 Limits of Assumption

The question of why pollution problems exist despite the natural processes of dilution and assimilation brings us back to the theme of balances. Wastes do not accumulate to undesirable levels if the production of pollutants is balanced by dilution and assimilation; in natural ecosystems, this is generally the case. Humanity's problem is not that the natural processes don't operate, but rather that the critical balance is exceeded. This comes about in three basic ways: (1) overproduction of wastes, (2) introduction of unique chemicals, and (3) reduction of assimilative capacity.

As a result of both growing population and increasing affluence, we produce wastes in much greater quantities than are found in natural ecosystems. In 1973 it was estimated that burning coal and oil contaminated with sulfur released 10 times more sulfur dioxide into the atmosphere than comes from all natural sources. Ethylene production from auto exhaust was estimated at 1000 times nature's production. Such overproduction of wastes occurs with countless other compounds.

4.1.3.1 Unique chemicals

Modern chemical technology now produces and markets some 70,000 organic chemicals for use in insecticides, herbicides, synthetic fibers, plastics, and so forth, and about 10,000 new chemicals are added each year. Many of these chemicals are completely unique to nature, that is, they do not occur in nature even in small amounts as do sulfur dioxide, ethylene, and carbon monoxide. Therefore, in many cases, nature has not evolved any way of assimilating them and consequently they accumulate. More importantly, some of these new compounds do not seem to have threshold levels—that is, no minimum exposure can

be considered safe. Any level above zero may have harmful effects, particularly when periods of exposure are prolonged. As air, water, and soil become generally polluted with these compounds, exposures may be lifelong.

4.1.3.2 Reduction of assimilative capacity

Alteration of the environment in many cases reduces its assimilative capacity. As we noted, soil microorganisms are highly effective in assimilating sulfur dioxide, carbon monoxide, and ethylene. Vegetation is effective in removing other air pollutants. However, how much vegetation and unpaved soil is left in our cities? We also noted that sediment, through destroying the aquatic life in streams and rivers, reduces the capacity of the streams to assimilate organic wastes. Dredging and filling wetlands destroys the enormous potential of these systems for assimilating nutrient wastes and hence preventing eutrophication or other undesirable effects.

Finally, it is interesting to note that nature does not oblige organisms to live in the center of natural pollution sources. Volcanic vents and hot sulfur springs, for example, have few, if any, inhabitants. By contrast, the structure of an industrial technological society obliges most of its members to live and work in cities, the very heart of pollution centers. Thus, we tend to maximize rather than minimize our exposure to pollutants. Put another way, we create a pollution soup in which we oblige ourselves to live.

To free ourselves from the ill effects of pollution, it is necessary to understand the relationship between particular pollutants and natural balances and processes in more detail. We will then have the basis for understanding how human activities must be adjusted to fit within the limits of natural balances.

4.2 ASSUMPTIONS APPLIED TO POLLUTION PROBLEMS

4.2.1 Air Pollution

4.2.1.1 Major air pollutants

A wide range of inorganic gases, organic compounds, inorganic metallic substances, and soot particles is discharged into the atmosphere by motor vehicles, factories, power plants, home furnaces, and waste incineration plants. Many of these compounds are known to have injurious effects on human health, and may cause death of both animals and plants. Principal compounds, their major sources, and their important health effects.

Table 4.1 Principal polluting compounds, their major sources, and their health effects***Gaseous Pollutants***

Pollutant	Human Sources	Effects
Sulfur oxides	Fossil fuels, especially high-sulfur coal.	<i>Health:</i> aggravation of respiratory problems. <i>Other:</i> corrosion of materials, formation of acid rain, injury to plants.
Carbon monoxide	Automobile exhaust; cigarette smoking.	<i>Health:</i> oxygen deficiency in blood; dizziness, headache, fatigue, loss of consciousness; impairment of fetal development.
Nitrogen oxides	Automobile exhaust; burning any fossil fuel.	<i>Health:</i> respiratory tract irritation.

Pollutants in Fine Particulate Matter

Pollutant	Human Sources	Effects
Sulfates (SO ₄)	Atmospheric transformation of sulfur oxides from fossil-fuel-burning power	<i>Health:</i> Aggravation of respiratory diseases including asthma, chronic bronchitis, and emphysema; reduced lung function.

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(Table 4.1 Contd.)

Pollutant	Human Sources	Effects	Control
	plants; oil refineries, etc.	lung function; irritation of eyes and respiratory tract; increased mortality. Effects seen at 8-10 micrograms/ cubic meter." <i>Other:</i> Corrosion of materials; impairment of visibility; formation of acid rain; leaf injury and reduced growth in plants.	
Nitrates (NO ₃)	Atmospheric transformation of nitrogen oxides from fossil-fuel combustion. (Fine particles-especially metal-bearing particulates-catalyze formation.)	<i>Health:</i> Aggravation of respiratory and cardiovascular illnesses; chronic nephritis. Effects seen at 2.16-3.8 micrograms/cubic meter." <i>Other:</i> Fading of paints and dyes; impairment of visibility; reduced growth and premature leaf drop in plants.	Reduction of nitrogen oxides.
Organic Substances	Condensation of organic vapors from	<i>Health:</i> Eye and nose irritation; suspected cause of	Reduction of reactive hydrocarbon and ni

(Table 4.1 Contd.)

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Pollutant	Human Sources	Effects	Control
	fossil-fuel combustion, evaporation, and industrial processes; atmospheric reactions which convert organic material to fine particulates.	cancer and mutagenicity. Class includes Particulate Polycyclic Organic Matter (PPOM)-known human carcinogens; organic alkylating agents-known or suspected carcinogens (e.g., nitrosamines, epoxides, lactones, etc.). <i>Other:</i> Effect on water quality; potential impact on ecosystems.	nitrogen oxide emissions; fine particulate control techniques.
Inorganic Metallic Substances: Lead (Pb)	Leaded gasoline (90%); combustion of coal and fuel oil; smelting of lead.	<i>Health:</i> Accumulates in body organs. Acute effects: serious damage to nervous system. Chronic effects: Impairment of hemoglobin-synthesis; possible effects on kidney and reproductive functions; possible brain damage; behavioral problems; neurological	Cleaning of residual gases by particulate control techniques; elimination of lead additives in gasoline.

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Pollutant	Human Sources	Effects	Control
Cadmium	Mining and smelting metals; manufacturing and industrial processes.	impairment, especially in children. Chromosomal abnormalities. <i>Health:</i> Chronic respiratory disease; anemia; hypertension; effects on the cardiovascular and nervous systems; suspect carcinogen. <i>Other:</i> Concentrates in vegetation and shellfish.	Cleaning of residual gases by particulate control techniques.
Nickel (Ni)	Industrial and manufacturing processes; combustion of residual oil.	<i>Health:</i> Dermatitis; pneumonitis; lung cancer; nasal and sinus cancer.	Particulate techniques at low temperature.
Beryllium (Be)	Mining; smelting of beryllium; coal combustion.	<i>Health:</i> Dermatitis; skin ulcers; inflammation of the lung. Possible bone and lung cancer.	Cleaning of residual gases by particulate control techniques.
Mercury (Hg)	Mining and refining of mercury; combustion of fossil fuels	<i>Health:</i> Accumulates in body organs; inhibition of enzymes; impairment of	Cleaning of residual gases by condensation; filtration through

(Table 4.1 Contd.)

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(Table 4.1 Contd.)

Pollutant	Human Sources	Effects	Control
	and refuse; smelting of ores.	nervous system; fetal malformations. <i>Other:</i> Toxic to birds of prey and other wildlife; leaf injury and reduced growth in plants.	impregnated charcoal; scrubbing with water.
Arsenic (As)	Copper, lead, zinc smelters; combustion of coal; burning of cotton trash;	<i>Health:</i> Bronchitis; other respiratory illnesses; dermatitis; skin cancer; lung cancer. pesticides.	
Vanadium	Industrial and metallurgical processes; combustion of fuel oil.	<i>Health:</i> Irritation of respiratory tract, and other sensitive tissues; chronic bronchitis, with or without emphysema; synergism with sulfur dioxide; possible cancer of the lung.	
Chromium (Cr)	Electroplating and manufacturing processes; combustion of coal and refuse.	<i>Health:</i> Dermatitis; skin ulcers; lung cancer.	Cleaning of residual gases by particulate control techniques.

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(Table 4.1 Contd.)

Pollutant	Human Sources	Effects	Control
Inorganic Fibers: Asbestos	Construction, deterioration, and demolition of buildings; erosion of brake linings and clutch facings; variety of consumer products (paint, spackle, etc.)	<i>Health:</i> Fibrosis, calcification; cancer of the lungs and pleural cavity.	Containment of asbestos processing and handling operations; cleaning of residual gases by particulate control techniques; cleaning of residual gases by condensation; filtration through impregnated charcoal; scrubbing with water. Elimination of asbestos in consumer products.
Talc and Fiber Glass	Building materials; insulation; consumer products.	<i>Health:</i> Possible involvement in lung cancer.	Containment of processing and handling operations; filtration through impregnated charcoal; scrubbing with water. Elimination from consumer products.

In large part, wastes are discharged into the atmosphere through exhaust pipes, chimneys, and vents with the simple assumptions that they will dilute to threshold levels and then disappear. Unfortunately, these assumptions are invalid for several reasons.

4.2.1.2 City air-limited dilution

When the outpouring of pollutants is concentrated in a limited area, as it is in a city, undesirable levels of air pollution are inevitably created at times. Wind and rising air currents flush the pollutants away, and mix and dilute them with large volumes of surrounding air, thus reducing problems. However, such air currents are not always present. In still air, dilution is limited to the rate of diffusion, that is, the natural movement of molecules from an area of high concentration to one of lesser concentration. Since particles such as soot diffuse rather slowly, remarkably high concentrations can build up in surrounding air.

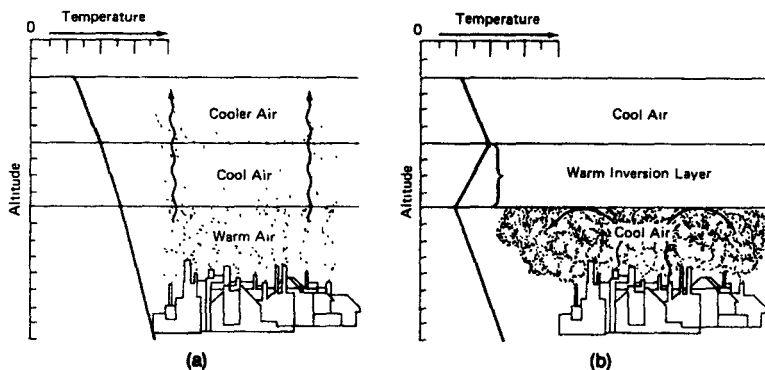


Figure 4.2 Temperature inversion. (a) Warm air rises, dispersing pollutants. (b) With a temperature inversion, a warm air layer overlying the cool air prevents pollutants from rising and being dispersed.

Aggravating the still-air condition is a weather phenomenon called a *temperature inversion*. Normally, air temperature decreases with increasing height above the ground. In this situation, the warm air near the ground rises (because warm air is lighter than cold air), carrying pollutants upward and dispersing them at higher altitudes. In a temperature inversion, the cold air is at the ground and warm air is above. This situation develops with the influx of a cold front during which the more dense cold air moves in under the warm air. With a temperature inversion, the upward currents of warm air are blocked and pollutants stay in the cold air near the ground. The effects

of a temperature inversion may be intensified by local topography, as in Mexico City and Los Angeles, where the surrounding hills or mountains prevent pollutants from moving horizontally.

Weather conditions which inhibit the dispersion of pollutants and hence result in their building up to high levels are referred to as air pollution *episodes*. Air pollution disasters which have resulted from such episodes include the following: London, 1150 deaths; London, 4,000 deaths; Donora, Pennsylvania, 20 deaths; New York, 400 deaths.

While such episodes are commonly cited to emphasize the seriousness of air pollution, they may actually distract us from the real issues. By associating air pollution and weather we tend to blame the pollution on the "terrible weather." This is a mistake. The weather patterns that produce episodes are quite normal. The tragedy lies in our failure to balance the volume of our pollutants with the air space available to receive them.

The citing of particular episodes and tragedies also tends to obscure the fact that average levels of pollutants in city air are manyfold higher than in clean air. Countless cases of eye and nasal irritation, coughing, fatigue, and asthma attacks are known to be associated with air pollution. Lungs are especially affected by pollutants in the air. In order to allow exchange of carbon dioxide and oxygen, they have a very large surface area of delicate body tissue. This tissue is intimately exposed to and affected by air pollutants. The most significant factor in lung diseases such as chronic bronchitis, emphysema, and lung cancer has been shown to be "personalized air pollution" cigarette smoking. However, more generalized air pollution certainly aggravates these conditions. Overall health costs resulting from generalized air pollution in the United States have been estimated as high as \$10 billion per year. When all this is considered, the loss in human health due to air pollution is much greater than particular episodes would suggest.

Air pollution also has severe effects on plants. It has killed countless trees and shrubs in cities. Many species can no longer be grown in cities and others are severely stunted.

City air pollution contributes to increased erosion of buildings and monuments, corrosion of metals, weakening of textiles and other fibers, and deterioration of paint. Also, the general dirt from air pollution demands increased washing of cars, windows, clothing, and so forth, and still it is difficult, perhaps impossible, to escape a perpetual dingy look.

Clearly, the volumes of pollutants we produce in cities, even given average dilution conditions, still accumulate to levels above the minimum thresholds for assuring human health, growing plants, and maintaining materials.

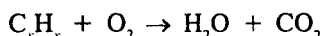
Another reason why we cannot assume that pollutants will simply dilute to threshold levels is the phenomenon of *synergistic interactions*. A synergistic interaction is that which occurs when two or more substances interact and cause an effect much greater than one would anticipate from the addition of their separate effects. You have probably heard of synergistic effects in connection with certain drugs and alcohol. Small doses of certain tranquilizers have a relatively mild effect, as do modest amounts of alcohol. However, when taking these drugs is combined with drinking alcohol, the effect may be fatal—tragically greater than would be anticipated on the basis of their separate effects.

Similarly, individual pollutants at existing concentrations might seem relatively harmless. However, in real life we are invariably exposed to many pollutants simultaneously and the potential for synergistic effects is virtually infinite. As time passes, scientists are discovering more and more synergistic effects involving pollutants. Three well-known effects are those relating, respectively, to photochemical smog, fine particles, and smoking.

4.2.1.2.1 Photochemical smog

In the early period of the Industrial Revolution, the commonest pollutants in most cities were particulate matter (smoke particles) and sulfur dioxide from burning coal. Most coal is contaminated with sulfur and, when burned, produces sulfur dioxide. As the use of coal gave way to cleaner-burning oil and natural gas during the first half of this century, air pollution was vastly lessened. However, in the 1950's and 1960's, virtually every city found itself increasingly enveloped by a brownish haze commonly called *smog*. It is more correctly referred to as *photochemical smog* because sunlight plays a role in its formation.

The worst culprit in producing photochemical smog is the automobile. Ideally, gasoline, which is a hydrocarbon (molecules made of hydrogen and carbon), should burn to carbon dioxide and water as the only waste products:



Unfortunately, gasoline burned in the cylinder of the internal combustion engine does not reach this ideal. Gasoline molecules are

incompletely burned, leaving various hydrocarbon molecules in the exhaust. Also, under conditions of combustion, some of the nitrogen of the atmosphere combines with oxygen to form various nitrogen oxides (NO , NO_2 , NO_3). Further, carbon monoxide (CO) also results from incomplete burning, as does some sulfur dioxide from sulfur contamination in the fuel, and lead, if present as an antiknock additive. All these wastes which leave the exhaust pipe along with carbon dioxide and water are to a greater or lesser extent toxic compounds and even by themselves are hardly desirable. However, their toxic effects are intensified by reactions between them, particularly between nitrogen oxides and hydrocarbons. Rather than being diluted further and gradually assimilated in the environment, these two compounds undergo a complex series of chemical reactions with each other, and with oxygen and water vapor in air to form ozone (O_3) and a wide variety of organic compounds consisting of various combinations of hydrocarbons with oxygen and nitrogen atoms. Sunlight provides the energy for these reactions; hence the resulting haze of this pollution is called *photochemical* smog. Ozone and many of the carbon-containing compounds, particularly one called *PAN* (for peroxyacetyl-nitrate), are extremely poisonous to both plants and animals. They are known to be responsible for eye, nose, and throat irritation and it is likely that they contribute to more serious disorders that develop over the long term. Thus, the interactions between nitrogen oxides and hydrocarbons are synergistic. Their end effect is a level of toxicity much greater than the effects of these compounds by themselves would suggest.

4.2.1.2.2 *Fine particles*

Synergistic reactions may also involve fine particles (less than 0.002 mm) of soot or smoke from burning any fuel or incinerating wastes. Such particles consist basically of nonreactive carbon. However, these particles, which are so small that they escape through most filters and remain suspended in the air for long periods, are potent adsorbers of metal atoms such as lead, hydrocarbons, sulfur, and nitrogen oxides. In other words, the fine particle collects and carries virtually every other pollutant. Many, perhaps most, of the chemical reactions resulting in the formation of more toxic compounds (as described in the formation of photochemical smog) may take place on the surface of fine particles. Then, when inhaled, these fine particles are drawn deep into the lungs where they may remain indefinitely. The lungs are equipped to filter out only relatively coarse

particles; they are not adapted to filter out these fine particles. Some authorities feel that the increasing frequency of lung cancer, emphysema, and other chronic respiratory diseases in urban areas may be partly attributed to the synergistic effect of metal atoms, hydrocarbon compounds, and so forth, being carried into and lodged in the lungs by fine particles.

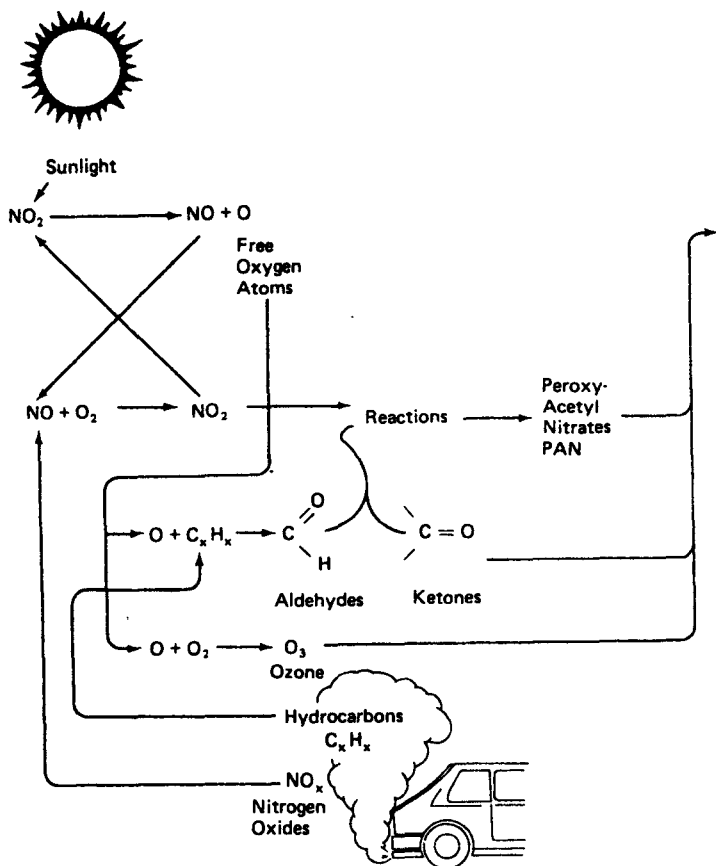


Figure 4.3 Formation of photochemical smog. Nitrogen oxides and hydrocarbons from auto exhaust interact in the atmosphere to form many compounds that are irritating and toxic to humans, animals, and plants. Only principal reactions are indicated here; there are actually more than 100 different reactions, involving hundreds of different compounds.

4.2.1.2.3 Smoking

Cigarette smoking has been clearly associated with increased risk

of lung cancer, heart disease, emphysema, and many other health problems. On top of this, there appears to be a synergistic interaction between smoking and general air pollution which increases the risk even more. For example, General air pollution has little significant effect on the incidence of chronic bronchitis among nonsmokers. However, among smokers, pollution results in a marked increase in the incidence of chronic bronchitis. It is fortunate that the prime contributing factor in this case, smoking, is one that we can choose to avoid.

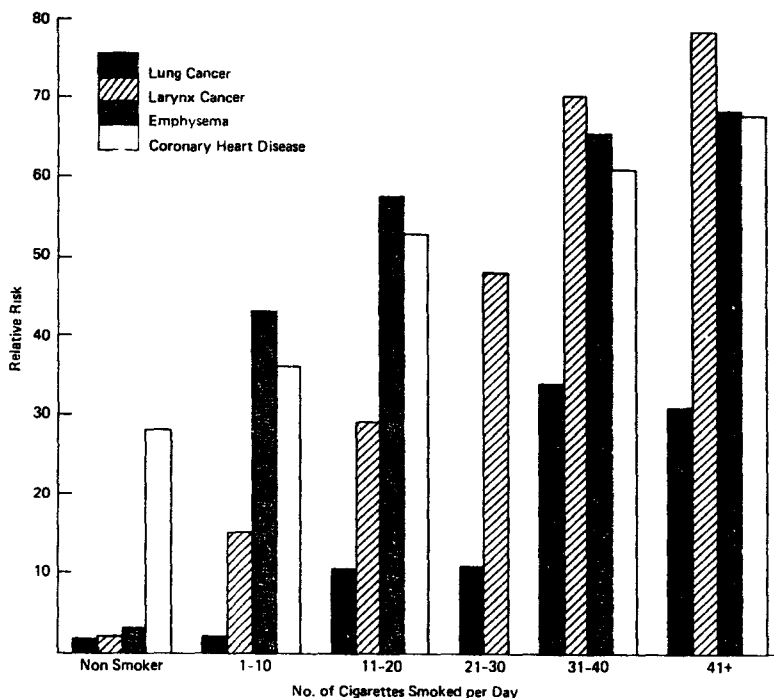


Figure 4.4 Many diseases and disease conditions are correlated with smoking.

4.2.1.3 Widespread effects

In the past, as described above, air pollution was generally considered basically an urban phenomenon. Consequently, the reasoning followed (and still persists among many people) that if urban pollutants could be diluted into the atmosphere at large, the final concentrations would be so low that they would cause no problems. This is the old assumption that "dilution is the solution to pollution." Therefore, taller smokestacks-up to 300 meters (1000 feet)-were

constructed for many industries and power plants in order to disperse pollutants more widely. Also, power plants have been constructed near coal fields in remote areas to remove their polluting effects from the concentrated areas of cities and instead to disperse them in areas of low pollution. However, much evidence has accumulated that this practice simply results in spreading harmful effects more widely. This is illustrated by sulfur dioxides leading to the formation of acid rain and the widespread effect of air pollution on plants.

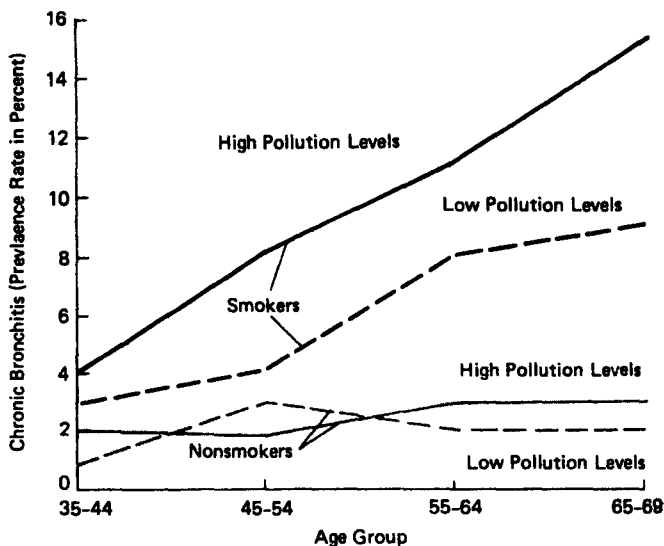


Figure 4.5 Synergistic effect between smoking and other air pollution. General air pollution by itself has little, if any, effect on the incidence of chronic bronchitis (compare the lines for nonsmokers). However, in combination with smoking, air pollution increases the risk markedly (compare the lines for smokers).

4.2.1.3.1 Sulfur dioxide and acid rain

Sulfur dioxide (SO_2) is a gas that is poisonous to both plants and animals. Sulfur dioxide is produced mostly by power plants which burn coal to generate electricity. A large power plant may burn 10,000 tons of coal a day; if this coal is contaminated with 3 percent sulfur, some 900 tons of sulfur dioxide per day will be discharged.

As was noted earlier, in the natural cycle sulfur dioxide may be removed from the air through assimilation by soil microorganisms. However, to avoid the toxic effects in the meantime, industries have attempted to dilute the sulfur dioxide by building taller smokestacks to disperse the gas. Ironically, this effort has largely circumvented

the natural process of assimilation and created a new pollution problem. For the natural process to work, sulfur dioxide must come in contact with the soil and its microorganisms. Tall smokestacks erected to promote dilution largely *prevent* this. But everything must go somewhere eventually. Airborne for long periods, sulfur dioxide gradually reacts with oxygen and water vapor in the air to form sulfuric acid (H_2SO_4). Thus 900 tons of sulfur dioxide from one day's operation of a single large power plant become some 1500 tons of sulfuric acid by the addition of oxygen and hydrogen to the molecule. The sulfuric acid is diluted by rainfall but even then the rain is commonly 10 to 100 times more acid than normal; in some cases it is even 1000 times more acid than normal. Nitrogen oxides contribute in a similar way by forming nitric acid (HNO_3). Rainwater containing such acids is called *acid rain*.

The effects of acid rain are numerous. Perhaps most striking is the dissolving of limestone and marble. Many statues and monuments have been eroded more in the last 50 years than they did in the previous 200. It also increases the corrosion rate of all metal structures, such as bridges. However, the most insidious long-term effect of acid rain is a gradual lowering of the pH of water and soil. This can lead to gross alteration of aquatic ecosystems and a greatly increased rate of leaching. For example, Cornell University biologist Carl Schofield has observed that more than half the lakes in the Adirondack Mountains (northern New York State) above 600 meters (1800 feet) have become highly acidic and 90 percent of these are devoid of fish. The death of the fish is due to both the acidity and the leaching effect of acid rain. In addition to decreasing pH, the acid precipitation leaches from the soil aluminum compounds which are toxic to fish. In another study, the water draining from a forest area in New Hampshire was monitored; it was found that leaching of nutrients had increased three- to tenfold because of acid rain. This constitutes a serious loss of fertility, which ultimately must be reflected in a decline in productivity.

Diabolically, the effects of acid rain are observed in what are generally considered unpolluted areas, hundreds of miles from pollution sources. The emissions which cause acid rain in the Adirondacks come from industries along the Great Lakes. The acid rain in New Hampshire comes from New York City. Similarly, sulfur dioxide originating in England has caused extensive acid rain damage to lake and stream ecosystems in Sweden. Almost everywhere that the pH of rainwater is measured, observers note some increase in acidity over that of pure rainwater. Therefore lesser effects can be presumed to extend even

more widely. A United Nations conference in the fall of 1977 recognized acid rain as a global pollution problem.

Federal air pollution laws restrict the sulfur dioxide emissions somewhat, but to prevent the impact of acid rain, regulations need to be much more stringent. Unfortunately, because of shortages of high-quality (low-sulfur) oil and natural gas, some leaders in industry and government are asking that air pollution regulations be relaxed to allow the burning of more coal and low-grade oil, which have high sulfur contents. If this occurs, acid rain problems can only become more severe. Obviously dilution is not the solution to pollution in the case of sulfur dioxide.

4.2.1.3.2 Air pollution and plant growth

There have been countless cases of vegetation-agricultural crops, ornamental plants, and forest species-being severely damaged or killed by air pollution. However, even more insidious than the outright visible damage, air pollution is also responsible for a general reduction in plant growth which can occur without other conspicuous signs of damage or abnormality. For example, a recent study in Yonkers, New York, showed that photochemical smog reduced sweet corn and alfalfa yields by 15 percent. Field experiments at Riverside, California, showed that yields of sweet corn were reduced by 72 percent, alfalfa 38 percent, radishes 38 percent, grapes 60 percent, navel oranges 50 percent, and lemons 30 percent as compared to similar plants grown in clean, filtered air. Another study in the San Bernardino Mountains of California showed that timber production had been reduced by 75 percent. Many other studies show similar results. Air pollution has forced the complete abandonment of citrus growing in certain areas of California and vegetable growing in certain areas of New Jersey-areas that were formerly among the most productive regions in the country.

The effects in most areas of the country are not this severe, for many important agricultural areas receive relatively little pollution, but nationwide the average loss of agricultural and forest production is estimated to be between 1 and 2 percent. This apparently small percentage is far from insignificant. With an annual corn production in the United States of about 6 billion bushels, a 2-percent loss amounts to about 120 million bushels.

Most importantly, the situation threatens to get worse. Air pollution control efforts of recent years have markedly reduced some pollutants in cities and undoubtedly the situation is better than if no pollution control had been exercised. However, more people driving

more miles, and industry burning more coal in place of cleaner burning oil and natural gas, as well as urban and industrial expansion in general, have been offsetting factors. Airline pilots report seeing the telltale haze of photochemical smog over wider and wider areas. For example, at times the smog extends continuously from Chicago to Washington, D.C., and continuously down the East Coast from Boston to Miami. The great concern is that if widespread air pollution gets gradually worse, reductions in crop production could occur with unanticipated, disastrous suddenness. The effect may be sudden, because reduction in growth (or any other pollution damage) is not necessarily a linear function of the pollution level. That is, one unit of pollutant does not produce one increment of damage, two units, two increments, and so on. Instead, plants will tolerate a given level of pollution with very little, if any, noticeable effect. But with a small increment in pollution above that level, the plant is pushed beyond its capacity to cope with the pollution insult and the damaging effect may increase drastically.

Walter W. Heck of the U.S. Department of Agriculture and North Carolina State University has stated, "An educated guess suggests that a doubling of present pollution concentrations on the East Coast could, under otherwise favorable environmental conditions, produce from 25 to 100 percent loss of many agronomic and horticultural crops and severe injury to many native species. . . . We are not far from pollution levels which could cause precipitous effects on agricultural production in the more humid areas of the United States." There are proposals to alleviate city air pollution by moving industries into rural areas, thereby aiding dilution of the pollution into the countryside. You can see that this could result in an unwitting and catastrophic sacrifice of important agricultural areas.

4.2.1.4 Global effects

Some waste products discharged into the air may affect the entire Earth. Pollutants that affect the ozone shield, and carbon dioxide and other pollutants affecting climate are two examples.

4.2.1.4.1 The Ozone shield

Earlier, we noted that ozone (O_3) produced in the lower atmosphere is a serious pollutant in that it is poisonous to both plants and animals. At the same time, paradoxically, ozone is absolutely essential in the stratosphere (upper atmosphere) in that it acts as a shield against ultraviolet radiation (UV). Ultraviolet is a part of the natural radiation from the sun; the wavelengths are just slightly shorter and have higher

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energy content than those of visible light. However, when UV penetrates living tissues, it is preferentially absorbed by proteins or nucleic acids such as DNA, and its high energy enables it to actually break the chemical bonds of these molecules. Consequently, UV is extremely destructive to biological tissues and is capable of causing mutations.

Some UV does penetrate to the surface of Earth; it is responsible for sunburns and is involved in some 200,000 to 600,000 cases of skin cancer per year in the U.S. However, we are spared the worst effects of UV because most of it is absorbed and hence screened out by the ozone in the stratosphere. Without this ozone "shield," the biological damage to both plants and animals would be disastrous. Indeed it is doubtful whether life could even exist on land without the protection of the ozone layer.

Interestingly, UV creates this shield itself by causing some oxygen molecules to split into separate oxygen atoms, some of which, in turn, combine with oxygen molecules to become ozone. Simultaneously, free oxygen atoms may combine with ozone, breaking it down to oxygen gas. Thus, a balance of ozone and oxygen is maintained in the stratosphere.

Certain pollutants diffusing gradually into the stratosphere from the lower atmosphere have damaging effects on the ozone layer. In particular, chlorine atoms catalyze the breakdown of ozone. By *catalyze* it is meant that a single chlorine atom can participate in the reaction repeatedly without itself being changed. Therefore a single chlorine atom can break down millions of molecules of ozone, upsetting the natural ozone balance.

A major potential source of chlorine reaching the stratosphere is the chlorofluorocarbons such as freon (CFC1₂) used as the propellant in aerosol cans. Chlorofluorocarbons liquefy under modest pressure and are relatively nontoxic and nonreactive. Thus, a small amount of liquid chlorofluorocarbon in an aerosol container can act as an inert ingredient that provides an even pressure over the life of the can. By 1974, the United States alone was spraying chlorofluorocarbons into the air at the rate of about 230 million kilograms (500 million pounds) per year.

Since the chlorofluorocarbons appeared relatively harmless no real concern existed about their being discharged into the atmosphere. It was assumed that they would be diluted and assimilated. However, in the mid-1970's a number of scientists reported that far from being assimilated, chlorofluorocarbons were diffusing into the stratosphere where they were breaking down and releasing free chlorine atoms.

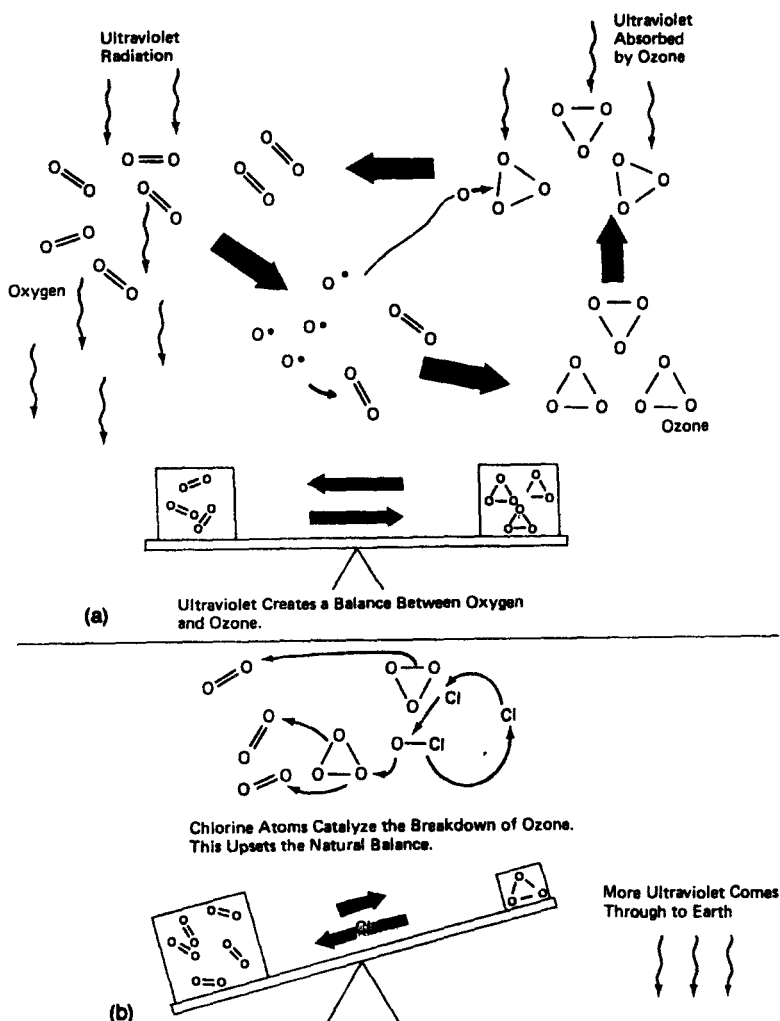


Figure 4.6 Ultraviolet radiation and the oxygen-ozone balance in the stratosphere. (a) Ultraviolet light causes the formation of ozone, which absorbs ultraviolet light. A balance exists between the formation and breakdown of ozone. (b) Chlorine atoms catalyze the breakdown of ozone; that is, a single chlorine atom may function over and over as shown to break down an infinite number of ozone molecules. Hence relatively little chlorine in the stratosphere may significantly shift the ozone-oxygen balance, permitting more ultraviolet to penetrate the atmosphere.

The chlorine is eventually removed from the stratosphere by combining with hydrogen to form hydrochloric acid (HCl), which finally returns to Earth by way of rainfall. However, this process is

very slow; therefore a relatively small amount of chlorine in the stratosphere can have a very large and prolonged effect. A report from the National Research Council concludes that a continuing release of chlorofluorocarbons at the 1973 rate would cause a reduction in the ozone layer of between 2 and 20 percent. Although the exact degree of the effects cannot yet be predicted, such a reduction in the ozone layer would produce substantial increases in human skin cancer and would have deleterious effects on plants and animals. While the extent of the damage to the ozone layer and the exact consequences of such damage are somewhat controversial, it is not wise to take a "wait-and-see" attitude. For one thing, it will take about 10 years for the chlorofluorocarbons released today to reach the stratosphere. Then, because of the catalytic nature of chlorine, the effects may endure for several hundred years.

The United States, in this instance, acted quickly by phasing out the use of chlorofluorocarbons in aerosol cans and is working on control of other uses of chlorofluorocarbons. Unfortunately, a number of other countries are still using chlorofluorocarbons in aerosol cans.

Additionally, chlorofluorocarbon compounds are not the only threat to the ozone layer. Carbon tetrachloride (CCl_4) is another substantial and perhaps even more significant source of free chlorine. In addition, nitric oxide (NO) can break down ozone in a manner similar to chlorine; high altitude aircraft, such as the supersonic transports (SST's), nitrogen fertilizers, and automobile exhaust are all direct or indirect sources of nitric oxide.

The lesson here is that when we do not definitely know what will happen to things dispersed into the environment, it is not safe to assume that they will be assimilated, that nature will take care of them. By so doing, we may be planting highly destructive time bombs which, once the fuses are lit, may be quite beyond our ability to control or stop.

4.2.1.4.2 Pollution and climate

So far we have stressed toxic or chemical effects of pollutants. However, such effects cannot be our only concern. Remember that the world ecosystem depends on subtle balances involving abiotic factors such as temperature and moisture, as well as biological factors. Disturbing the abiotic factors can be as destructive as direct poisoning of ourselves or agricultural crops. In this regard carbon dioxide and suspended particles have special significance.

Both carbon dioxide (CO_2) and suspended particles are natural

constituents of the atmosphere. We have already discussed the indispensable role of CO_2 in the carbon cycle between photosynthesis and respiration. Suspended particles include ash and soot from volcanoes and natural fires, dust (clay particles) blown from deserts, and water droplets from condensation of water vapor (clouds and mist). Therefore, it might appear that additions by humans of carbon dioxide, suspended particles from burning fuel, and other materials would not change anything. However, both suspended particles and CO_2 have marked effects on energy radiated to and from the Earth. Hence they are critical factors in determining overall temperature and thus climate. There is much evidence that our contribution of suspended particles and CO_2 to the atmosphere is already affecting climate and that the effects may become much more severe in the future.

As the sun's radiation strikes the atmosphere, it may simply be reflected into space or it may penetrate down to the Earth itself. Only the energy that penetrates the atmosphere actually adds to the energy balance of the Earth; what is reflected does not. The more reflection, the less energy received by Earth. This is where suspended particles play an important role. Light is reflected from the upper surface of clouds, haze layers, dust particles, and so forth. Therefore, the more suspended particles, the more reflection and the less energy penetrating to the Earth, with resulting cooler temperatures. Climatologist Reid Bryson states, "An increase of one percent in the normal reflectivity of the Earth from perhaps 37 to 38 percent would lower the mean temperature of the Earth about 1.7°C , or 3.1°F ." There is some direct evidence of this phenomenon: Times when exceptional volcanic activity increased the dust in the atmosphere have been correlated with periods of cooler temperatures.

Carbon dioxide affects the opposite side of the Earth's energy balance, namely, the radiation of heat. Energy reaching the Earth is largely in the form of light. Upon striking the Earth, most of the light is absorbed and in one way or another converted to heat. The heat is eventually reradiated from the Earth in the form of infrared (heat) radiation. Carbon dioxide in the atmosphere is transparent to light radiation but it tends to absorb and thus impede the passage of infrared radiation. This means that energy can get in but has trouble getting out. Therefore, atmospheric and surface temperature increases until there is enough heat "pressure" to overcome the resistance. The more CO_2 in the atmosphere, the more blockage of heat outflow and hence the greater the increase in temperature. This phenomenon is called the

greenhouse effect, because of its similarity to what occurs in a greenhouse or in a car left sitting in the sun. Light energy enters through the glass and is absorbed and converted to heat. The glass impedes the exit of infrared radiation; hence the interior temperature increases. Carbon dioxide is not the only molecule that works in this way: Water vapor, ozone, and certain organic molecules have a similar effect.

Humans seem bent on altering both sides of the Earth's heat balance. Since the beginning of the Industrial Revolution, ever-increasing quantities of CO_2 have been added to the air through the burning of fossil fuels (coal, oil, and natural gas). It is estimated that an equal quantity of CO_2 has been added by the cutting and burning of forests to make way for agriculture and the oxidation of organic matter in the soil due to agriculture. At least half the CO_2 has been assimilated in oceans or in other ways, but the other half has simply remained in the air, gradually raising the CO_2 concentration of the atmosphere. Since 1860 the concentration has increased about 13 percent, from about 290 to 331 parts per million (from 0.029 to 0.033 percent).

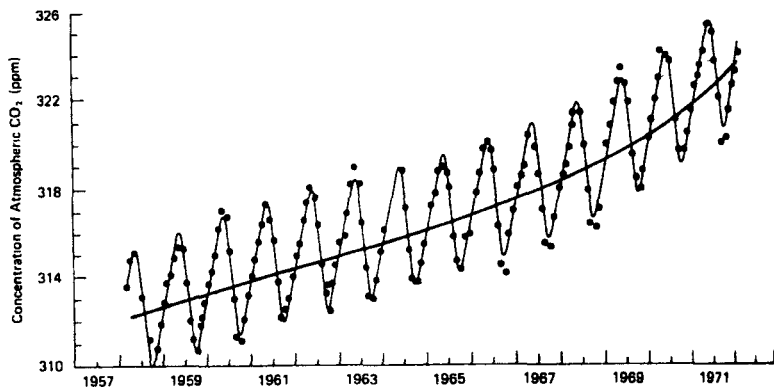


Figure 4.7 Carbon dioxide concentration in the atmosphere fluctuates between winter and summer due to seasonal variation in photosynthesis. But the *average* concentration is gradually increasing owing to human activities, namely, burning fossil fuels and oxidation of soil organic matter. This trend may lead to an increase in global temperatures which will result in other widespread effects.

Paralleling this increase in CO_2 from the 1880's to the 1940's was a gradual increase in the average world temperature of about 0.4°C (0.7°F).

However, since 1940 and continuing into the 1960's and 1970's, average temperatures have declined, largely reversing the previous increase. This has occurred in spite of the continued increase in CO_2 .

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Bryson believes that this decrease in temperature is due to an increase in suspended particles since World War II, overshadowing the CO_2 effect. You will recall that suspended particles tend to cool the atmosphere, while CO_2 tends to warm it. The increase in suspended particles is the result of human activities. Important sources are dispersed photochemical smog and other pollutants from cities and industry, aircraft exhaust, smoke from "slash and burn" agriculture (burning forests to clear land for agriculture, a common practice in the tropics), and increased wind erosion of soil because of desertification. It is estimated that the total quantity of suspended particles traceable to human activities approximately equals that produced by natural sources. Further, man's potential to pollute could increase the suspended particle concentration so greatly over the next 50 years that global temperatures could drop by as much as 3.5°C (4.3°F) enough to trigger another ice age.

However, another ice age in the near future is considered unlikely, because the CO_2 greenhouse effect is still operating. In this regard it is interesting to contrast the Northern and Southern hemispheres. The increase in suspended particles is largely a phenomenon of the Northern Hemisphere. They are mostly produced in the Northern Hemisphere and tend to settle out of the atmosphere before they reach the Southern Hemisphere. Carbon dioxide, on the other hand, diffuses evenly through the atmosphere of the entire globe. In keeping with their respective effects, it is found that the recent cooling trend is a phenomenon observed only in the Northern Hemisphere. Measurements in the Southern Hemisphere show that the warming trend observed prior to 1940 has continued unabated. It is predicted that the CO_2 effect will soon counterbalance the suspended particle effect in the north as well, and general warming will resume.

A 1977 report from the National Academy of Science also stresses that the CO_2 greenhouse effect has the most dire implications for the future. According to the report, unconstrained use of fossil fuels over the next 200 years would cause a four- to eightfold increase in atmospheric CO_2 . In turn, this could increase average world temperatures by 6°C (10.8°F) or more. According to the report, this temperature increase would probably not lead to a massive melting of the polar ice caps and subsequent flooding of all coastal and lowland areas, a fear that has often been stated. However, the temperature change, in the words of the report, "would exceed by far the temperature fluctuations of the past several thousand years and would very likely, along the way, have a highly significant impact on global

precipitation.”

The connection between temperature and precipitation is most important. Bryson points out that very modest shifts in temperature, whether toward warmer or cooler, dramatically alter the pathways of major air currents. In turn, this drastically alters patterns of precipitation: Some regions receive more; others receive less. Agricultural crops and practices the world over are intricately attuned to average local moisture conditions. Therefore any change in precipitation is more than likely to have severe disruptive effects on agricultural production. While scientists may debate the direction, extent, and timing of temperature changes, there is little doubt that they are occurring, and the implications should be clear.

Thus, even given virtually perfect dilution and pathways of assimilation, the Earth is not large enough to handle carbon dioxide in the volumes that we are producing, without upsetting fundamental balances. Again it points to a desperate need for us to recognize limits and attune our activities to what the Earth can sustain.

4.2.2 Water Pollution

Natural waters receive numerous pollutants from a wide variety of sources: nutrients from sewage outlets and fertilizer runoff; pesticides and herbicides from agricultural runoff; oil, grease, and numerous chemicals from street and highway runoff; chemicals from the fallout of air pollutants; chemicals leached from landfills and other dumps; chemicals from industrial processing; and waste heat. Historically, we have tended to hold the same assumptions about dilution, threshold levels, and assimilation of these pollutants by water as by air. As with air pollution, we have found that these assumptions are not fully valid and we are therefore confronted with many pollution problems. We shall discuss only a few of the areas that present significant problems.

4.2.2.1 Nutrients and eutrophication

The *eutrophication* is the series of events caused by additions of nutrients and leading to excessive growth of algae, then to depletion of dissolved oxygen by bacteria decomposing the algae, and finally to kills of fish and other aquatic organisms because of lack of oxygen. Eutrophication is one of the critically important forms of water pollution and, in many areas, it threatens to become worse. It is a classic example of humans exceeding the assimilative capacity of the natural system. Even though the nutrients are natural substances, the ecosystem balance is upset in such a way that a chain reaction which

disrupts the entire system is initiated.

4.2.2.2 Thermal pollution

Waste heat is a byproduct of many industrial processes. Waste heat must be dissipated into the environment, where it may raise temperatures to an undesirable extent; hence waste heat is referred to as *thermal (heat) pollution*. Particularly troublesome are electric power plants in which fuel is used to produce steam to drive turbogenerators. In such plants about two-thirds of the heat released from the fuel is dissipated into the environment in the process of recondensing the steam. The most convenient and economical way to dissipate the waste heat is to pump water from a lake, river, or other natural body of water over the cooling coils and return the warmed water to the natural body.

The water going through the cooling system itself gets hot enough to kill most organisms. However, intake pipes are screened to prevent the entrance of fish and dilution factors are calculated so that the overall temperature increase in the receiving body will not be enough to harm organisms. So much for the theory! There are many cases of fish being killed by being drawn against intake screens. Also, planktonic organisms (microscopic free-floating organisms) which are critical in many food chains are not screened out but go through the system and are killed. Finally, experience and experiments have shown that even modest changes in temperature can have farreaching repercussions on an ecosystem. Some of the possible effects include:

- (1) Increasing temperature may promote or intensify the latter phases of eutrophication in which oxygen depletion leads to fishkills. This occurs because warmer water holds less dissolved oxygen than cooler water. At the same time, increased temperature raises the metabolic rate and hence the rate of oxygen consumption by both bacteria and fish. Thus, more oxygen is being consumed by these organisms at the same time that less is available. The result may be large numbers of fish killed by oxygen deprivation.
- (2) Increasing temperature may affect the species composition of the producer level and hence the entire food chain. Many valuable species, namely green algae and diatoms, have lower optimum temperatures for growth than do noxious blue-green algae. Thus, thermal pollution can lead to a replacement of desirable algae by the undesirable blue-greens.
- (3) Increased temperatures may disrupt critical predator-prey

relationships. For example, trout have a lower optimum temperature than the minnows they feed on. Consequently, increased temperatures enable the minnows to escape from the trout more easily. Hence the minnow population proliferates, while the trout population starves.

- (4) Many synergistic effects come into play as a result of increased temperatures. Fish that are resistant to diseases at lower temperatures may become highly susceptible at increased temperatures. Also, increased temperatures may render fish more sensitive to other pollutants such as heavy metals and pesticides.
- (5) Fish may be attracted to the warmer temperatures of a thermal discharge, but then may be killed by the sudden drop in temperature when the discharge is turned off, as it must be for periodic maintenance of a power plant.

Since our consumption of electrical energy continues to increase, we must be exceedingly wary about increasing the impact of thermal pollution. Heat cannot be dissipated into natural bodies of water without potentially wide-ranging effects. An alternative is to dissipate waste heat into the air by means of cooling towers. While discharging heat into the atmosphere may have some local climatic effects, so far these have not been shown to be significant. Curtailing our profligate use of energy is also an alternative which deserves more consideration.

4.2.2.3 Chlorinated Hydrocarbons, Heavy Metals, and Bioaccumulation

Many chemicals discharged into water are diluted and assimilated; however, in some cases quite the reverse occurs. Instead of becoming ever more diluted and finally disappearing, some chemicals “reappear” in organisms at much *higher* concentrations. This *phenomenon of chemical buildup or accumulation to higher concentrations in a biological system* is known as *bioaccumulation*, or *biomagnification*.

Bioaccumulation occurs when a substance is taken in by an organism but cannot be metabolized or excreted. Therefore the organism accumulates the substance. The effect of bioaccumulation becomes magnified when several steps of a food chain are involved. The first organisms in the food chain accumulate a modest level of the substance. However, the second-level organisms accumulate much more, because in the course of its life an animal must eat many times its own weight in food to compensate for energy use. All the polluting substance contained in the ingested food is concentrated in the bodies of the

feeders. Since their biomass is only about one tenth the biomass of what they eat, the concentration of the polluting substance is increased tenfold. This concentrating effect is repeated throughout the food chain, each step increasing the bioaccumulation tenfold or more. A four-step food chain, thus, may produce a biomagnification of ten-thousandfold. Chlorinated hydrocarbons and heavy metals are two classes of compounds that have proven particularly susceptible to bioaccumulation and hence are particularly dangerous as pollutants.

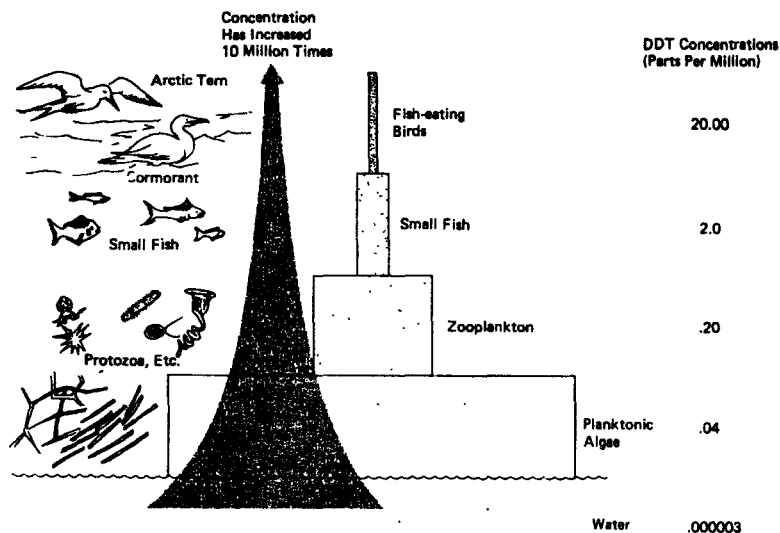
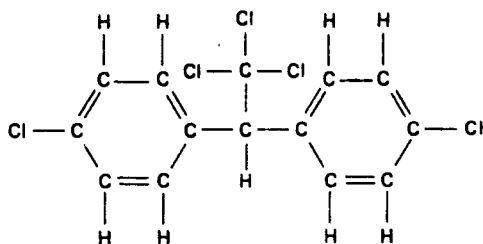


Figure 4.8 Biomagnification. Through food chains, certain substances may become highly concentrated in the lesser biomass at higher trophic levels. Biomagnification will occur with any stable substance that is absorbed but not excreted by biological organisms. Many chlorinated or other halogenated hydrocarbons and heavy metals are in this category.

4.2.2.3.1 Chlorinated hydrocarbons

Chlorinated hydrocarbons, also called *organochlorides*, are synthetic organic compounds in which one or more hydrogen atoms have been replaced by chlorine atoms. Bromine and fluorine atoms, which are chemically similar to chlorine, may also be substituted, giving rise to brominated or fluorinated hydrocarbons, respectively. Chlorine, bromine, and fluorine all belong to a chemical group known as *halogens*. Therefore this entire group of substituted hydrocarbons is known as *halogenated hydrocarbons*. Such compounds are widely used in plastics, electrical insulation, pesticides, flame retardants, wood preservatives, and many other products.

Many chlorinated hydrocarbons have two features which render them particularly susceptible to bioaccumulation: extreme chemical stability and high solubility in fat but relatively low solubility in water. Extreme chemical stability means that these chemicals persist almost indefinitely. They do not break down in the environment nor can they be metabolized by organisms. The high fat-solubility but low water-solubility means that they are readily absorbed by organisms because organisms contain virtually the only fat in the environment. Once in organisms they tend not to be excreted because excretion again demands solubility in water. The result is bioaccumulation.



DDT (DICHLORODIPHENYLTRICHLOROETHANE)

Figure 4.9 DDT. This pesticide is a classic example of a chlorinated hydrocarbon that is subject to bioaccumulation. Note that the structure consists basically of carbon and hydrogen but that chlorine atoms have been substituted for hydrogen atoms in several locations.

A classic case of bioaccumulation of a chlorinated hydrocarbon involves the pesticide DDT (dichlorodiphenyltrichloroethane). The insecticidal (insectkilling) properties of DDT were discovered shortly before World War II and it was subsequently used in huge quantities through the late 1960's for the control of virtually all kinds of insect pests, particularly disease-carrying insects such as malaria mosquitoes and fleas which carry typhus. It was assumed that any excess DDT would simply be diluted by the environment and thus disappear. It was therefore a great shock when it was discovered that, far from disappearing, DDT was accumulating through food chains and was responsible for the reproductive failure and/or death of countless birds, including our bald eagle, which held positions at the tops of food chains. DDT was also found to be accumulating in humans; however, no specific harmful effects have been identified.

For these and other reasons, DDT has been banned for most uses in the United States and some other countries. However, DDT continues to be exported for use in a number of other areas of the world. And,

just as significantly, the DDT story is repeated by numerous chlorinated hydrocarbon chemicals and other halogenated hydrocarbon compounds as well.

For example, PCB's (polychlorinatedbiphenyls) are widely used in plastics, electrical insulation, and carbonless printing paper, and escape into the environment from these and other sources. Like DDT, PCB's have been found to be accumulating in many species, and are present in many human food sources. Even more ominous, PCB's are much more toxic to humans than DDT. Even low doses have caused reproductive failure in monkeys and higher doses are conspicuously carcinogenic in rats. With the discovery of PCB's in many species of fish in the Great Lakes and in the Hudson and Mississippi rivers, these waters have been declared hazardous and as a result commercial fisheries have been closed. PCB's are now being phased out of certain uses.

In 1976, an episode occurred involving yet a third kind of chlorinated hydrocarbon. Kepone, an insecticide, had been allowed to escape into the James River from a manufacturing plant located in Hopewell, Virginia. Potentially toxic amounts of kepone accumulated in fish, forced the closing of all commercial fisheries on the James River, and threatened fishing in Chesapeake Bay. A study concluded that the exceedingly high stability of kepone and the supply of it in the river sediments will force commercial fisheries on the James River to remain closed for at least several decades and perhaps for as long as 100 years.

Many other such episodes might be cited and new episodes seem almost certain to occur in the future, because literally thousands of halogenated hydrocarbons are in use and new ones are continually being introduced. Many have the basic characteristics of chemical stability and fat solubility which lead to bioaccumulation.

4.2.2.3.2 Heavy Metals

As the name implies, heavy metals include that group of metallic elements with relatively high atomic weights, such as lead, mercury, copper, cadmium, and zinc. These particular heavy metals have received the most attention as pollutants but many others may yet be added to the list. In general, heavy metals tend to bind strongly with protein molecules which in many cases are enzymes. You may recall that the functioning of many enzymes actually depends upon a specific protein-metal ion combination, thus giving rise to nutritional requirements for certain trace minerals. However, the wrong kinds of metals, such as

mercury or lead, or even too much of an essential trace element, such as zinc or copper, can upset this critical protein-metal ion balance, thus impairing or even stopping the action of certain proteins. Frequently the wrong protein-metal bonding is quite specific. Mercury and lead, for example, have a strong tendency to combine with certain enzymes in the central nervous system. Hence, they readily lead to nervous disorders including insanity, mental retardation, coma, and death. Mercury, in addition, has been shown to combine specifically with a protein that functions closely with the genetic material, DNA. This may explain why mercury poisoning often leads to severe birth defects. Tragically, once these effects occur, they are in most cases irreversible.

This protein binding capacity of heavy metals leads to bioaccumulation as well as toxicity. Bound to a protein, the metal atom cannot be excreted. Hence very small doses over a period of time can gradually accumulate in the body to reach damaging, if not lethal, levels. A classic instance of this phenomenon is the "Minamata" disease, named for a small fishing village in Japan.

In the mid 1950's, cats in Minamata began to show spastic movements followed by partial paralysis and later coma and death. At first this was thought to be a peculiar disease of cats and little attention was paid to it. However, concern escalated quickly when the same symptoms began to occur in people; such additional symptoms as mental retardation, insanity, and birth defects also were observed. Scientists and medical experts diagnosed the problem as acute mercury poisoning. But what was the source of the mercury? It was found that a chemical company near Minamata was discharging waste containing mercury into the river that drained into the bay where the Minamata villagers fished. Mercury deposited in the sediments was absorbed by bacteria and biomagnified through the food chain to the fish. Then, villagers who subsisted on a diet high in fish accumulated toxic, and even lethal, levels of mercury. By the time the situation was brought under control, some 50 people had died and 150 had suffered serious bone and nerve damage. Even now, the tragedy lives on in crippled bodies, retarded minds, and children with severe birth defects.

A worldwide search for mercury prompted by the Minamata tragedy revealed dangerous levels of mercury in fish of many other areas, including our own Great Lakes. Subsequent investigations revealed another aspect of the problem. Previously, mercury was not considered to be a threat because the metallic form of mercury is not

particularly poisonous. Most mercury goes through the digestive tract, without ever being absorbed. However, bacteria living in bottom sediments not only absorb mercury, they put it through a chemical reaction in which mercury atoms become attached to organic compounds, giving rise to what is called "organic mercury." Of particular importance is a reaction known as *biomethylation*, in which the mercury is attached to a methyl ($-\text{CH}_3$) group to yield a compound called methyl mercury ($\text{Hg}-\text{CH}_3$). Unlike mercury itself, methyl mercury is absorbed nearly 100 percent; then it is nearly 100 times more toxic than metallic mercury and is not readily excreted.

With these discoveries, efforts have been made to sharply reduce discharges of waste mercury. Thus the hazard of future episodes of poisoning from environmental mercury has been greatly reduced. However, mercury remaining in sediments from past discharges continues to be a problem in some areas. For example, it was discovered in 1977 that fish from two Virginia rivers contained dangerously high levels of mercury. The source of the mercury was past industrial discharges. Although the factories and the discharges themselves had been shut down 27 years previously, the mercury was still leaching from sediments and accumulating in food chains.

Having recognized and corrected for the hazards of mercury should not make us complacent. It should make us much more wary of the danger inherent in heavy metals. For example, tin and other heavy metals also undergo biomethylation reactions that increase their toxic potential. Tin has been shown to have a very specific and negative effect on a particular kidney enzyme. Such specific effects mean that very low doses can be quite damaging because all the atoms are accumulated in a single system. Furthermore, as with air pollutants, synergisms may occur between heavy metals. For example, copper and zinc in combination have been shown to be more than 10 times as toxic to fish as either element alone.

Thus, as our industry and technology use greater and greater amounts of metals (tin use has doubled in the last 10 years); the potential for future Minamata-type disasters on perhaps an even larger scale is distressingly high. This potential can be offset if we get over the idea that these metals will simply dilute and disappear in the environment and instead take precautions to limit their escape.

It should also be noted that water and food are not the only sources of human exposure to heavy metals. The air is another major source of exposure because these metals are also discharged into the

air as we incinerate trash which contains such things as mercury batteries, as we burn coal which contains various heavy metals as contaminants, and as we burn gasoline containing lead additives. Regarding the latter, studies show that strikingly high percentages of urban children have elevated levels of lead in their blood, much more than can be explained by ingestion of paint chips which contain lead and which have been a prime source of lead poisoning in the past.

4.2.3 Solid Wastes and Accidents

The assumption of dilution obviously holds only for gaseous or liquid wastes discharged, respectively, into air or water. For solid wastes disposed of in or on the ground, we tend to hold the converse assumption—they will stay where they are put. Many cases prove that this assumption is equally invalid.

4.2.3.1 Leaching from municipal and industrial landfills

The leaching from municipal landfills may pollute ground water. A similar but even more serious threat exists with respect to dumps of industrial wastes. Most notorious are wastes from the chemical industry. In the course of manufacturing synthetic organic chemicals for plastics, pesticides, solvents, and other uses, extraneous chemicals are also produced in reaction vessels. Many of these chemical wastes are halogenated hydrocarbons, which, we have observed, are often highly stable, toxic, carcinogenic, and subject to bioaccumulation. Indeed, they are frequently referred to as *hazardous wastes*. Unfortunately, they have not been treated with the respect that they deserve. In large part chemical companies have simply put such wastes in steel drums and buried them in landfills. What happens twenty or thirty years later as the drums rust through? The potential for tragedy is vividly illustrated by what happened at Love Canal.

Love Canal was an abandoned canal bed near Niagara Falls, New York. Years ago it served as a convenient burial site for thousands of drums of waste chemicals. When the canal was filled, homes were subsequently built along the old banks and life went on normally—until 1978. In 1978, residents in the Love Canal area observed that they were experiencing an unusually high rate of miscarriages, birth defects, liver disease, and other health problems. They also observed that after rains, strange black chemicals oozed out of the ground and through their basement walls. They called in health authorities to ask if there was any connection, and indeed there was. The chemicals were identified as various toxic chlorinated hydrocarbons. The “time bomb” in Love Canal had gone off.

Insidiously, there are many similar time bombs ticking away in various parts of the country. In the last 30 years the use of synthetic organic chemicals has increased manyfold, and the volume of hazardous wastes has increased likewise. Much of this waste has been and still is disposed of in the ground. The Environmental Protection Agency estimated in 1978 that close to 90 percent of such disposal was inadequate and that 1200 to 2000 dumps were leaking hazardous chemicals into soil and ground water. This is not an encouraging thought when we recall that ground water is directly or indirectly the source of water for nearly all of us. Indeed, there are already hundreds of reports of well water contaminated with at least traces of hazardous chemicals, and more such reports are coming in all the time.

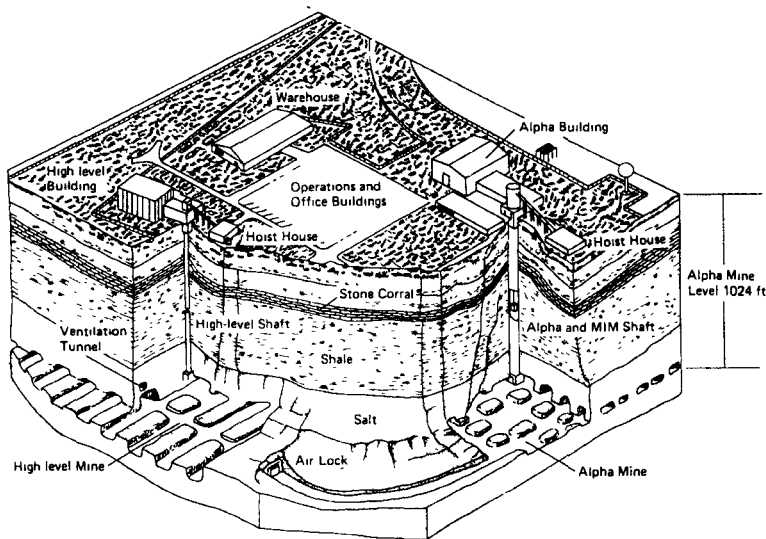


Figure 4.10 Disposal of radioactive wastes from nuclear power plants. These wastes must be isolated from the environment for thousands of years. Elaborate plans have been made for their disposal, but will this assure that they will stay where they are put?

In 1979 the Environmental Protection Agency estimated that the cost of cleaning up dumps of hazardous chemical wastes—action imperative to prevent further contamination of ground water—could be as high as 50 billion dollars. Even this expenditure would not purify the ground water that is already contaminated; we can only wait for the ground water system to gradually flush itself out—which, in some cases, may take hundreds of years. Clearly, burying hazardous wastes in the ground, with the tacit assumption that they will stay put, has

been a tragic and costly mistake. Safe alternatives must be put into effect.

4.2.3.2 Nuclear wastes

As we proceed to generate more 'and more of our electricity by means of nuclear power, there is a corresponding increase in the production of nuclear wastes. These nuclear wastes consist of highly radioactive elements which are extremely potent in causing mutations which may lead to birth defects and/or cancer. Some of the wastes may retain their radioactivity for periods up to 100,000 years. Therefore, the safety of nuclear power depends not only on the safe operation of the power plants themselves, but also on isolating these wastes from the biosphere for very long periods.

The nuclear industry and various government experts are confident that suitable techniques are available to keep nuclear wastes where they are put. However, the public is quite well aware that elaborate waste containment facilities and plans for monitoring do not, in fact, give assurance that this is the case. There is still the possibility, indeed the probability, of human failure. In 1973 a leak occurred in a tank at the Hanford nuclear waste storage facility in the State of Washington. The leak went unnoticed for six weeks despite the fact that both the loss and the increasing radioactivity in the ground were being recorded on automatic monitors over the entire period. The problem of safe disposal of nuclear wastes is the basis for much of the public reaction against nuclear power plants.

4.2.3.3 Accidents

The fallacy of the assumption that things stay where they are put may be extended to include the general tendency to assume that things will go as planned, or said another way, that accidents won't happen. The shortcoming of such an assumption is self evident: people will make mistakes and accidents will happen. As technology uses increasingly toxic compounds and greater and greater amounts of almost everything, the stage is set for very simple mistakes or accidents to result in widescale disasters.

As an example of such an event, in 1973 a few sacks of a fire retardant chemical got mixed up with an animal feed additive by a distributor in Michigan. If the chemical had been of low toxicity the amounts that were fed to the animals would have had little, if any, effect. However, the fire retardant chemical was PBB, a highly stable, bioaccumulating halogenated hydrocarbon closely related to PCB but

some five times more toxic. The results of this accident: Numerous people, mostly farm families, became sick, suffering varying degrees of nervous disorders; some 500 farms had to be quarantined; 30,000 cattle, 1.5 million chickens, thousands of sheep and hogs, and tons of cheese, milk, and eggs had to be destroyed because of the contamination, resulting in economic ruin to many farmers. The damage was estimated on the order of 100 million dollars, not including any compensation for individual human suffering. Moreover, the chemical is remaining and recycling in the Michigan ecosystem. Several years after the initial incident, reaccumulation from "unknown" sources was still causing sporadic occurrences of PBB poisoning.

In another incident, this one in the town of Seveso, Italy, in 1976, a safety valve in a chemical plant malfunctioned, and about a kilogram (2.2 pounds) of material was released into the air—a seemingly minor mishap. But in this case the material was dioxin, a chlorinated hydrocarbon and one of the most toxic substances known. The entire town of 100,000 residents had to be evacuated; hundreds of people suffered severe skin ailments; animals died by the thousands; and consumption of all local food was banned. A year later an area around the factory was still uninhabitable and there is much concern that birth defects may occur in the next generation.

Even relatively nontoxic materials take on disaster potential if the volume is large. Oil is a case in point. Crude oil is a mixture of natural organic compounds and in modest quantities is broken down by organisms and assimilated. However, the huge amounts which may come from an accident involving a supertanker can result in enormous ecological disasters. In March 1978, the supertanker *Amoco Cadiz* went aground off the French coast, spilling 220,000 tons of crude oil. Some of the results: 200 miles of one of Europe's most picturesque coastlines affected; over 20,000 birds, including a whole colony of rare puffins, wiped out; 9,000 tons of oysters made inedible and their culturing grounds ruined; marine worms which are essential in the food chain for commercial fish obliterated; tourism of the region cancelled out, affecting the economic lives of thousands. The longer-term effects are not yet known, but scientists believe they will be severe and last for many years.

Unfortunately the *Amoco Cadiz* was not the first such disaster, nor is it likely to be the last. With more and more oil being shipped in supertankers, more and even worse such disasters become increasingly probable in the future.

4.3 COPING WITH POLLUTION

Given all the problems and potential problems of pollution, it is tempting to call for an immediate moratorium on all further polluting. However, a moment's thought reveals that this is hopelessly simplistic. In manufacturing anything, only a fraction of the raw material consumed ends up in the product; the remainder becomes waste. In turn, the use of any consumable product is invariably synonymous with the release or discharge of waste products into the environment. Thus, stopping the output of wastes cannot be done short of closing out all human activity on Earth.

But pollution is not to be passively accepted, either. Somewhere between "closing out" humanity and accepting all pollution as inevitable, there is a long and laborious pathway of developing and implementing both technological and behavioral changes which will lead to controlling or managing wastes. With such control, the polluting impact of wastes can be reduced even if they can't be eliminated altogether. Then perhaps we can enjoy the benefits of both technology and a clean environment.

But, as mentioned, the pathway is laborious and ultimately it involves not just "they" who make laws or manufacture products. Ultimately it must involve all of society. The overall process can be divided into three steps: (1) recognizing threats of pollution, (2) devising methods of control, and (3) implementing controls.

4.3.1 Recognizing Threats of Pollution

The threats of pollution to human health, plant life, and global ecology in general should be clear from the preceding discussion. However, a few points deserve emphasis.

First, it should be apparent that we can no longer assume that pollutants will simply dilute to threshold (safe) levels and then disappear by assimilation. This is particularly true of synthetic organic chemicals and heavy metals that are subject to bioaccumulation.

Second, we need to revise our thinking as to what threshold levels are or even if they exist at all. Historically we have tended to think of threshold levels in terms of short-term exposures and assume that if it doesn't hurt today, it won't hurt tomorrow. But now we face lifelong exposures to various pollutants. More and more, scientists are finding that long-term exposure to low levels of pollutants may be just as disastrous, or more so, than short-term, high doses. The carcinogenic potentials of cigarette smoking and asbestos fibers are prime examples. Whether or not there is a safe level for long-term exposures is difficult

to determine experimentally. To learn the effect of a given exposure over a period of 40 years could require 40 years. However, based on general genetic theory, most scientists now concede that any substance that is mutagenic or carcinogenic in experimental organisms has *no* threshold level. According to this view, any exposure above zero produces some risk of inducing cancer and the risk simply increases with increasing exposure.

Compounding the problem of determining the threshold levels for a given compound may be an almost infinite number of possibilities for synergistic interactions among and between various pollutants and environmental factors. Many maladies of "unknown cause" from which we presently suffer may in time be shown to be due to such synergisms and/or long-term exposures to what we thought were harmless compounds.

Finally, it should be emphasized that some pollution effects may have worldwide impact and be irreversible once we have allowed them to occur. The only choice will be to suffer the long-term consequences. Potential destruction of the ozone shield and altering the climate by means of the CO₂ greenhouse effect are included in this category.

In conclusion, we need to develop a new point of view, one in which we evaluate pollutants against the background of natural nutrient cycles and balances. Unless our pollutants in kind and amount clearly fit into this background of natural processes and balances, we should assume that the biosphere *will not* take care of them. Sooner or later they will build up or accumulate in one or another part of the cycle, upsetting the overall balance and producing far-reaching consequences of indeterminable magnitude.

4.3.2 Methods of Control

Approaches toward reducing pollution can be divided into four general areas: (1) trap the wastes and manage where they go; (2) chemically change objectionable wastes to nonobjectionable compounds; (3) modify or change the production method so that undesirable wastes do not result; and (4) discontinue the use of the product or operation that causes undesirable amounts of pollution.

4.3.2.1 Trapping wastes

Exhausts from furnaces, incinerators, smelters, and so forth can be passed through various types of filters or electronic precipitators which trap and remove particulates, such as smoke particles. Such devices do not remove polluting gases, such as sulfur dioxide (SO₂), which exist as individual molecules, or very fine particles. However,

"chemical filters" can be used to remove specific compounds. For example, sulfur dioxide may be removed by "scrubbers," devices in which the exhaust is passed through a spray of lime which chemically combines with the sulfur dioxide and causes it to precipitate as a sludge of calcium sulfite/calcium sulfate ($\text{CaSO}_3/\text{CaSO}_4$). Similarly, organic compounds can be removed by passing the air or water through activated carbon (charcoal) filters. Additional types of filters may be designed to remove other specific compounds. More than one device may be required to remove all the contaminants from the waste stream.

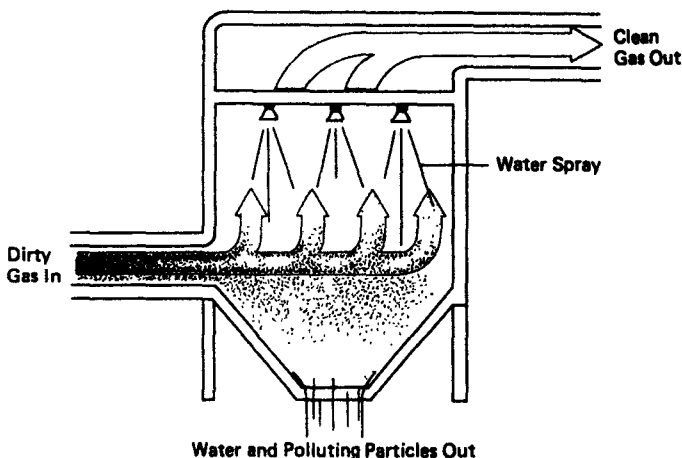


Figure 4.11 Scrubber. Exhaust gases may be passed through a chemical and/or water spray to remove certain gases such as sulfur dioxide.

Trapping the pollutants, however, is only half the problem. They still must go somewhere. Little is really solved if trapped pollutants from one source are dumped somewhere else; this only trades one pollution problem for another. For example, disposal of sludges from sulfur dioxide scrubbers can present problems. Materials collected from air filters and precipitators are frequently washed down the drain, resulting in water pollution problems, and we noted the problems resulting from disposal of waste chemicals in landfills. However, trapping wastes at least provides the potential for an acceptable, nonpolluting means of disposal. In addition, some wastes may be recycled or made into another useful product. For example, captured waste mercury can be reused. Trapped sulfur dioxide (SO_2) can be made into sulfuric acid (H_2SO_4), a widely used industrial chemical. Particulate ash may be made into building materials. However, such recycling or reuse won't tend to take place unless it is cost competitive.

That is, sulfuric acid will not be made from waste sulfur dioxide unless it can be done at least as cheaply as obtaining sulfuric acid from other sources.

4.3.2.2 Chemical change

In many cases noxious chemical wastes can be chemically changed to innocuous compounds. This is the function of the catalytic converter used to control pollution from cars. As exhaust passes through the converter, a catalyst causes more oxygen to react with the carbon monoxide and unburned hydrocarbons, thus oxidizing them to carbon dioxide and water vapor. (Lead destroys the catalyst. Can you see why leaded gasoline should not be used in cars equipped with such converters?) The principle of chemical change can also be applied to all the hazardous chemical wastes in the halogenated hydrocarbon category. By use of high-temperature incinerators such wastes can be oxidized to carbon dioxide, water, and other harmless compounds.

4.3.2.3 Change the process or operation

Instead of adding filters, converters, or other devices, it may be possible to change the operation itself so that the same product is obtained without the noxious byproducts. For example, a Japanese auto manufacturer (Honda) introduced what is commonly called a *stratified combustion engine*. The engine has a modified combustion chamber which provides for more complete burning of fuel and hence produces relatively little carbon monoxide and hydrocarbon fragments. Several techniques exist for removing sulfur from coal before it is burned. Although mercury is used in the production of most chlorine today, methods do exist for producing chlorine without using it, thus eliminating discharges of waste mercury. Increasing safety standards to minimize the chance of accidents may also be put in this category.

4.3.2.4 Discontinue use

The ultimate way to eliminate pollution by an offending product or substance is to discontinue its production, or use. However, this assumes that suitable substitutes exist or that society is willing to forego whatever advantages the product offers. There are a number of examples of this approach. Sale of high-phosphate detergents has been banned in some areas where eutrophication is a problem and low- or zero-phosphate detergents have been substituted in their place. DDT and some other chlorinated hydrocarbon pesticides have been banned from general use and other pesticides have been substituted. In the United States, chlorofluorocarbons have been discontinued from use in aerosol cans, and other propellants have been substituted. Although

substitutes for a particular product may be possible, they need to be regarded with caution since it is quite possible for the substitute to create pollution problems just as bad or worse than those of the original. For example, one proposed substitute for phosphate in detergents was found to be highly carcinogenic.

An example of society choosing to forego the advantages of a product was seen in the decision of the American people through Congress to abandon development of the supersonic transport (SST), although we did end up with the British-French Concorde anyway. The widespread public attack on nuclear power is another example of this approach in progress although the final decision here is not yet made. Additionally, it is not entirely clear that people who object to nuclear power really appreciate or have accepted the alternatives.

There are many proposals for decreasing air pollution in various cities by reducing traffic. These proposals range from such techniques as increasing city parking fees through the outright banning of all private vehicles from certain areas. The generally low acceptance or outright rejection of such proposals shows that the public may be unwilling to make the tradeoff in many cases.

4.3.3 Implementing Controls

We have discussed the threats of pollution and we have seen that there are methods for controlling pollution. Next is the need to choose and implement the controls to do the job.

4.3.3.1 *The need for laws*

Many people feel that industry should control its own pollutants on the basis of good conscience. However, good conscience or not, the following argument shows why it is effectively impossible for an industry to clean up its pollution unilaterally.

Whatever method of pollution control is used costs money. In trapping wastes or chemically changing them to less toxic compounds, the cost of filters, precipitators, catalytic converters, and so forth may be considerable. Then there is additional expense in operating and maintaining such devices. In producing a product by a new method to avoid a polluting byproduct, a company must write off the capital invested in the old production equipment, make a substantial investment in new production equipment, and perhaps face a more expensive production procedure. In discontinuing a product, a company again must abandon its investment in production equipment as well as sacrifice all income from the product. Only in rare and exceptional cases does pollution control lead to cheaper methods or valuable byproducts that create an overall cost savings.

Suppose a company were to undertake pollution control unilaterally. It has basically two choices: It can pass the costs on to its customers in the form of higher prices for its products, or it can pay for the costs itself and hence sacrifice some of its profits. In a competitive system, both choices are basically untenable. If the costs are passed to the customer, the higher-priced products lose out to competing products because, other factors being equal, consumers will choose the lower-priced product. Alternatively, if costs are taken out of earnings there is less money available to replace equipment, develop new products, expand marketing, and so on. Here again, the company will lose out to competitors. Therefore, by virtuously undertaking pollution control, the company succeeds only in sacrificing itself to its competitors who don't adopt similar controls. Simply dropping a product because it pollutes, particularly if it is a major source of revenue, is an even more conspicuous economic loss for the company and its investors.

These economic realities dictate that industrial interests will vigorously attempt to avoid pollution control as far as possible because it is a cost that does not contribute to production or sales. They will fight even more vigorously against the banning of any product from which they derive significant profit. Examples of such actions abound. Therefore laws and means of enforcing compliance with the laws are necessary. Interestingly, when companies are finally forced into taking pollution control measures, they frequently make the best of it by extensively advertising whatever steps they have taken. Such advertising presents a virtuous public image and hides the fact that the industry vigorously opposed and may still be opposing the regulations on the legal level.

4.3.3.2 Laws and compliance

People often comment, "Why don't *they* pass a law . . . ?" It is important to recognize that in a democracy laws are not passed by edicts of the President or anyone else. They are passed by Congress, state legislatures, city councils, and other governing bodies. In turn legislators respond to their constituents, who are individuals like you and me. If we want laws, we need to make our voices heard.

Public interest can be brought to bear on government in various ways. In the elective process one can support those candidates who share one's views. Representatives can be written or called to support or not support particular legislation. Through membership in environmental interest groups, one can support professional lobbyists, lawyers, and others who work to pass and enforce environmental

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legislation. These avenues of participation exist at local, state, and federal levels.

Through the 1960's and early 1970's a wave of ecological public interest and awareness did result in the formation of politically active environmental organizations and many environmental laws were passed. Most significant was the National Environmental Policy Act of 1969 (NEPA), which set the stage for many laws which followed. Most significant in the area of pollution are the Clean Water Act of 1972, the Clean Air Act of 1970, the Safe Drinking Water Act of 1974, and the Toxic Substances Control Act of 1976. Additionally, many states and local governments have laws which extend or expand upon the provisions of federal laws. Under these laws billions of dollars have been spent by both industry and government to control various pollutants and significant progress has been made in many areas. Certainly the situation is much better than it would have been if no action had been taken.

However, the existence of these laws and the fact that some progress has been made should not make us complacent concerning the future. First, these laws, as all laws, are subject to change by amendment or outright repeal. For example, in 1977 under mounting industrial pressure and with environmental zeal fading, important provisions of the Clean Air Act, which prevents further deterioration of air quality in many regions, were nearly lost. The granting of delays in the time by which the auto industry must meet certain standards on auto emissions has become almost routine.

Second, the process of reaching compliance (actually meeting the standards and requirements set forth by the laws) will continue well into the 1980's and probably far beyond. Here again, progress toward compliance will proceed only as far and as fast as public pressure demands. Without continuous public pressure there is plenty of continuing pressure from industrial interests to delay compliance indefinitely.

Finally, scientific investigations are really just beginning to reveal the magnitude and seriousness of the more subtle pollution problems such as those involving bioaccumulation and long-term exposures, various synergistic interactions, acid rain, the CO₂ greenhouse effect, and the ozone shield.

To prevent backsliding where progress has been made, to continue toward compliance of existing laws, and to meet new challenges, there will be a continuing need for public interest and involvement.

4.3.3.3 *Benefit-cost ratio*

As environmentalists promote higher degrees of pollution control, industry counters by pointing out the high costs involved. There is no question but that pollution control does cost money and that these costs are passed on to consumers in the form of more expensive products, higher utility bills, and so on. Thus it appears that we might save money by tolerating the pollution and not having controls. This is not necessarily so. Industries would save money, because they do not pay many of the hidden costs of pollution; however, the public does. The hidden costs of pollution include: higher health insurance premiums to cover the costs of pollution-related illnesses; higher product costs to pay for absenteeism because of pollution-related illnesses; higher maintenance and cleaning costs because of increased corrosion and dirt from pollution; higher food and wood-product costs because of crop and timber losses caused by pollution; higher fish and shellfish costs because of reduction of populations as a consequence of pollution; higher transportation costs for traveling to more distant recreational areas because nearby areas are polluted. Therefore, as citizens, our choice is not between paying for pollution control and not paying for pollution control; the real alternative is between paying the costs of pollution control or paying the many hidden costs that result from pollution. The question is: What are the relative costs in the two areas?

In attempting to arrive at concrete answers regarding relative costs, professionals perform *cost-benefit* analyses. In such analyses, professionals estimate as accurately as possible the *costs* of controlling or eliminating various pollutants. These costs are compared with the monetary *benefits* that may be achieved, such as reductions in health-care costs, maintenance and cleaning costs, food and wood-product costs, and so on. The result is a *benefit-cost ratio*. If benefits are greater than the costs, pollution control is economically justified. On the other hand, if costs are estimated to be *greater* than benefits, the effort is not worthwhile.

The problem in determining a benefit-cost ratio is that values assigned to many factors that enter into costs and/or benefits are crude estimates at best. Depending on one's point of view, one may come to quite different conclusions. For instance, industry is prone to maximize cost factors and minimize benefit factors, at least for controlling its own particular pollutants. On the other hand, environmentalists are likely to underestimate costs and place high

values on potential benefits. Workers who stand to lose their jobs if a polluting factory is closed will undoubtedly perceive relative costs and benefits differently than residents who are only affected by the pollution and have no vested interest in the factory. Further, certain benefits may be purely aesthetic—for example, the pleasure of having clear air and distant views. What monetary value should be placed on these? Here again, viewpoints will differ greatly.

Decisions, therefore, will be based not only on scientific data regarding the effects of pollution, but also on how individuals like you and me perceive and express our values. For example, the environmental movement of the late 1960's and early 1970's took place because enough people valued its benefits more than they feared its costs. The result was the passage of the aforementioned and many other environmental laws and the progress in pollution control that has been made to date. Indeed, cost-benefit analyses performed by the Environmental Protection Agency show that benefits derived from pollution cleanup have outweighed the costs. However, in spite of such analyses, it appears that the values of our society may now be shifting and that people are seeing the costs of pollution control as greater than the benefits. The result has been a decline in movement toward environmental goals, if not some backsliding.

It is necessary to reemphasize the hidden costs of pollution—costs which we all pay, whether or not we suffer direct health effects or other inconveniences from pollution. Also, much more emphasis should be placed on *deferred costs* which result from not controlling pollution or not implementing proper methods of waste disposal. For example, disposal of chemical wastes in landfills may have been the least expensive alternative in the short run. However, inherent in such decisions was the deferred cost of billions of dollars which we must spend to take care of those dumps, since they now are threatening our water supplies. Proper disposal of the materials in the first place would have been much cheaper. The same may be said regarding today's pollution. Improving pollution control may seem too expensive and not worth the cost. However, by not exercising better pollution control, we may well be deferring incalculable expenses into the future. Consider, for example, the cost that may come from reducing productivity of both natural and artificial ecosystems through the effect of acid rain that leaches nutrients, or the enormous medical expenses that may come from the bioaccumulation of more and more halogenated hydrocarbons, heavy metals, and so on. Until we recognize

and respond to the basic limits of what the biosphere can dilute and assimilate, and keep our output of pollutants within these limits, it is inevitable that we will be setting the stage for future tragedies.

4.3.4 Pollution and Lifestyle

Once decisions have been made to reduce pollution, there remains some choice in the methods to be used. We tend to consider pollution control in terms of add-on devices or processes such as filters or converters. However, do such devices really solve the problem? Recall that pollution is the inevitable result of excessive material and energy flow demanded by present lifestyles. Whenever there is a one-direction flow of materials, as opposed to recycling, materials will inevitably accumulate at certain points and present pollution problems. Add-on pollution control devices may redirect the flow and make it more tolerable for a time, but they don't get at the underlying problem, the flow itself. In fact, they may actually increase it. Filters, converters, and so on themselves must be manufactured and hence represent a further flow of materials. In addition, they require more

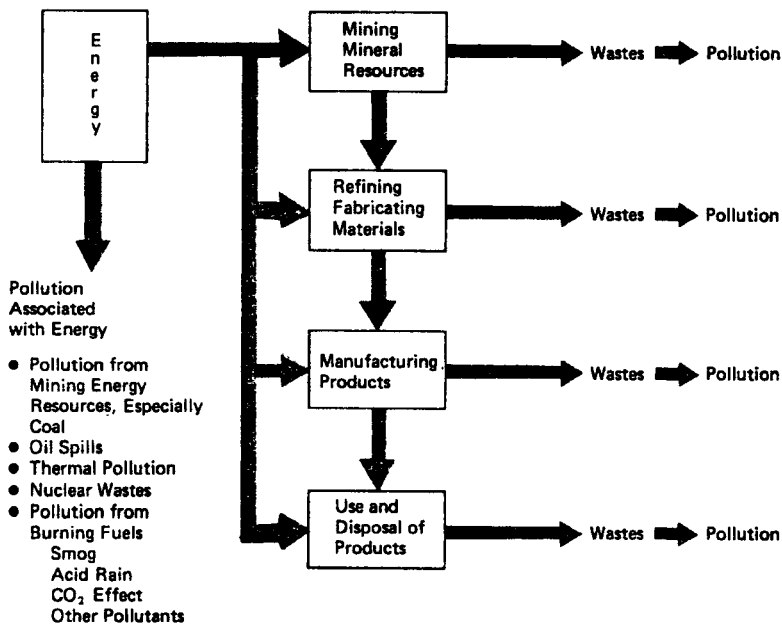


Figure 4.12 Use and disposal of products is the end of a long series of events with pollution occurring at every step. Reducing consumption at the end would reduce pollution at all the intervening levels.

energy to operate, which requires more flow of fuel and waste products of combustion and, in turn, more pollution control; and so the vicious cycle goes on.

Action which can be exercised by individuals and which should be given more serious consideration in national planning and policy-making is the development of lifestyles which use fewer materials and less energy, thereby lessening the flow and the fundamental output of pollutants. Actions such as product reuse, extending product lifetimes, and reducing consumption which were discussed at the end of Chapter 6 are just as or even more important in connection with reducing industrial pollution.

5

Sewage Treatment

Waste is any movable material that is perceived to be of no further use and that is permanently discarded. Once in the environment, wastes frequently cause damage to ecosystems and/or human health and therefore act as pollutants.

Successful waste management can largely avoid such pollution. This chapter introduces the more widely available strategies and technologies that can be effective in this area. The first three sections deal with the approaches used in the management of the relatively low-hazard wastes that are generated in bulk by industrial, commercial and domestic activity. Consideration of the options available for the safe treatment and disposal of high-hazard wastes is given in the fourth section. The chapter closes with a brief introduction to the concepts of waste minimisation, cleaner production and integrated waste management. If more widely adopted, these ideas have the potential to greatly improve current waste management practices.

5.1 WASTES FROM FOSSIL FUEL COMBUSTION

The main wastes generated during the combustion of fossil fuels are sulfur dioxide, NO_x , carbon monoxide, unburnt hydrocarbons, particulates, residual solids (including ash) and carbon dioxide. The technologies that are available for the management of these wastes are briefly reviewed in this section.

5.1.1 Sulfur Dioxide

Fossil fuels contain both organic sulfur (e.g. in thiophene rings) and inorganic sulfides (principally H_2S in natural gas and FeS_2 in coal). During combustion these react with atmospheric oxygen (O_2) to produce sulfur dioxide (SO_2).

The sulfur content of fossil fuels varies considerably. For example,

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coals and fuel oils generally contain 1-4%, and 3-4% S respectively. However, there are naturally occurring low-sulfur fuels (e.g., coals <1% S and fuel oils <0.5% S). Clearly, burning these preferentially is one of the options available for diminishing the emissions of sulfur dioxide. Unfortunately, this is of only limited applicability as supplies of these low-sulfur fuels are comparatively small.

Another alternative is the dilution and dispersion of the sulfur dioxide produced, principally by building taller chimneys. This has found favour in the past and has had noticeable success in the reduction of local levels of pollution. Unfortunately, it has had no impact on overall contamination; in effect, 'what goes up must come down'.

Fuel cleaning processes that remove sulfur are routinely applied to natural gas, oil and coal. These are now considered in turn.

Natural gas contains variable amounts of hydrogen sulfide (H_2S). This may be effectively removed by a number of processes including adsorption onto zeolites (a type of aluminosilicate mineral). The hydrogen sulfide may then be oxidised *in situ* with hot sulfur dioxide to yield sulfur vapour and regenerated zeolite adsorbant. The sulfur is then condensed and sold, while the zeolite is reused.

The desulfurisation of oils is desirable for a number of reasons that are unrelated to the lowering of sulfur dioxide emissions. These include avoiding the deactivation (poisoning) of platinum catalysts used during oil processing. Consequently, oil desulfurisation was practised before the environmental need to reduce sulfur dioxide emissions was recognised. The main process involved is hydrosulfurisation. During this the oil is reacted with hydrogen (H_2) at elevated temperatures, under pressure and in the presence of a catalyst. This converts the sulfur to hydrogen sulfide which can then be separated as a gas.

An important consequence of oil desulfurisation is that motor spirit (petrol, gasoline) has a very low sulfur content (between 0.026% in US Premium grade and 0.040% in the UK). As a result, transport makes very little contribution to the total anthropogenic emissions of sulfur dioxide.

Coal is cleaned by the separation of the organic fuel from the inorganic ash-forming mineral impurities that it contains. This may be done on the basis of density, for the fuel has a lower specific gravity (1.1 to 1.8) than the impurities (from about 2 to about 5). In one process the raw coal is finely ground, so that most of the mineral particles become distinct from the fuel. The ground raw coal is then agitated in a mixture of air, water, oil and surfactant. The denser

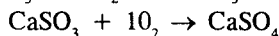
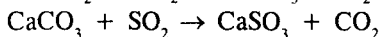
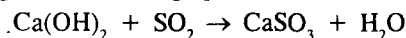
particles sink, while the others are held by surface tension at the interface between the liquid and the air. The cleaned coal is then isolated in a settling tank, where the air/oil/water mixture is allowed to separate, causing the fuel to sink.

Processes such as this may remove much of the inorganic sulfur fraction (FeS_2 has a specific gravity of 4.5). In a typical British coal, about half of the sulfur is inorganic, the rest forming part of the organic matrix of the fuel. It is now technologically possible to remove some of this also, though it is currently not economically viable to do so.

Vast amounts of coal are consumed worldwide, particularly during the production of electricity. This, coupled with the relative inefficiency of the routine coal cleaning process, makes this fuel by far the largest single contributor to anthropogenic sulfur dioxide emissions. There is therefore considerable interest in the removal of sulfur dioxide prior to the release of the flue gases.

Sulfur dioxide removal rates of 90% can be achieved from the combustion zone in boilers that are based on *fluidised bed combustion* (FBC) technology. In such systems the fuel is added in a pulverised form to a bed of inert material (e.g. sand or coal ash). This is kept in a state of agitation (i.e. fluidised) by a strong updraught of air, which acts as the oxidant. Such systems allow the coal to be burnt efficiently at relatively low temperatures ('900°C).

As an alternative, sulfur dioxide can be removed downstream of the boiler after the fly ash has been removed, a process called flue gas desulfurisation (FGD). FGD can be highly efficient: 90% removal rates are generally achievable. In a typical system, an aqueous slurry of an alkaline absorbant, commonly lime, or limestone, is passed in a fine spray through the flue gases. Sulfites and sulfates are therefore generated during this 'scrubbing' process:



The last of these reactions can be encouraged by the injection of air into the sump of the scrubbing tower. This yields high-quality gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$) which can be sold for use in plasterboard and other building materials.

5.1.2 NO_x , Carbon Monoxide and Unburnt Hydrocarbons

The burning of fossil fuels in air produces nitric oxide (NO) and,

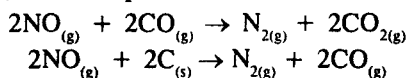
to a lesser extent, nitrogen dioxide (NO_2); these are collectively known as NO_x . They are formed by the reaction of atmospheric oxygen with nitrogen at the high temperatures reached during combustion. The nitrogen may originate from either the air or the fuel, thereby producing thermal- NO_x and fuel- NO_x respectively.

The problem of fuel- NO_x is primarily associated with coal because it has relatively high levels of nitrogen (1-2%) compared with other fossil fuels. For example, natural gas is virtually nitrogen-free, while fuel oil contains <0.5% N.

Clearly, thermal- NO_x formation occurs whenever fuels are burnt in air. This allows transport to be a major contributor to NO_x emissions. For example, in the UK about half of NO_x is traffic-related. The remainder originates from stationary producers, particularly electricity generating stations.

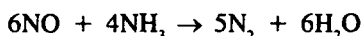
Reduction in the emissions of NO_x can be achieved by alterations to the combustion process. The reactions that produce thermal- NO_x are endothermic and are therefore favoured by high temperatures. Lowering the temperature of combustion by, for example, recycling exhaust gases will therefore diminish NO_x emissions. Unfortunately, this will also reduce the Carnot efficiency of any heat-to-work device driven by the fuel. If used in a motor vehicle, NO_x reduction by this method will therefore be at the expense of fuel economy.

Fuel- NO_x emissions can also be controlled by adjustments to the combustion process. Fuel nitrogen that has been oxidised to nitric oxide may then be reduced to molecular nitrogen by either fuel-derived volatiles or char, for example:

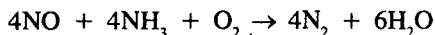


These reactions can be encouraged by allowing the early stages of the combustion process to be carried out under fuel-rich conditions, followed by an injection of air into the flame when it is more mature, allowing the char to be oxidised. This approach, called staged combustion, when used alone can result in the removal of up to 50% of NO_x in coal-fired stations.

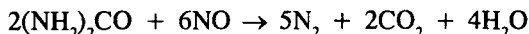
The treatment of flue gases can also lead to NO_x removal. The approach used is dependent on whether the source is static or mobile. In the former case, either ammonia, NH_3 (with or without a catalyst), or urea, $(\text{NH}_2)_2\text{CO}$, is injected into the stack gases, causing the NO_x to be reduced:



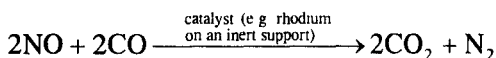
and



or



The treatment of vehicular emissions may be achieved by the catalytic reduction of NO_x to molecular nitrogen at the expense of carbon monoxide (CO) present in the exhaust gases:



Then, air may be injected and the gases allowed to pass over an oxidation catalyst such as platinum or palladium on an inert support. This will facilitate the conversion of any residual carbon monoxide to carbon dioxide and any unburnt hydrocarbons present in the waste stream to carbon dioxide and water.

5.1.3 Particulates

Both stationary sources and Diesel-powered vehicles produce significant amounts of particulates. Where attempted, the recovery of these contaminants from the stack gases of the former source is generally very successful. The technologies used are based on cyclones, electrostatic precipitators and/or fabric filters (bag filters).

During the operation of a cyclone, the exhaust gases enter the top of its essentially cylindrical body, at a tangent. This causes them to move downwards in a helical fashion, generating centripetal forces that drive the particulates to the walls, from where they fall, exiting the cyclone at the bottom. The cleaned gases then leave the top of the cyclone via the pipe at its centre.

Electrostatic precipitators (ESPs) operate by virtue of a potential difference of 30 to 60 kV between the wires and plates that they contain. This causes a very steep gradient in the electric field around the wires and a concomitant high concentration of ions. These charge the particles of the effluent stream, which are then accelerated towards the plates by the potential difference. The dust may then be dislodged from the plates by agitation, allowing it to fall into a collection hopper.

Fabric filters (bag filters) physically remove particulates from the exhaust gases that are made to pass through them. The filters may be of many designs, although tubular constructions are common. The dust burden is periodically removed by either mechanical shaking and/or the reversal of the direction of gas flow.

There is increasing concern over the sooty particulates from Diesel

engines, as epidemiological studies indicate that these contaminants may cause a range of health problems including heart disease. Currently, in the UK, Diesel engines account for about 40% of all black smoke emissions. It seems likely that this percentage will go up as the popularity of Dieselpowered vehicles increases.

Control of Diesel particulates is technologically difficult, though two approaches seem promising. The first involves improving the homogeneity of the fuelair mixture at the time of firing, so ensuring a more complete burn. The second relies on ceramic filters that may be cleaned either physically, by compressed air, or chemically, by heating in the presence of air.

5.1.4 Residual solids

The combustion of finely ground coal in electricity generating stations produces very large quantities of residual solids. These are ashes and, more recently, the products of limestone-based desulfurisation.

Two types of ash are generated, namely pulverised fuel ash (PFA) and furnace bottom ash (FBA); together these amount to about 12-13 Mte a' in England and Wales alone. PFA is collected as a particulate from the flue gases and accounts for 80% of the total. Both of these products are used in cementitious materials. Despite this, in areas where production outstrips demand, considerable quantities are sent to landfill.

As previously mentioned, lime- or limestone-based desulfurisation post PFA removal can yield highquality gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$). Clearly this has commercial value. However, the vast amounts produced may be sufficient to swamp the market, necessitating other disposal routes including landfill.

5.1.5 Carbon Dioxide

All fossil fuel combustion leads to the generation of carbon dioxide. Many exotic means of diminishing the contamination of the atmosphere with this gas have been suggested. Included amongst these is the possibility of increasing the primary productivity of the oceans. It is thought that this may be achieved by adding relatively small amounts of iron to areas that are deficient in this element. According to this hypothesis, the consequent increased rates of photosynthesis will result in the absorption of carbon dioxide. Recent large-scale experiments in the Pacific demonstrated that a single addition of iron salts did indeed promote productivity, at least in the short term. However, a fully concomitant net consumption of carbon dioxide did not occur. One

possible explanation of this is that an increased biomass of photosynthetic plankton encouraged the activity of grazing zooplankton, and that the respiratory activity of these organisms recycled a large proportion of the carbon dioxide originally absorbed. It seems doubtful that the seeding of oceans with iron represents a feasible means of controlling atmospheric levels of carbon dioxide. A more practicable approach is to look for improved fuel efficiency. This would be of even greater efficacy if coupled with a switch to low-carbon fuels such as methane, or even non-carbon fuels including hydrogen (H_2), which may be generated by hydroelectric power.

5.2 LOW-HAZARD SOLID WASTES

Solid wastes (refuse) may be categorised by source into mining, agricultural, industrial and urban (municipal) waste. The last of these includes wastes generated by commerce, local authorities and domestic households.

On a global basis, data concerning the amounts of solid waste generated are inadequate. The problem of insufficient data is compounded by variations in the definition of waste from country to country, making comparisons difficult. However, it is clear that the problem is enormous. For example, for the period of the late 1980s it has been estimated that the OECD countries generated in excess of 1.85×10^{12} kg of solid waste per year. What is more, in some respects the situation appears to be getting worse, particularly in the developing countries. For example, in the more developed world, *municipal* solid waste generation increased from about 3.2×10^{11} kg a^{-1} in 1970 to 4×10^{11} kg a^{-1} in 1990 ($\sim 25\%$). During the same period, the production of refuse in the developing nations underwent an even more rapid rate of increase from 1.6×10^{11} kg a^{-1} to 3.2×10^{11} kg a^{-1} ($\sim 100\%$).

Most solid waste is of low intrinsic hazard. Nonetheless, if mismanaged even this has the potential to cause a diversity of problems, ranging from aesthetic deterioration of the environment through to significant increases in the incidence of disease and the pollution of drinking waters.

Of economic necessity, mining waste is usually disposed of on land near to the mine workings, often forming large spoil heaps. Agricultural solid waste, including crop residues and dung, have fertiliser and soil-conditioning value. Therefore, they are generally disposed of *in situ*. This leaves industrial and urban wastes to consider. The main disposal options for low-hazard waste from these sources

are, in approximate order of increasing desirability: indiscriminate dumping, landfill (organised dumping on land), incineration (if organic) and reuse.

Indiscriminate dumping is an almost ubiquitous problem. However, it is particularly acute in many of the cities of the developing world. This is despite the relatively low *per capita* generation of domestic refuse in these cities ($\sim 145\text{--}330\text{ kg a}^{-1}$) compared with the production rate in the cities of the more-developed countries ($\sim 255\text{--}655\text{ kg a}^{-1}$). The main cause of the problem is that, in the less-developed nations, only about 50-70% of urban solid waste is collected. The remainder accumulates in the streets and open spaces, where it becomes a breeding ground for vermin, spreading disease. Where collection does occur, it frequently results in the formation of open tips that support large numbers of waste-pickers who derive an income from the reusable articles that have been discarded.

Unlike open tips, properly managed landfill sites are a very effective means of low-hazard solid waste disposal. If the waste is covered with soil on a daily basis, odour release is controlled and vermin are discouraged. Under these conditions, these facilities are called 'sanitary landfill' sites and need not be a source of either public nuisance or health hazard. Once full, anaerobic degradation of the material within the capped landfill occurs over a 3-10 year period. During this time the site is of little use as the ground settles and gas is evolved. This is mainly carbon dioxide and methane, controlled removal of which is desirable as this avoids the danger of explosions.

The incineration of low-hazard solid waste with high organic contents in large purpose-built facilities is attractive for several reasons. Principal among these is the considerable reduction in the volume of solid material achieved by this process. In the case of domestic refuse this is generally about 75%. What is more, the residue does not undergo anaerobic digestion when placed in landfill; consequently, settlement and gas generation do not occur, allowing the site, once full, to be built upon.

The major drawback of incineration is the generation of flue gases and particulates. These can be minimised by the application of technologies that are essentially the same as those used to clean the stack gases of static fossil fuel burning facilities.

Solid wastes sent for incineration frequently include chlorine-containing organic substances, such as polyvinylchloride (PVC). The burning of these leads to the formation of trace amounts of

polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDDs and PCDFs), some of which are highly toxic. The presence of these materials in the emissions and ashes produced by incinerators of all kinds has generated considerable opposition to this type of waste treatment facility.

There are several ways in which low-hazard solid waste can be reused. The main processes available are the recycling of individual materials, the generation of refuse-derived fuel, composting, and thermochemical treatment.

Urban refuse from an industrialised country may be expected to have a composition. Virtually all of the components listed would have a reuse value, if they were collected separately. Unfortunately, this is largely impracticable and economically unviable. However, in recent years there has been a move towards the separate collection of the more valuable items, particularly paper, glass, aluminium, steel, plastics and fabric. In the UK this has been achieved largely by the willingness of the public to take these items to specialised receptacles ('banks'), often situated in car parks or at household waste disposal facilities.

An alternative approach is to separate the mixed waste after collection. In the case of the ferrous metals this is readily achieved by magnetic means. While the separation of the other components is more difficult, it can be achieved, to some extent, on the basis of density.

The recycling of waste has other environmental benefits besides those directly associated with direct waste reduction. Waste recycling generally consumes fewer resources and produces less pollution than the winning of materials from virgin sources.

There is evidence to suggest that the recycling of some solid wastes is becoming more significant. For example, on a worldwide basis, in 1971 recycled aluminium formed about 16% of the total yearly consumption of this metal; by 1987 this had grown to over 23%. What is more, during the same period, total consumption of aluminium increased from about 1.2×10^{10} to approximately 2.2×10^{10} kg a⁻¹.

The success of recycling activity varies considerably from one country to another. For example, while the UK recycles 14% of its glass, the Netherlands recycles 62%. Ironically, because of the activities of waste-pickers, lessdeveloped countries frequently have high rates of refuse reuse. This is despite the generally lower levels of valuable material contained in the solid waste of these countries.

Density-based solid waste separation can generate an inorganic fraction (containing metals and glass for recycling) and an organic fraction of sufficiently high calorific value to use as a fuel. This refuse-derived fuel may be used in either a shredded or a pelletised form. Recently, waste tyres have been used to fire cement kilns; one advantage of this process is that the ash becomes integrated with the product, obviating the need for its disposal.

Composting of refuse, under aerobic conditions, offers another way of producing a useful product. The material generated finds use as a soil conditioner and low-grade fertiliser.

Finally, solid waste can be degraded by a variety of thermochemical processes, including pyrolysis (i.e. chemical breakdown achieved by heating in an anaerobic atmosphere). The product of most of these processes is a solid residue together with fuel gas and oil. The attraction of such processes is that they simultaneously reduce the mass of the solid that has to be disposed of, while producing fuels with good handling properties.

5.3 LOW-HAZARD WASTE WATERS (SEWAGE)

The water within the sewerage system of a community is called sewage. It consists of the outflow from domestic and industrial premises and, in some cases, the run-off from roads. This waste water is usually greatly diluted by the ingress of ground water through leaking pipe joints.

TABLE 5.1 The composition of typical urban refuse derived from an industrialised country.

Component	Proportion/% by weight
Paper	35
Garden waste	16
Food waste	15
Metals	10
Glass	10
Plastics, rubber and leather	7
Rags	2
Miscellaneous	5

Only a very small fraction of sewage (0.05%) is waste material, the rest being water. Despite its apparently low waste content, the discharge of untreated sewage into surface waters can lead to gross pollution.

Sewage treatment is primarily aimed at lowering the pathogen content of the waste. Additional objectives include a decrease in its biochemical oxygen demand and solids content. These objectives may be achieved in a series of stages.

Preliminary treatment removes the larger objects within the raw sewage (lumps of wood, bottles, sanitary towels, toilet paper, etc.) and the grit. This may be the only treatment, if any, that is given to sewage prior to discharge in the sea. The processes used are mechanical. Screens constructed from iron bars remove the larger objects. In addition, macerators may be used to break up the more friable lumps so that they may proceed for further treatment. Grit separation is achieved using gravity under conditions where the less dense organic matter remains in suspension.

In all but the most rudimentary plants, the effluent from preliminary treatment is then subjected to primary treatment. During this process the sewage is allowed to slowly traverse a tank, allowing about half of the suspended solids to fall to the bottom. This produces primary sludge and settled sewage (also called primary effluent). The sludge is digested and the settled sewage then enters secondary treatment.

Secondary treatment is a biological process. Three designs of reactor are in common use, namely trickling (biological) filters, activated sludge tanks and oxidation (stabilisation) ponds. The last of these is only appropriate in warm climates.

TABLE 5.2 The potential savings of recycling.

	Potential saving/%			
	Aluminium	Glass	Paper	Steel
Water used	0	50	58	40
Energy used	90-97	4-32	23-74	47-74
Mining wastes	0	80	0	97
Polluting emissions to the atmosphere 95	20	74	85	
Polluting discharges to watercourses	97	0	35	76

A trickling filter is a tank filled with inert solid particles, typically in the size range 3.8-5.0 cm. These are covered with a mixed, essentially microbiological community, mainly developed from the sewage. Settled sewage is sprinkled over the top of the tank via moving booms. The sewage percolates down the filter, where its organic content is

largely oxidised by organisms established on the solid support. Oxygen for this process is provided by air that is passively drawn into the tank. In contrast, activated sludge tanks are actively aerated. In these the settled sewage is oxidised by a suspension of micro-organisms.

Both trickling filters and activated sludge tanks produce secondary sludge that is removed downstream in final settlement tanks. In the case of the activated sludge process most of this is returned to the aerated tank in order to maintain its biological community. The remainder may be added to the primary settlement tank, as may all of the sludge from trickling filters.

Oxidation ponds are large shallow (~ 1 m) tanks through which settled sewage may slowly pass. Microbial action releases nutrient species (CO_2 , NH_3 , NO_3) that sustain algal growth. The algae generate molecular oxygen during photosynthesis, which sustains the activity of the bacteria. An anaerobic sludge forms on the bottom of the ponds in which methane is produced. An advantage of oxidation ponds is that they can be harvested. The algae generated can be fed to animals or burnt. What is more, oxidation ponds can be used to raise fish, although there is a risk of pathogen transfer if the fish are used for human consumption.

The preliminary, primary and secondary treatments outlined above are highly successful. When in combination, they are capable of producing an effluent with less than 30 mg l^{-1} of suspended solids and a BOD of less than 20 mg l^{-1} (typical sewage contains 600 mg l^{-1} of total solids, of which 200 mg l^{-1} are suspended, and has a BOD of 3000 mg l^{-1}). Pathogen populations are also greatly reduced. For example, the population of *Salmonella paratyphi* can be decreased by 84-99% by the use of trickling filters.

The final effluent from the treatment works is discharged into a river, lake or sea. Tertiary treatment is seen as desirable in locations where the degree of dilution of the effluent is small, or where potable water is to be withdrawn downstream for treatment and distribution. The purpose of this treatment is to further reduce the BOD and/or concentrations of suspended solids, nutrients, toxicants (such as heavy metals or poisonous organics) and/or pathogens. A wide range of technologies have been developed to facilitate the desired improvements, including oxidation ponds, sand filters, microstrainers, adsorption onto activated carbon, ion exchange, chemical precipitation, microfiltration, and disinfection. None of these, when operated alone, can bring about the desired reductions in all of the parameters listed above.

Conventional sewage treatment generates sludge from both the primary and secondary stages, to which may be added any sludge produced during tertiary treatment. These are mixed and digested in a two-stage process, the aim of which is to produce a material of reduced volume and acceptable odour that does not attract harmful insects or rodents. The first stage is anaerobic and is carried out at 27-35°C. It produces a gas that is approximately 72% methane and 28% carbon dioxide; this may be collected and used to generate heat (to warm the digester) and electricity.

The second stage is carried out in the open. The sludge is allowed to settle (thicken), producing the final product, digested sludge. Disposing of this is problematic. Options include dumping at sea, incineration, landfill, and use on land as a fertiliser/soil conditioner. The last of these is restricted by both transport costs and the heavy metal burden of the sludge. Landfill sites are increasingly scarce and dumping at sea is becoming restricted by legislation; in the US this practice was banned in 1991, and in the UK it will cease in 1998. Therefore the percentage of sludge that is sent for incineration appears likely to increase, at least in the more-developed countries.

For economic reasons, many households are not connected to mains sewerage systems. A commonly practised, but inferior, alternative is waste water treatment based on the septic tank. This acts as a combined sedimentation tank and anaerobic digester. The liquid effluent is allowed to soak away into the soil, while the sludge is periodically removed from the tank to be treated in a conventional sewage treatment plant.

Despite the existence of well-established technologies for the treatment and disposal of sewage, these are denied to many people. The situation appears to be getting worse; estimates indicate that by the year 2000 the number of people without sanitation facilities will reach 1880 million.

5.4 HIGH-HAZARD WASTES

Assessment of the amount of high-hazard waste generated on a global basis is problematic. This is in part because of inadequate record keeping, but also because there is no uniformly accepted definition of high-hazard waste. This makes international comparisons very difficult as some countries use definitions that are much more all-embracing than others. By way of illustration, it is interesting to note that 41% of the solid industrial waste generated in the USA is categorised as hazardous. This compares with 33.5% in Hungary, 3% in the UK and 0.3% in Japan and Italy. To a considerable degree,

the enormous disparity between these figures reflects the differing regulatory frameworks within which the data were collected.

There are strong indications, however, that the production of high-hazard wastes is vast and expanding, both absolutely and as a proportion of industrial wastes as a whole. Global production of hazardous waste is estimated to be at least 3.38×10^{11} kg a⁻¹, about 80% of which is generated in the USA. In some countries the rate of increase appears to be phenomenal. For example, estimates of South Korean hazardous waste production for 1985 and 1989 are 1.2×10^{10} and 2.1×10^{10} kg a⁻¹ respectively.

In addition to the hazardous wastes currently being produced, considerable amounts have been inappropriately disposed of in the past. Consequently, a large number of sites have been contaminated and are potentially hazardous. For example, 32000 such sites have been identified in the USA alone. The remedial treatment of these is likely to be extremely costly.

There is also a legacy of materials that are now known to be hazardous, but that were once in common usage. Disposal of these substances is likely to cause problems for some time to come. Notable amongst these are the polychlorinated biphenyls (PCBs), which found extensive use as dielectrics in transformers and asbestos, which was widely used as a building material.

For the purposes of the discussion here, highhazard wastes may be considered to be those that, when released in relatively small amounts, are capable of producing severe and/or long-lasting damage to human health or the environment. Included in our definition are materials that contain pathogens or radioactive isotopes, along with substances that are corrosive, toxic, flammable, violently reactive or explosive.

5.4.1 Treatment and Disposal

Strategies for the treatment of high-hazard wastes can be divided into those aimed at reuse, at destruction or at immobilisation.

Options for reuse include purification followed by recycling. This approach is frequently applied to solvents, as recovery of pure material from waste solvent is often achievable by distillation. An alternative approach is to use the waste from one process as a feedstock for another. For example, some waste oils may be mixed with fuel oils and burnt in industrial boilers.

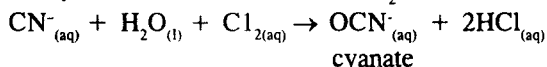
There are instances where the waste from one process can be used to treat the waste from another. For example, prior to painting

or electroplating, the oxide coat on steel is removed using acidic pickling liquors. Once spent, these may be reused as precipitating agents, removing phosphate from waste waters.

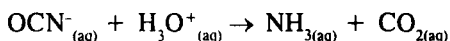
Reuse within the facility that generated the waste is desirable as the need for transport is minimised.

However, this is not always possible. In such cases, certain types of waste, including metals and solvents, may be passed on to commercial reclaimers. These then treat the wastes and sell them on as useful products. Alternatively, the wastes generated by one manufacturing company may be used directly by another. In some areas this has been encouraged by the establishment of 'waste exchanges'. These produce databases that list the wastes available in a given region, so facilitating trade in these commodities.

Destruction of high-hazard wastes is only applicable to those that are hazardous by virtue of molecules that they contain rather than their constituent elements. For example, the cyanide ion (CN^-) is found in wastes from metal processing industries. It is highly toxic even though both of its constituent elements are essential for life (Chapter 5). It is toxic by virtue of its affinity for the active sites of enzymes involved in respiration. Consequently ingestion of sufficient amounts of this ion results in rapid death. Wastes containing this species may be detoxified by treatment with chlorine (Cl_2), thus:



followed by



Compare this process with the approach used in the treatment of waste waters containing toxic metals. These are generated by a number of industries including mining, metal-plating and ceramics manufacture. Unlike cyanide, metals cannot be destroyed. Consequently, treatment of waste streams contaminated with these elements involves the removal of metals from the aqueous phase. Clearly, the contaminants vary from source to source; however, they may include copper, nickel, cadmium, lead, chromium, mercury and/or zinc. These and many other heavy metals can be largely removed from the water by the addition of an anion that causes the precipitation of the metal as an insoluble salt. Anions used for this purpose include sulfate (SO_4^{2-}), sulfide (S^{2-}) and hydroxide (OH^-).

As in the case of cyanide destruction, discussed above, many of the destructive treatment methods are waste specific. However, there

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are others of more general applicability, notably thermo-chemical and biological processes.

The main thermo-chemical treatment used is incineration. In the case of wholly organic wastes, this method is also a means of complete waste disposal as the products are relatively harmless gases, principally $\text{CO}_{2(g)}$ and $\text{H}_2\text{O}_{(g)}$, which are subsequently vented into the air. The high temperatures reached also result in sterilisation. This is seen as a great advantage in the disposal of materials, such as hospital wastes, that may be contaminated with pathogens.

Incineration of high- and low-hazard wastes share the same drawback, namely potential air pollution. However, this may be minimised by a combination of:

- 1 high-temperature combustion (ideally $> 1000^\circ\text{C}$);
- 1 a long residence time of the waste in a hot oxidising environment (> 2 or 3 seconds, depending on the waste);
- 1 rapid stack gas cooling (to avoid the formation of toxic dioxins and furans: Box 16.3);
- 1 flue gas cleaning.

By such means, modern plant is capable of achieving burnouts in excess of 99.99%.

Unfortunately, there are still a great number of incinerators that do not incorporate all of the features listed above. This has led to concern in recent years. In the UK, much of this has centred on the incineration of clinical waste as, until recently, this fell outside the reach of all environmental law.

The incorporation of organic high-hazard wastes into the input stream of cement kilns has been used for many years as an ultimate disposal system. This has several advantages including high-temperature incineration with long residence times and the incorporation of any ash into the cement product, thus avoiding disposal costs.

Other thermo-chemical treatments applicable to high-hazard wastes include pyrolysis and wet air oxidation. The latter of these involves heating the waste in a water slurry at high temperatures and pressures in the presence of air or pure oxygen (O_2). The products of this process are similar to those generated by combustion.

Biological methods of high-hazard waste disposal have been used for some time. In one system, known as land farming, oily wastes are spread onto the soil. Decomposition may be enhanced by the addition of inorganic fertilisers and the periodic disturbance of the

land using conventional agricultural implements. This generates the right conditions for the breakdown of the wastes by the naturally occurring soil microorganisms.

Wastes that are neither recycled nor destroyed must be disposed of. This can be done with much greater safety if the waste is immobilised first. The technologies used to do this involve either the incorporation of the waste into a solid matrix or its encapsulation within an impermeable polymeric cover. In addition to immobilisation these processes are variously referred to as stabilisation, solidification or fixation.

Solid matrices within which waste can be incorporated may be formed of cementitious or organic polymeric material. Alternatively, inorganic wastes may be turned into a glass (vitrified) or incorporated into ceramic artifacts such as bricks. Vitrification involves the formation of a melt at around 1300°C and is therefore highly expensive. Consequently, it is generally reserved for the treatment of highly hazardous materials and may become the preferred option for the treatment of highly radioactive wastes.

Historically, the bulk of high-hazard waste has been disposed of to landfill, often with little or no pretreatment. In 1985, in the UK about $2.75 \times 10^9 \text{ kg a}^{-1}$ of chemical waste was sent to landfill; this compares

with a total of about $4.2 \times 10^8 \text{ kg a}^{-1}$ that was treated chemically, fixed or incinerated.

It is clear that ill-considered landfill practices have caused and continue to cause environmental damage at a large number of sites. Nonetheless, when carefully managed, landfill is still seen as a highly appropriate means of disposal for many highhazard wastes.

Modern secured landfill facilities are located in areas where groundwater contamination is unlikely. They are covered and lined with impermeable membranes and leachates are collected, monitored and treated. The site is divided into a number of areas called cells, into which wastes of known characteristics are placed. This avoids the codisposal of incompatible materials and facilitates future removal of waste for recycling or further treatment. Ideally, an extensive programme of air and groundwater monitoring should be undertaken prior to the establishment of the facility and during and after its operation.

Heightened public concern and the increased commercial pressure on land means that the continued use of landfill as the primary means

of high-hazard waste management seems in doubt, at least in the more-developed countries. Programmes of waste minimisation and waste treatment are likely to become more prevalent.

Other procedures that are used for the disposal of high-hazard wastes include dumping at sea. This has been a matter of controversy for some time. In *relative* terms the amounts of waste disposed of by this means are small; nonetheless, the absolute quantities are significant. For example, in 1985 the UK disposed of about 2.3×10^8 kg a⁻¹ of chemical wastes in this way. However, there have been political moves to curb this practice. It was agreed by the 13th Consultative Meeting of the London Dumping Convention that all sea-dumping of non-inert industrial waste should cease by 31 December 1995.

High-hazard wastes have also been disposed of by placement at depth within the Earth, well out of the reach of potable aquifers. This has been done both within disused mines and by deep-well injection.

5.4.2 International Trade in High-hazard Wastes

In recent years, in the more-developed countries, there has been a progressive tightening of the legal frameworks that regulate the disposal of high-hazard wastes. In many cases this has increased the financial cost of disposal within the countries of origin, spawning an international trade in noxious waste. For example, legal exports of hazardous waste from Europe to less-developed countries total about 1.2×10^8 kg a⁻¹.

In some cases, waste is imported by countries that have appropriate facilities for its treatment and disposal. For example, in 1992, clinical waste was imported by the UK from Germany for incineration in a specialised facility near Heathrow airport. Unfortunately, there have been a number of instances where high-hazard waste has been exported to countries that do not have the necessary facilities to deal with it adequately.

5.5 WASTE MINIMISATION, CLEANER PRODUCTION AND INTEGRATED WASTE MANAGEMENT

Historically, industrial waste producers have relied on the cheapest means of disposal. This frequently involved discharges of untreated noxious material into water bodies or dumping on unsecured landfill sites. It is now evident that such inappropriate waste disposal practices

have left a legacy of problems. The consequent economic, environmental and social costs are huge, but difficult to estimate. Most of these costs have been absorbed by society as a whole. However, there is evidence that attitudes are changing as the 'polluter pays' principle becomes more widely established.

Appropriate treatment and disposal of wastes can greatly ameliorate their environmental impact. However, this 'end-of-pipe' technology cannot reduce the amount of waste generated. This can only be achieved by in-process modifications targeted at cleaner production. The concept of cleaner production involves the application of integrated strategies aimed at avoiding unnecessary waste production and ensuring that the remaining wastes produced are innocuous. In order to be fully effective, this concept must be applied throughout the life-cycle of a product, from the extraction of the raw materials from which it is made through to its ultimate disposal.

Large manufacturing organisations are under increasing legislative and consumer pressure to limit the impact of their operations on the environment. There are now many examples of corporate initiatives that are aimed both at waste minimisation through cleaner production and at appropriate waste treatment. These can have direct economic as well as environmental benefits. For example, the 3M Corporation introduced its Pollution Prevention Pays, or '3P', programme in 1975. This concentrates on waste reuse and the reduction of pollution at source. The corporation believes that between its inception and 1989 the 3P programme directly resulted in a saving of US\$408 million. Other examples include Polaroid's TUWR (Toxic Use and Waste Reduction) programme, started in 1987, and Dow's WRAP (Waste Reduction Always Pays) policy initiated the year before; excellent accounts of these measures are given by Buchholz.

Since the mid-1970s national and international agencies have been instrumental in promoting responsible waste reduction, treatment and disposal. The then EEC took an early interest, organising in 1976 one of the first meetings to discuss 'Low and NonWaste Technologies' (LNWT). By 1978 compendia of these technologies were available. Other initiatives include PRISMA (Project on Industrial Successes with Waste Prevention) in the Netherlands, the Environmental Management Company (CETESB) in Brazil, and UNEP's International Cleaner Production Information Clearing House (IPIC). For further information about these and other schemes.

Clearly there is still enormous scope for improvement. In many

cases this can be achieved by simple means, such as waste segregation. To cite but one example, in Britain each hospital bed generates about 18 kg per week of waste. This includes everything from used dressings to flowers, all of which is classified as clinical and incinerated as if it were hazardous. In Germany, by careful segregation of truly hazardous materials from the rest, the amount of clinical waste is reduced to about 18 kg per year per bed.

6

Environment of Microorganisms

Microorganisms are usually not studied in their natural habitats, and their modes of life may be drastically changed before the microbiologist can gain specific knowledge of particular types. Only a small percentage of bacteria have been studied at all, and the overall ecology of microorganisms has been changed only slightly by man's span on earth. Micro-organisms usually change their environments, and changes may occur quite rapidly under favourable conditions microorganisms can survive wide variations in temperature, pH, and other changing conditions.

Ocean waters have high salt contents but do not produce halophilic conditions. Marine microorganisms, in general, are not halophilic. Marine sediments form a different environment from that of marine waters. Organisms that inhabit the sea floor are called *benthic*. Organism ecologies found in open oceans. If strictly marine organisms exist, they must be found in open oceans and nowhere else. Few, if any, specific marine bacteria have been identified. Bacteria, fungi, flagellates, ciliates, diatoms, and various algae play important roles in marine ecology. Bacteria probably play the biggest role on bottom environments, and photosynthetic diatoms, in the photic water zone. Gram-negative rods predominate in ocean environments, although large numbers of *Sarcina*, *Micrococcus*, and other gram-positive forms are found.

Soils provide the widest variation of habitats for microorganisms and, in turn, contain the most varied array of microorganisms. Microbial modes of life have changed through geological time, and

microbial metabolism has accounted for most changes that have occurred. Primitive forms of life were probably simple and unicellular (if cellular at all) and existed under conditions quite different from those of present-day life.

Forms of elements are changed by microbial metabolism. Some important examples are found in cycles of carbon, oxygen, nitrogen, and sulfur. Some microorganisms produce organic carbon compounds from carbon dioxide, and others breakdown organic carbon to carbon dioxide. The process of changing organic to inorganic substances has been termed *mineralization*. Some microorganisms change ammonia to NO_2 , and others convert NO_2 to NO_3 . On the other hand, some utilize NO_3 molecules for electron acceptors and reduce it. Both oxidation and reduction of sulfur also occur. Nitrogen and sulfur both occur in amino acids. Some bacteria fix atmospheric nitrogen directly, but other nitrogen fixers live symbiotically in roots of plants.

Principles of microbial ecology have been well presented by Brock (1996) and Wood (1965), and marine microbiology, including much material on ecology, was logically outlined and evaluated by ZoBell (1946). A symposium on marine microbiology compiled and edited by Oppenheimer (1963) and a treatment of deep sea marine microbiology by Kriss (1963) contain much information concerning microbial life in the ocean environment. MacLeod (1965) has dealt with the existence of specific marine bacteria. These references have been used extensively in the preparation of materials on marine ecology, and Brock (1966), Lamanna and Mallette (1965), Stanier, Doudoroff, and Adelberg (1963), and Thim-ann (1963) have served as principal sources for information related to terrestrial ecology.

6.1 MICROORGANISMS AND ALL LIFE'S ACTIVITIES

On orientation, relations of microorganisms to essentially every vicissitude of life were pointed out. In succeeding chapters different morphological and biochemical types and variations in reproductive processes have been evaluated. Although extremes in both morphology and biochemistry are evident in the world of microorganisms, likenesses and kinships have been emphasized. A topic of paramount importance in the biology of microorganisms is *the life of organisms in their natural habitats*. Both aquatic and terrestrial habitats provide excellent opportunities for studying microorganisms in relation to each other.

Although microorganisms probably inhabited ocean waters for long period previous to their appearance on land and then inhabited the land mass for other aeons prior to the appearance of higher forms, neither the sea, land, nor the atmosphere above them was what it is today. One point deserves emphasis: *Man has disturbed the overall ecology of microorganisms only slightly, and even less in ocean water than elsewhere!* The ways of life of a few have been altered, but the general mass of microorganisms proceed as though man did not exist.

6.2 FLUCTUATING MICROORGANISMS

An extremely important biological consideration is the relation of organisms to their environment. Ecological relations are easily observed in cases of large plants and animals but may be overlooked in the area of microorganisms, although relations are most important at the microorganisms level. The most obvious example of change of environment of some organisms is that brought about by the human race. Changes have been melodramatic and exciting during the last century. A less obvious change has been that observed in other animals. There is, of course, an obvious difference between the ecology of the human race and that of other animals, and also between higher animals and microorganisms. *Microorganisms and plants plan absolutely nothing!* Everything depends on nature and the environment with which nature surrounds the organism.

When we consider the habitats of microorganisms, we must conclude that the environment selects mutants and also causes induced enzyme synthesis among microorganisms. In this manner we see that in a very real sense microorganisms are victims of their environments! Aeons of time are not required for one microbial population to die out and be replaced by an entire different type or by mutants from the original. Changes often occur rapidly. It is often possible to isolate organisms with apparently similar morphology from different environments, but their biochemistries may be vastly different. If we were able to examine organisms in nature in minute detail, we should probably observe that there were some points at which each strain differed from others. In other words, the deoxyribonucleic acid (DNA) of each strain is probably unique! On the other hand, if we could hunt enough strains, we may find a multiplicity of similar DNA arrangements. At present these are speculative questions but ones that will probably be answered by future researchers.

Habitats of microorganisms are difficult to define because many microorganisms are motile. In addition to their own movements, water,

wind, animals, and other factors may be instrumental in changing the habitats of organisms. Habitats may change in the soil with rain, snow, or other moisture changes, and within rivers, lakes, oceans, and other bodies of water the habitat is in a continuous state of change.

Microorganisms may live under drastically changing condition in nature. Microbial cells may contain certain elements when grown under one set of conditions and contain different elements when grown under different conditions. An organisms can contain different elements when grown under different conditions. An organism can assimilate only substances that are present in its environment, but the mere fact that a substance is assimilated does not mean it is essential to the well-being of the organism. In natural environments, microorganisms may grow in temperature extremes ranging from below 0 C in frozen foods and icy climates to above 100 C in hot springs. The pH growth range may vary from near 0 with *Thiobacillus* to 12.0 to 13.0 in other organisms. Hydrostatic pressure may vary from 0.0 to 1400 atmospheres and the oxidation-reduction potential (E_h) from -400 to +850 millivlts. The fact that a microorganisms grows on a certain medium when isolated does not mean that growth factors in the isolation medium are the same as those utilized by the organism in nature. Adaptive enzyme formation or mutation with selection may change the strain drastically from characters it possessed in its natural habitat. Only recently has the nutrition of microorganisms in nature been studied to any appreciable degree, and scant data are available on the subject. The most successful studies of microbial nutrition in nature have been accomplished by means of the radioautographic technique. Organisms either in culture or in nature usually have nutritional preferences but may utilize other nutrients in absence of the preferred types.

6.3 MARINE ENVIRONMENTS

Variation in the salt content of ocean waters is usually between 33 and 38 parts per thousand but may be less in shallow areas near shorelines and river mouths. The oceanic environment is relatively constant at a specific depth in a given locality. There are obvious variations in temperature between equatorial and polar regions and in pressure between the surface and great depths. Variations in temperature and light-penetrating powers accompany changes in depth, but oceanic variations are usually gradual.

Although the ocean contains an extensive microbial population, nutrition variations are much less extensive than those found on land.

Since there are more limitations on available nutrients, fewer variations in the nature of chemical reactions by marine microorganisms would be expected. Nitrogen fixation and denitrification, the part played by bacteria to other forms of life, and the role played by bacterial slime in the formation of the ocean floor have been cited by Waksman (ZoBell, 1946) as significant marine processes in which bacteria appear to take an active part.

Sea water remains alkaline with usual pH ranges between 8.0 and 8.3. Concentrations of calcium, potassium, chloride, sulphate, bromine, and many other ions remain fairly constant in ocean waters.

6.4 MARINE SEDIMENTS

There is considerable exchange of inorganic salts between ocean sediments and overlying waters, but sediments are selective by means of microbial activity and phenomena. Exchange may be caused by animal movement or water turbulence. Both pH and E_h values of sediments are usually lower than those of waters that cover them and these differences produce corresponding differences in microbial flora between sediments and overlying waters. Organic matter and microbial population are much more concentrated in sediments. Marine organisms that inhabit the sea floor are known as *benthic* organisms, or *benthos*, and those that live in the water above the floor are termed *pelagic*. *Biotic zones* of the ocean are determined by the types of life that inhabit them. *Neritic* organisms are those that live near the shore (the area outlined by the continental shelf), and *oceanic* organisms are those that inhabit open waters. The *photic* layer of sea water, which constitutes about 5% of the ocean, contains enough light to promote photosynthesis. The vast underlying *aphotic* zone contains an abundance of bacterial life, which is particularly abundant on the sea floor. Less than 5% of the light falling on the clear ocean waters penetrates below 20 meters, and in coastal regions the depth of penetration is much less.

6.5 MARINE ECOLOGY

Bacteria, fungi, flagellates, ciliates, sarcodina, diatoms, and unicellular and multicellular algae all play important roles in marine ecology. Diatoms, Radiolaria, Foraminifera, Silicoflagellates, and a few other microorganisms contain calcareous or siliceous parts that persist after death of the organisms and form bottom oozes, which are important constituents of sediments. Bacteria characteristically catalyze reactions at low temperatures that would otherwise occur only at very

high temperatures or, in some cases, under extreme pressure. Examples of microbial action are seen in coal and probably in oil formation.

Bacteria probably play the most active role of all organisms in aqueous environments. They inhabit sediments where few other organisms can live and reproduce because of the anaerobic modes of life of some forms, but they are often depleted to some extent by ciliates that feed on them. Bacteria rapidly alter pH and E_h , convert organic to inorganic materials, synthesize, organic from inorganic materials, and both oxidize and reduce compounds. Significant genera in marine environments are *Vibrio*, *Micrococcus*, *Sarcina*, *Bacterium*, *Pseudomonas*, *Corynebacterium*, *Spirillum*, *Mycoplan*, *Nocardia*, and *Streptomyces*. Gram-negative rods predominate in the sea in contrast to the gram-positive forms of soils. Most cocci are isolated from continental shelf waters. Pleomorphism is common among marine bacteria. Both autotrophic and heterotrophic forms have been identified, and some types appear to be facultative and variable in relation to the use of organic compounds.

The tremendous importance of diatoms in marine environments have long been recognized. Near shorelines they often form enormous masses, called blooms, and remove large quantities of nutrients from water. Water is thus depleted of phosphate, nitrogen, silica, and other important constituents. Plankton, however, serve as food sources for small ocean animals.

6.6 CLASSIFIED MICROORGANISMS

Most species of marine bacteria listed in Bergey's manual have been isolated from marine environments near the shorelines or from the soil. Only a few genera can be classified as strictly marine. It appears that workers who isolated organisms from the open sea probably attempted to identify them in relation to existing data taken from land forms. Specific names of marine organisms, therefore, have the same names as soil forms in the majority of cases. Since both marine and soil species are cultured for many generations under conditions far different from their natural habitats, tendencies to change from their natural modes of life are apparent. Organisms from any natural habitat may change considerably between natural growth conditions and condition of growth that provide criteria set up by microbiologists for identification. In order for an organism to be considered strictly marine, it should be found in appreciable numbers at great distances from land or other nonmarine influences. Since conditions in the sea show some homogeneity, there is a corresponding reduction in the number of marine microorganisms

types below the number of types isolated among land dwellers. According to Kriss (1963) there is no proof of the existence of truly marine taxonomic groups of microorganisms. There is no proof that organisms similar to any of those in the ocean could not be found somewhere on land if the search were pursued far enough. One peculiar, threadlike, non-branching microorganism that was isolated from the Arctic, Pacific, and Atlantic Oceans, however, was considered as probably strictly marine. All possible terrestrial habitats for this particular microorganism (*Krassilnikovia*), however, have not been explored. Present concepts of classification make the designation of an organism as being strictly marine rather complicated. The particular organism considered as being marine did not grow into colonies on laboratory media, but it was studied by direct microscopic examination of slides that had been submerged and on which organisms had grown. Isolation was from widespread areas, however, and organisms were morphologically homogeneous by light microscopical studies, regardless of the locations of isolation. The organism was homogeneous, unbranched, and nonseptate, and it contained a head at one end that consisted of round refractile bodies. Marine microbial reproduction, although difficult to study, has been shown to be low, and reproduction rates decrease with an increase in water depth and pressure. Actinomycetes and fungi were not found in deep sea explorations but were observed in shallow waters, and they are considered terrestrial in origin. Details of isolated marine microorganisms are presented by Kriss, and the interested student is urged to consult that work for further information. Growth requirements, bacterial types, metabolic pathways, growth conditions, taxonomic position, and relations to indigenous flora as they pertain to marine bacteria are described by MacLeod (1965).

Bacterial and plankton populations show parallels, and it is thought by some workers that a large per-cent of open ocean bacteria characteristically live attached to plankton. Dissolved substances secreted by phytoplankton that inhabit the photic zone probably contain various bacteria and account for a major portion of bacteria of that zone. Bacteria are also associated with zooplankton, but to a lesser degree than with phytoplankton. The influence of animals on bacterial life is pronounced on the sea floor. Bacteria utilized dead animals for nutrients and also inhabit some living forms. Certain protozoa and other forms, in turn, utilize bacteria as food, and population of bacteria and animals apparently reach states of equilibria by these processes.

Blue-green algae have a wide range of habitats. They form carpets in shallow ocean waters. *Oscillatoria* and *Nostoc* are especially

important in marine environments. Blue-green algae are numerous in tropical waters.

Dinoflagellates occur in both fresh and salt water and appear to tolerate most ocean environments. They may outnumber diatoms in tropical waters. Photosynthetic flagellates, not usually classified with the dinoflagellates, are found mostly in photic zones of water and surfaces of sediments. Colourless flagellates may be more abundant than pigmented forms in the open ocean.

Ciliates inhabit regions just above sediments and are heterotrophic. They do not contain chlorophyll and just obtain energy by chemosynthesis. Foraminifera and Radiolaria are important Sarcodina. Their shells form ocean oozes from which limestone and jasper are obtained. Green, red, and brown microscopic algae serve as food source for some marine animals that feed near shores. Algae are usually confined to near-shore areas, bays, lagoons, and fresh water. The role of fungi in marine environments has not been adequately explored, but they probably carry on significant conversions of organic compounds in many marine locales. The role of yeasts in marine ecology has not been extensively investigated; some ocean water yeasts are apparently of terrestrial origin, but some workers believe that some yeasts are typically marine.

6.7 EFFECTS OF WATER AND SEDIMENT

Bacteria may reduce food supplies beyond the minimum requirement for other organisms in some ocean environments. Bacterial metabolic products may also inhibit the growth, or even cause the death, of other organisms. Bacterial growth, on the other hand, may be inhibited by metabolic products of fungi and other bacteria. Concentrations of organic matter in sea water are below the minimum required for many bacteria, and this low concentration of organic constituents is one of the most important factors in controlling marine population.

Marine sediments are extremely high in calcium carbonate deposits in the form of limestone. Large amounts are deposited by the remains of animals and calcareous algae, and heterotrophic bacteria also contribute to the limestone accumulations. Deposits are built up more rapidly where organic matter is abundant and are sparse in deeper areas of the ocean. A fairly large number of bacteria and their reactions are involved in precipitating calcium carbonate. Iron and manganese are also deposited by bacterial action. Autotrophic bacteria are active in iron deposits. Autotrophs characteristically oxidize iron and manganese to hydroxides, and heterotrophs precipitate iron and

manganese as sulfides; heterotrophs also deposit organic iron compounds. Microorganisms are active in altering pH, redox potential, gas tension, and other physical and chemical conditions in bottom sediments. Production of CO_2 and organic acids, oxidation of H_2S to H_2SO_4 , reduction of S to H_2S , conversion of NH_3 to NO_2 and NO_3 , and liberation of phosphate from organic compounds all lower pH values. Opposed to these bacterial actions are utilization of CO_2 , oxidation or decarboxylation of organic acid salts, reduction of sulfate to H_2S , conversion of NO_2 or NO_3 to N or to NH_3 , and formation of NH_3 from nitrogenous compounds. All the preceding metabolic processes are accomplished by deposit dwelling microorganisms.

Bacterial growth in general tends to lower E_h values and utilizes oxygen. The greatest area of oxygen consumption is just above bottom deposits, and the transformation of other gases is also most rapid in the same area. Large amounts of methane and hydrogen result from fermentation microbial metabolism of organic compounds in ocean mud. Anaerobic liberation of nitrogen and hydrogen sulfide also occurs. Certain protein and carbohydrates are rapidly metabolized by microorganisms, but the more slowly changing materials settle to form deposits. Lignins, complex protein, chitins, fats, and other complexes known as humus fall to the bottom to form most deposits. The formation of petroleum in ocean bed deposits by present-day microbial action is an unanswered but tremendously important question.

6.8 ARRAY OF MICROORGANISM

Aside from certain types of living animal tissues, it would be difficult to find a nonmarine habitat that is not duplicated in the soil. As has been just suggested, many marine habitats can also be approximated in certain soil environments. Heterogeneity in soil habitats and also in their inhabitants is tremendously complex. Variations, which are evident nowhere else in nature, occur in soil microorganisms. The terrestrial habitat is the master. Springs, rivers, streams, small lakes, and other inland water bear soil microbes unless polluted with those from artificial habitats. Most plant species come from the soil, and there are few strictly animal parasites.

A small number of microorganisms, especially bacteria and viruses, have become adapted to living as saprophytes in the *alimentary tracts of animals*. *It is important that the student understand, however, that microbes which inhabit animals form only a very small part of the microbial populations.* Adaptation of microorganisms to life with plants and animals was a late step in ecological development, but probably a

simple and gradual one, as plants and animals development, but probably a simple and gradual one, as plants and animals developed. Specialized developments of microorganisms to the point of parasitizing plants, animals, or even each other have occurred but represent reaction of only a *very small* section of the microbial world.

6.9 CHEMICAL REACTIONS

Since microorganisms in all probability are by far the earth's oldest inhabitants, chemical changes through geological time have probably been carried out by them. The *biosphere* is the part of our sphere that supports life, and it consists of the oceans, a few feet of top soil, the atmosphere above the earth, and some areas below the few feet of earth ordinarily inhabited. As was mentioned earlier, microorganisms alter their habitat, and in so doing, they have changed constituents of the atmosphere and prepared the way for all present living forms. Although microorganisms at first doubtless inhabited ocean water they have inhabited all areas of terrestrial life and adapted to interconversions of almost every available compound.

6.10 MICROBIAL MODES OF LIFE

In his presentation of the concept of *biopoiesis* (the origin of life) Pirie (1957, 1960) listed five theories. The theory of inevitable natural causes to bring about evolutionary changes is most widely accepted among scientists, but the question of whether there was only one occurrence or whether there were many is debatable. In the beginning of Pirie's scheme, early chemical reactions were carried out in the absence of oxygen. Inorganic photosynthesis and related accompanying processes were included in chemical reactions. Chemicals were numerous, structures were simple, and chemical evolution accompanied by biochemical selection resulted in the formation of a probiotic mass. A period of biochemical uniformity followed that of uncertain reactions. A narrow group of biochemical reactions, now in the presence of oxygen, produced an array of morphological variations. Oxygen available for biochemical reactions possibly arose from inorganic photolysis. Short-wave radiation, probably in the ultraviolet region, furnished energy for photolysis, and the earth's atmosphere was such that ultraviolet radiation was not shielded out. As life developed, organic photosynthesis came into being, and more oxygen became available. Present-day photosynthetic and chemosynthetic organisms are probably somewhat different from those of earlier specimens.

Many hypotheses as to the origin of bacteria have found their way

into present-day thinking. Since fossil remains are very scant or absent, these educated guesses can be neither substantiated nor disproved. The hypothesis that earlier forms were aquatic spirilla, cocci, or other types is less important geochemically than are metabolic patterns carried out under primitive conditions.

As we review geological time factors and chemical processes in which microorganisms have been involved, we may say that for long geological periods *all organic chemical processes were carried out by microorganisms*. As microorganisms modes of life developed, metabolic patterns became more complicated, more energy-yielding processes became available, and groups of organisms that could build inorganic substances into organic substances and those that, in turn, could convert organic substances into minerals both arose. In later development, microorganisms furnished the atmosphere in which both plants and animals could develop. Throughout the history of living on earth, microorganisms have been the principal agents of compound conversion; they still play the leading role.

6.11 CHEMICAL CONVERSIONS

Microorganisms account not only for the great *quantity* of metabolic changes but also account for many *qualitative* changes that are carried out nowhere else in nature. Furthermore, microorganisms are distributed to essentially all areas of our biosphere. They decompose essentially all organic compounds to inorganic substances and are also responsible for most photosynthesis. Microorganisms (especially bacteria and fungi) possess a high ratio of surface area to volume, and this permits and exceedingly rapid transfer of substrate (nutrients) into metabolites (waste products). The total combined action of bacteria and fungi probably converts as much as nine tenths of the earth's organic matter back to inorganic constituents. Bacteria and fungi in the soil of the yards and gardens of a crowded city are able to convert more organic material than the city's human population. In addition, heterotrophic microorganisms multiply rapidly, and an enormous metabolizing population can quickly arise when proper conditions are available. Each strain of microorganisms, however, is limited in the *qualities* of materials that the clone can mineralize or convert into inorganic constituents. For the mineralization of a wide array of compounds, therefore, a wide variety of microorganisms is essential.

6.12 MICROBIAL ECOLOGY

In present-day ecology, microorganisms carry on processes that

were not part of their original conversion patterns. Since the appearance of plants and animals, photosynthesis is generally carried on by plants and chemosynthesis by animals. Cycles of some important elements will be outlined in this section. In all cases the entire cycle could be completed by microorganisms, and much of it is. The role played by present-day plants and animals, however, is considerable and will be included.

Microorganisms eventually degrade or mineralize the bulk of organic matter synthesized by green plants, algae, are other microorganisms in nature. Organic compounds synthesized by autotrophic plants may be interconverted to organic compounds of animals or to organic compounds of microorganisms themselves by ingestion and metabolism. Conversion to compounds in microorganisms may be through metabolizing plants, animals, or structures contained in other microorganisms. Eventually, most organic compounds of presently living forms will be mineralized or be converted into inorganic forms. Reconversion of inorganic to organic compounds also occurs simultaneously.

Carbon dioxide, derived from the breakdown of organic matter, is found in the earth's atmosphere or in water in the form of dissolved carbonates and bicarbonates. Photosynthesis and mineralization on earth appear to balance each other and maintain a concentration of about 0.03% carbon dioxide by volume in the atmosphere. A balance between carbon dioxide in the atmosphere and bicarbonates and carbonates in the waters of the earth is maintained.

It should be noted here that, although we have accounted for only the carbon dioxide produced by microorganisms, a large amount results from animal and plant respiration and direct combustions. Examples of the latter are the burning of methane to carbon dioxide and water in the presence of oxygen and the burning of organic compounds in wood and coal.

Molecular nitrogen makes up about 80% of the earth's atmosphere but, in this form, is available for use to only a few living forms of life. In terrestrial habitats the combined or fixed nitrogen in the soil below the atmosphere, however, constitutes only a very small percentage of the total nitrogen. Combined nitrogen varies tremendously in different soils, but in any soil it is probably present in concentrations of less than 1 to 100,000 of that in the atmosphere above it. The amount of available nitrogen is usually the factor that limits the growth of vegetation in a soil, but salinity, pH, temperature, iron content,

and other environmental conditions may also be limiting factors. Forms of nitrogen that are useful for organisms other than nitrogen fixers are ammonia, nitrates, and organic nitrogen compounds. In aquatic habitats the amount of dissolved or organic nitrogen is also far below that of the atmosphere which covers the water. Nitrates are required by most green, red, brown, and other eucaryotic algae and may be the limiting factor in their growth in certain marine areas. The degree of nitrate concentration does not vary in oceanic environments to the extent that it does in soil, but it is more concentrated in cooler than in tropical waters. In tropical waters, for example, eucaryotic algae blooms are less frequent, and those of blue-green algae, which utilize molecular nitrogen or ammonia as nitrogen sources, are more frequent. Nitrate is more concentrated toward the bottom of oceans. Many algae and phytoplankton can utilize nitrites and continue the nitrogen cycle in the absence of *Nitrobacter*, which characteristically converts nitrite to nitrate. Nitrogen fixation in the sea and in the soil appears to follow similar processes and is carried out by similar organisms, except *Azotobacter* is apparently absent from most marine waters, and a few other types of nitrogen fixing microorganisms have not been identified in marine habitats. Some others that appear in marine waters probably come from nearby land environments.

6.13 FIXATION OF NITROGEN

Microorganisms play the leading role in nitrogen conversion, although plants assimilate it and animals, along with microorganisms, break down organic compounds that contain it. Briefly, the cycle can be described as follows:

1. Microorganisms convert molecular nitrogen and ammonia to compounds that can be assimilated by plants and eucaryotic algae.
2. Plants and eucaryotic algae assimilate available useful forms of nitrogen into amino acids and eventually into protein. Blue-green algae can carry on both processes.
3. Animals and microorganisms denitrify amino acids to produce ammonia, urea, or compounds shown in amino acid metabolism. These three processes may be referred to as *nitrogen fixation*, *nitrogen assimilation*, and *denitrification*.

Nitrogen may be converted by artificial means into forms that can be assimilated by plants, but all methods employed for this process so far are accompanied by drastic conditions of temperature, pressure,

etc. Most conversion by microorganisms, on the other hand, proceed in an orderly, controlled manner.

6.14 FREE-LIVING MICROORGANISMS

For a number of years it has been known that certain microorganisms fix nitrogen, although they do not live in conjunction with legumes or other plants. A large number of anaerobic spore-forming rods, designated as clostridia, have been isolated and shown to fix nitrogen in soils or in cultures. Increasing the amount of available carbohydrate increases the amount of nitrogen fixation by *Clostridium pasteurianum* and other anaerobic organisms. Fermentation yields less energy per unit of carbohydrate utilized, however, than respiration. The less energy per unit of carbohydrate utilized, however, than respiration. The nitrogen-fixing free clostridia can utilize either molecular nitrogen or ammonia for conversion into nitrate, but both carbon monoxide and nitrous oxide inhibit anaerobic fixation.

As has been stated on several previous occasions, blue-green algae are capable of both photosynthesis and nitrogen fixation. Both hydrogen gas and carbon monoxide will inhibit fixation. The purple bacterium, *Rhodospirillum*, can fix nitrogen rapidly, and nitrogen gas can be utilized as a nitrogen source by this organism if biotin is present. The sulfur bacteria Thiorhodaceae are also nitrogen fixers and can utilize molecular nitrogen as a source. *Rhodopseudomonas*, *Rhodomicrobium*, and other members of the Rhodobacteriineae are able to carry out the process. *Aerobacter* and *Methanobacterium* species, along with many others, have been shown to be weak nitrogen fixers.

The free-living form that is most often thought of in connection with independent nitrogen fixation is the *Azotobacter*. Several species have been described and can be differentiated by minor morphological characteristics and colony types when grown on glucose or mannitol agar. The best known species are *chromococcum*, *agilis*, and *indicum*. Carbohydrate is essential in growth media, and growth is aerobic. Molybdenum is essential for fixation with some forms and is highly stimulatory to others. Ammonia appears to be an intermediate in fixation, but neither hydroxylamine (NH_2OH) nor hydrazine (H_2NNNH_2) has been definitely shown to be involved.

6.15 FIXING NITROGEN IN ROOTS OF PLANT

Controlled experiments will demonstrate that leguminous plants

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will usually add nitrogen to soils. The efficacy of nitrogen addition to the soil was naturally considered as a function of leguminous plants until recent years. Conversion by bacteria instead of plants has been established, and the process also occurs in some plants other than legumes.

The root hair of a legume may be infected by micro-organisms, which are connected in a trade like formation that runs through the hair. Infecting organisms can be cultured and are seen as bacilli or coccobacilli during active growth. In the infected root nodule, however, there are many pleomorphic forms, which take on different sizes and shapes.

When nodules were first observed on roots of legumes, their significance was not understood. Even after the discovery of bacteria in root nodules, it was not known that bacteria produced the nodules and that bacteria-containing nodules were essential for leguminous nitrogen fixation. Combined roles played by legumes and their infecting bacteria in nitrogen fixation were later termed *symbiotic nitrogen fixation*.

Experiments which illustrate that microorganisms, and nodules formed by them, are essential for symbiotic leguminous nitrogen fixation are as follows:

1. Either leguminous or nonleguminous plants grown in nitrogen-free sterile soil will grow poorly and show nitrogen deficiency.
2. If, however, some fresh soil is added, the legume will grow well, bear nodules on its roots, and fix nitrogen; the nonlegume shows no change. An additional point should be noted. Some nonleguminous plants have nodules that may result from infection by various organisms. Most of these infected plants do not fix nitrogen, but some have been shown to be nitrogen fixers, and plants for the preceding experiment must consist of nonleguminous plants that do not fix nitrogen.
3. The legume transplanted to new sterile nitrogen free soil, will flourish but the nonlegume will not.

Bacteria that infect roots of various legumes have been grouped into the genus *Rhizobium* (*rhiza* means root). There is specificity in some cases as to the host plant, although antigenically all groups are closely related. In general, *Rhizobioum* species names are derived from plants that particular microorganisms infect. Some species with plants that they characteristically infect are as follows:

1. *R. trifolii*, clover
2. *R. leguminosarum*, peas, vetch
3. *R. meliloti*, alfalfa

4. *R. phaseoli*, beans
5. *R. lupini*, bluebonnet, or lupine
6. *R. japonicum*, soybean

There are some smaller groups in which differentiations are not very clear. Growing *Rhizobium* in culture reduces specificity for host plants, and the question of species specificity is unclear.

Approximately 1 million microorganisms per milliliter are apparently necessary for the infection of root hairs, and only a very small fraction of infections in root hairs develops into nodules. Root hairs of plants that do not form nodules are often infected.

Both legumes and rhizobia appear to be essential for efficient nitrogen fixation. Adequate carbohydrate nourishment for the plant is helpful, and excess nitrogen in the soil or hydrogen gas in the atmosphere may be inhibitory to symbiotic nitrogen fixation. Both carbon monoxide and combined nitrogen have been shown to exert inhibitory effects. Aspartic and glutamic acids appear as the first products of nitrogen fixation by rhizobia, and glutamic acid is found as a product of fixation by nonsymbiotic or free-living nitrogen fixers. The importance of relations of rhizobia to plants becomes apparent because adequate supplies of keto-acids are essential. Keto-acids are plentiful in photosynthetic plants, and it is in these plants that fixation of nitrogen by rhizobia finds its peak.

Molybdenum and cobalt are apparently necessary to support symbiotic fixation, although both are active in oligodynamic quantities and usually present in soils. A red substance, resembling hemoglobin of mammalian red blood cells, is present in active nodules. This hemoglobin material combines with oxygen or carbon monoxide, as is true in cases of mammalian hemoglobin. It is possible that molecular nitrogen might combine with nodule hemoglobin as the first step in fixation. Hemoglobin is not functional in the respiration of plants.

6.16 UTILIZING AMMONIA MICROORGANISMS

For less than a century conversion of ammonia to nitrate has been associated with living organisms. This process has been termed *nitrification*. The process of nitrification proceeds rapidly in the soil. Plants convert nitrates to nitrogen constituents of amino acids or back to ammonia and usually contain only small quantities of $-\text{NO}_2$ or $-\text{NO}_3$ in their tissues. Nitrification proceeds in soils with lower pH levels than those in which nitrifying organisms will live and carry out

the process in cultures. Nitrification in soils possibly proceeds on particles where local pH values are favourable. The growth of microorganisms on particles where conditions are more favourable may also help to explain why their metabolism is not inhibited by the presence of organic matter that is ordinarily toxic to lithotrophs. The yield of nitrite from ammonia is slow and is relatively low compared to the amount of oxygen consumed by converting microorganisms.

6.17 NITRATES AND MICRO-ORGANISMS

If nitrogen-fixing and nitrifying microorganisms lived in the soil and ocean beds with no organisms carrying out the reverse process, all soil and ocean deposits would become extremely rich in nitrate, which, in turn, would be available for plant food. For the benefit of animal life on earth and in the sea, unfortunately, this is not the case. It can usually be said of both terrestrial and marine habitats that for each action or process there is an opposite reaction, although opposite reactions may not be, and usually are not, equal. Reactions of nitrification and nitrogen fixation balanced against denitrification in soils determine to a large degree the amount of nitrogen available for plant growth and, thus, soil fertility.

The process proceeds under anaerobic conditions because some organisms involved will utilize oxygen if available. An adequate source of hydrogen must also be present for reduction. In some soils denitrification proceeds during the wet seasons, and most available nitrate is converted into ammonia. During the dry season, when aerobic conditions prevail, the reverse process occurs, and a high concentration of nitrate is present in the same soil. Products of microbial denitrification (reduction of nitrates) are mostly NO_2 , N_2O , and N_2 , and the process is inhibited by the presence of free oxygen because bound oxygen instead of free oxygen must be used as a hydrogen acceptor in nitrate reduction. Microbial reduction of N_2O to N_2 has been demonstrated, and a number of intermediates between NO_3 and N_2 have been postulated. Extreme reduction of nitrate to ammonia by microorganisms has also been demonstrated. Hydroxylamine is apparently an intermediate in some of these conversions.

6.18 MICROORGANISMS AND SULFUR COMPOUNDS

Inter conversion of sulfur, both in marine and land environments, constitutes a series of processes of great importance in geochemistry. Both chemical and biological conversions occur, and sulfur in some

form is an essential constituent in all living organisms. Interconversion steps, in brief, consist of the oxidation of H_2S and sulfur to $\text{SO}_4^{=}$, assimilation of $\text{SO}_4^{=}$ by plants, metabolism of plant tissues by animals or microorganisms, and eventual reduction of plant and animal sulfur (usually in amino acids) to H_2S . The $\text{SO}_4^{=}$ may be reduced directly to H_2S by certain microorganisms, particularly the *Desulfovibrio*. Hydrogen sulfide is present in sulfur springs, mostly as a result of volcanic action, but it is oxidized when exposed to atmospheric oxygen. The reduction of sulfur to H_2S and its oxidation may occur as spontaneous chemical reactions, or they may result from biological activities.

Soluble sulfates furnish available sulfur for most living organisms. In living material, however, sulfur is *reduced* and appears in its reduced form in sulfur containing amino acids (cysteine, cystine, and methionine). It will be recalled that green sulfur bacteria and purple sulfur bacteria can utilize H_2S as a sulfur source, but this type of metabolism, of course, is limited in nature to areas of availability of H_2S . Where the sulfate atom is limited in nature to areas of availability of H_2S . Where the sulfate atom is utilized by plants, for example, only the atoms actually incorporated into cell substance are reduced, and reduced sulfur products are not formed by side reactions. *Desulfovibrio*, however, grows anaerobically and *utilizes sulfate as an electron acceptor*, with H_2S appearing as an end product of respiration. Reduced sulfur thus formed is not incorporated into sulfur amino acids of the microbe's proteins, but the $\text{SO}_4^{=}$ molecule serves merely as an electron acceptor for the oxidation of organic substrates or of hydrogen. The activities of *Desulfovibrio* are quite apparent on oceanic floors near shorelines and in the bottoms of streams, lakes, and ponds. High concentrations of sulfate reducers form a very important link in the chain of mineralization. Iron sulfide accumulates where both H_2S and iron are present, and H_2S is in evidence along some coastal areas where sulfates are abundant. Black mud results from iron sulfide, and the resulting odours are characteristic.

7

Soil Microorganisms

Life began on earth. There were no biological Big Bangs, nor extraterrestrial cultivators, just evolution from plausible nonliving beginnings." That is how John Scott, a biochemist at the Manchester Medical School in England, stated the basic assumptions scientists generally make about the origin of terrestrial life.* The question is, How did evolution from nonliving beginnings proceed? This chapter will attempt to answer this question, following theoretical developments that have gained widespread support from the scientific community since the late 1970s.

The story begins with the physical and chemical events that are believed to have taken place on the surface of the young Earth roughly 4 to 4½ billion years ago. At that time violent and nearly incessant volcanic eruptions occurred at many places, while at the same time the final accretion phase of the planet's formation drew to a close and its surface layers began to reach some semblance of equilibrium. The eruptions spewed forth large quantities of water, carbon dioxide, molecular nitrogen, and many other molecules, from which the first atmosphere and the juvenile ocean formed. Driven by energy from sunlight, lightning, volcanic heat, and meteorite impacts, the inorganic molecules reacted chemically with each other and produced a great variety of organic molecules - amino acids, sugars, lipids, the bases of nucleic acids, and many more. Gradually these molecules accumulated in the waters of the Earth until, in the words of John Haldane, "the primitive oceans reached the consistency of hot dilute soup". Today scientists believe that the temperature of the primitive ocean was probably close to the freezing point of water. Furthermore, its content of organic molecules may not have been as concentrated as Haldane had envisioned it, at least not throughout most of its volume.

Nevertheless, many people still refer to it as the "primordial soup." At present we are far from understanding all of the chemical reactions that took place on the young Earth. It seems that simple organic molecules assembled into larger and more complex molecules similar to those found in today's organisms, as suggested by laboratory experiments. For instance, amino acids probably assembled into peptide chains, and nucleotides assembled into short strands of RNA and other kinds of nucleic acids. Surfaces of clays, lava, rocks, sand, and other readily available substances may have served as catalysts facilitating these assembly reactions.

According to the theory of the origin of life that I am presenting here, short strands of RNA were the first molecules in the primordial soup that carried information, albeit very little, and they are regarded as the starting point of the evolution toward cellular life. (For alternative theories favored by some biochemists, see Cairns-Smith 1985 and Dyson 1985.) The short strands of RNA were capable of self-replication. In the process mistakes were made, so that the copied RNAs frequently differed from the original ones with regard to nucleotide sequence and length (recall that RNA nucleotides are of four types, with the bases A, G, C, and U). Some of the RNA molecules were more successful than others in surviving and replicating themselves under the prevailing conditions. Thus, the two components that form the basis of the Darwinian theory of evolution - random creation of variation and natural selection - may have been introduced very early among the chemical reactions in the primordial soup. As chemical evolution continued, different sets of RNA molecules coupled together into cooperative units. Some of the RNAs carried instructions for the assembly of primitive enzymes (peptide chains), while others acted as catalysts and contributed to the actual assembly of enzymes. Enzymes, in turn, helped in the replication of RNAs. Eventually, some of the coupled units of RNAs and their enzymes became enclosed by membranes and the first primitive cells-the protocells-were born. Life had emerged from among the random and spontaneous chemical reactions in the primordial soup.

This brief summary outlines the key components of, the events that many biologists and chemists believe may have been central to the origin of life on Earth, and the remainder of this chapter will fill in some of the details. At present this theory is based on many assumptions and contains many gaps. It is not based on any direct evidence dating back to the primordial soup, for none has survived.

Furthermore, laboratory experiments that attempt to simulate early Earth conditions and to reproduce the chemical reactions that took place then have been only partially successful in telling how the protocells arose. Scientists think they understand in general terms how the Earth's atmosphere and ocean came into existence. They believe they know something about the formation of organic monomers from inorganic raw materials and the assembly of polymers from monomers. However, they still have only a very limited understanding of the emergence of order and information among the chemical reactions in the primordial soup. And they know virtually nothing about the evolution from those initial chemical reactions to the formation of the first cells. The Nobel prize-winning German biochemist Manfred Eigen characterized very aptly our current state of knowledge of how life began: "Anyone attempting [to re-create life] would be seriously underestimating the complexity of prebiotic molecular evolution. Investigators know only how to play simple melodies on one or two instruments out of the huge orchestra that plays the symphony of evolution."

We should not be discouraged by this lack of knowledge. Let us accept the problem of the origin of life as one of the great challenges facing science today. Let us also accept the fact that time inevitably diminishes and sometimes erases evidence of long-ago events. Hence, our first task is to discover the fragments of evidence that have survived. Our second task is to make good use of them. That is how Darwin and Hubble confronted their scientific challenges, which also dealt with events of long ago and for which much of the original evidence had been erased by time. Darwin deduced his theory of the origin and evolution of the species mainly from data gathered on a single trip around the globe, and Hubble based his proposal about the expansion of the Universe on measurements of recession velocities of about two dozen distant galaxies. Thus, there are precedents in the history of science that fragmentary information is no barrier to the development of feasible theories. Let us be optimistic that this will also be true of the current scientific attempts to reconstruct the events that led to life on Earth.

7.1 GEOLOGIC ACTIVITY ON THE YOUNG EARTH

When the Earth was formed roughly 4.5 billion years ago, it was a hot, partially molten mass without an ocean or much of an atmosphere. Most of the heat came from gravitational energy that was released

when planetesimals collided and fell together to form the Earth, as discussed in chapter 4. Additional gravitational energy was released when the high-density iron and nickel of the proto-Earth sank toward the center to become the core of our planet, and lighter rocky material rose toward the surface to form the mantle and crust. Some of the energy also came from the decay of radioactive isotopes. These energies were liberated much more rapidly than they could be radiated away, and consequently they accumulated as heat. Only a fraction of this heat has been lost during the intervening eons. Even today, our planet's central temperature is still approximately 4300 K.

During the final accretion phase, Earth acquired a surface layer of low-density rocks rich in many kinds of volatiles including water, carbon dioxide, molecular nitrogen, and organic compounds. This rocky material was probably derived from carbonaceous chondrites, which bombard the Earth to this day, along with other types of meteorites, though at a much reduced rate. The outermost layers of the Earth radiated their heat into space and cooled to the point where they crystallized and hardened. They became the basaltic and granitic rock layers that form the crust of the Earth and float like rafts on the denser underlying mantle.

The crust is primarily responsible for maintaining the Earth's high internal temperature. It has a very low thermal conductivity, which slows the rate of heat flow from the Earth's interior to the surface. This can be seen in some of the desert caves in the western United States where the snow and ice that drift in during the winter stay throughout the hot summer months, even though they are separated from the surface by only a few meters of rock. Another feature that contributes to the maintenance of the Earth's high internal temperature is the presence of long-lived radioactive elements - uranium-235 and -238, thorium-232, and potassium-40-in the crust. The decay of these elements steadily releases heat and is the source of much of the geothermal energy that flows to the Earth's surface. Thus, the crust, enveloping the Earth, acts like an electric blanket: Its low thermal conductivity corresponds to the insulating qualities of the wool or polyester, and its radioactivity corresponds to the electric heat output of the blanket.

One consequence of the Earth's high interior temperature is that the outer part of the core and the mantle have never hardened into rigid structures, but have remained in molten or "pasty" states, resembling fluids of high viscosity.* Another consequence is that powerful

convective currents are generated in the mantle, which relentlessly push and pull on the overlying layers and prevent them from settling into a permanent configuration. That is why our planet's surface features continuously change over geologic time. Continents converge and break up, ocean basins come and go, and mountain ranges are lifted up and weathered down.

The pushing and pulling on the Earth's lithosphere by currents in the underlying mantle involve enormous forces and energies. Usually we are not aware of those geologic activities because they happen so slowly; but earthquakes, volcanic eruptions, geysers, and hot springs are reminders that we live on a restless and dynamic planet. This restlessness must have been much more severe when the Earth was first formed than it is today. The Earth's interior was hotter then and its temperature had not yet had time to adjust to a smooth gradient from the center to the surface. Consequently, the currents in the mantle must have been stronger. The lithosphere was still crystallizing and had not yet achieved its present thickness and rigidity. This crystallizing was slowed by the steady release of heat from the decay of the radioactive elements, which were initially much more abundant than they are today. Because it was thinner and less rigid than it is now, the lithosphere of the young Earth was more easily deformed by the mantle currents than it is today. As a result, earthquakes and volcanic eruptions, accompanied by huge lava flows, must have occurred almost incessantly and with great intensity over large areas of the young planet's surface, much as they occurred on the Moon, Mercury, Mars, and, perhaps, on all planetary bodies of intermediate size.

In addition to earthquakes and volcanism, which are processes created by conditions within the Earth, there also was violence from outside. When our planet was formed and had reached approximately its final mass and size, there was still plenty of interplanetary debris - planetesimals, comets, rocks, and dust - left from the original protoplanetary disk. For hundreds of millions of years this debris kept falling onto the Earth at a high rate until most of it had been swept up. Even today some traces remain, as indicated by the roughly 30 tons of matter that fall onto Earth every day in the form of "shooting stars" and meteorites. The largest of the planetesimals that bombarded the young Earth probably weighed many billions of tons and were comparable in size to the asteroids that still orbit the Sun today. On impact, they shattered the crust, carved out huge impact craters, and threw molten and pulverized crustal material across the planet's surface.

TABLE 7.1. Partial listing of Molecules

CH ₄	methane	H ₂ O	water
CO	carbon monoxide	H ₂ S	hydrogen sulfide
CO ₂	carbon dioxide	NH ₃	ammonia
CO ₃	carbonate	NO ₃	nitrate
C ₂ H ₄	ethylene	N ₂	molecular nitrogen
C ₂ H ₆	ethane	O ₂	molecular oxygen
HCN	hydrogen cyanide	PO ₄ ⁼	phosphate
H ₂	molecular hydrogen	SO ₄ ⁼	sulfate
H ₂ CO	formaldehyde		

7.2 ORIGIN OF THE EARTH'S ATMOSPHERE AND OCEAN

The earthquakes, volcanic outbursts, and bombardments by meteorites, which ravaged the young Earth so regularly, did not just churn the crust and produce large lava flows. As hot lava reached the surface and meteorites heated their impact areas to incandescence, gases that had been trapped in the rocks burst into the open in huge amounts -gases of water (H₂O), carbon dioxide (CO₂), molecular nitrogen (N₂) and, in lesser amounts, of molecular hydrogen (H₂), carbon monoxide (CO), methane (CH₄), ammonia (NH₃), hydrogen sulfide (H₂S), and many others. Our planet was acquiring its first atmosphere.

This kind of outgassing (releasing of gases) can still be observed today, although at a considerably diminished rate, in the hot springs and geysers of Yellowstone National Park, active volcanoes such as Mount St. Helens, and many other places of geothermal activity. For example, volcanic eruptions are usually accompanied by the emission of thick and often foul-smelling clouds of gases that billow for miles into the atmosphere. The gases originate from within the lava. They escape into the open when the hot lava reaches the surface of the Earth and is no longer subjected to the high pressures deep below the ground. Quite often the gases bubble forth in ways that give the resulting rocks a frothy appearance and make them lighter than water. During some eruptions the gases burst forth so violently that they shatter the lava into fine-grained dust known as ash, which may be thrown hundreds or even thousands of kilometers -into the surrounding areas.

7.2.1 Water

Despite the heat that accompanied outgassing on the early Earth, the average temperature of the atmosphere was probably not far above

TABLE 7.2 Relative Abundances (in Percent) by Mass of the Most Common Elements in the Sun, Entire Earth, Earth's Crust, and Human Body

<i>Element</i>	<i>Sun</i>	<i>Entire Earth</i>	<i>Earth's Crust</i>	<i>Human body</i>	
Hydrogen	73.6	—	—	10.	(63.)
Helium	24.8	—	—	—	—
Carbon	0.29	—	—	18.	(9.5)
Nitrogen	0.093	—	—	3.1	(1.4)
Oxygen	0.77	30.	46.	65.	(25.)
Neon	0.12	—	—	—	—
Sodium	0.0029	—	2.1	0.1	(0.03)
Magnesium	0.046	13.	4.	0.04	(0.01)
Aluminum	0.0049	1.1	8.	—	—
Silicon	0.069	15.	28.	—	—
Phosphorus	0.0007	—	—	1.	(0.2)
Sulfur	0.038	1.9	—	0.3	(0.05)
Chlorine	0.0011	—	—	0.2	(0.03)
Argon	0.018	—	—	—	—
Potassium	0.0003	—	2.3	0.4	(0.06)
Calcium	0.0057	1.1	2.4	2.	(0.3)
Iron	0.16	35.	6.	—	—
Nickel	0.0084	2.4	—	—	—

the freezing point of water, for the energy output of the Sun—our planet's major source of heat—was then only about two-thirds of what it is today. Hence, the water, which was the most abundant molecular species emitted by volcanism and meteorite impacts, did not remain in gaseous form for long. It condensed into rain or snow and fell to Earth. Very likely the early rains came down in torrents, accompanied by storms, lightning, and thunder. The first rivers began to flow, glaciers and ice caps accumulated in the polar regions, and low-lying basins filled with liquid water to become our planet's juvenile ocean.

7.2.2 Carbon Dioxide

Volcanic outgasings on the early Earth poured forth not only water but also many other gases. The most abundant gas after water was carbon dioxide. This presented a potential danger for the eventual

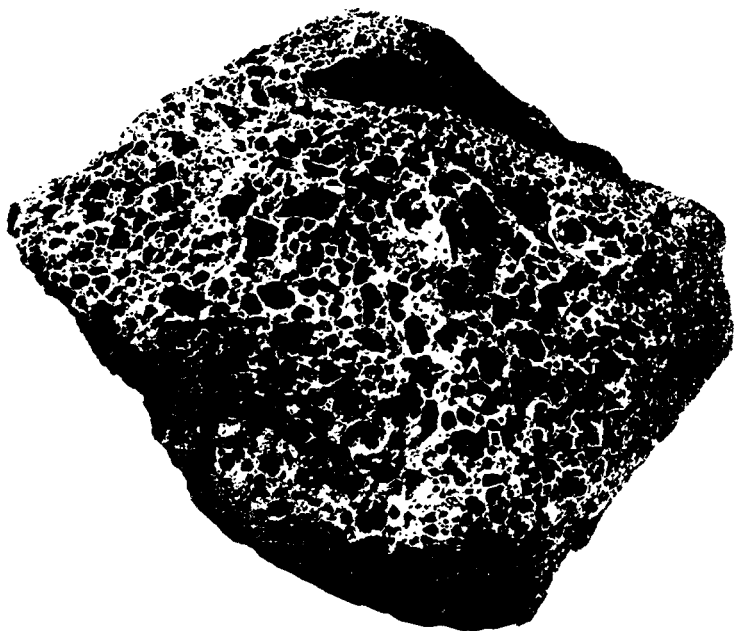
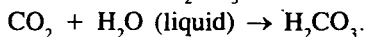


Figure 7.1 Basaltic volcanic rock filled with bubble holes.

origin and evolution of life on Earth because a planetary atmosphere that contains substantial amounts of CO_2 heats up to high temperatures by the *greenhouse effect*. This effect comes about as follows. Solar radiation, whose wavelengths correspond mainly to the colors blue through red (see introduction to Part One), penetrates relatively easily through the atmosphere and is absorbed by the ground. The absorbed energy heats the ground, which reradiates it in the infrared part of the spectrum. Infrared (IR) radiation has long wavelengths and, unlike the original solar radiation, much of it becomes absorbed by the CO_2 in the atmosphere. The CO_2 molecules reradiate this absorbed radiation, with some of it being sent back toward the ground. The net result is that the IR radiation does not readily escape into space but becomes trapped in the atmosphere, heating it as well as the ground.

Fortunately, the greenhouse effect had only a small effect on the young Earth's surface temperature because the rains washed the CO_2 out of the atmosphere before it could accumulate to dangerous levels. This happened because CO_2 dissolves in liquid water (but not in gaseous water) and forms carbonic acid (H_2CO_3):



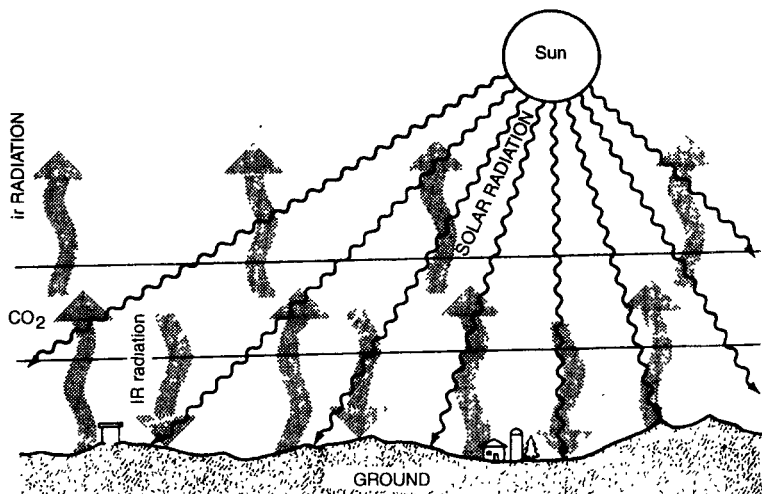


Figure 7.2 The greenhouse effect. Molecules of carbon dioxide (CO_2) in the Earth's atmosphere act somewhat like the glass roof of a greenhouse. They are transparent to the radiation from the Sun and let it pass to the ground. The ground heats up and reradiates energy as long-wavelength IR radiation. However, CO_2 molecules are much less transparent to IR radiation. They absorb most of the IR radiation, then reradiate some of it upward into space and the rest downward toward the ground, thus trapping much of the radiant energy and raising the temperature of both the atmosphere and the ground.

The rains and rivers brought the carbonic acid in contact with the ground, where it leached positive ions of calcium (Ca^{++}) and magnesium (Mg^{++}) out of rocks and combined with them to form limestone (CaCO_3) and dolomite [$\text{CaMg}(\text{CO}_3)_2$]. The limestone and dolomite then became deposited as sediments on the ocean floor. Thus, most of the original CO_2 that had been released into the atmosphere became locked up in sedimentary rocks. (Note that after shale and sandstone, limestone constitutes the most abundant sedimentary rock type of the present-day terrestrial crust.)

The third most abundant gas released by volcanism and meteorite impacts on the early Earth was molecular nitrogen, N_2 . It is chemically rather inert and did not condense into liquid form at the temperatures that prevailed on Earth, nor did it combine with water. It stayed in the atmosphere and, in the course of time, accumulated to become its dominant molecular constituent.

7.2.3 Oxygen

Interestingly, molecular oxygen (O_2) - which today makes up 21% (by number) of our atmosphere and is essential for the survival of all life (except some bacteria) was present in only very low concentrations

in our planet's original atmosphere. Had it been abundant, it would have rusted the iron and other metals. The oldest known rocks show no consistent evidence of such rusting. Only rocks younger than about 2 billion years do and, thereby, attest to the abundant arrival of molecular oxygen in the atmosphere by that time. Molecular oxygen was released in appreciable quantities into the atmosphere only after some organisms acquired the ability to split water molecules as part of their photosynthetic activities.

The absence of O_2 from the early atmosphere was a fortuitous circumstance. Had it been present in large amounts, its high chemical reactivity would have made the development of life very difficult, if not impossible. Even today O_2 is inherently poison to all organisms. They can withstand its reactivity only because they possess special molecules that chemically bind with O_2 and let it react in slow, controlled steps.

Without O_2 , the atmosphere of the young Earth did not have the ozone (O_3) layer that it has today. This layer is crucial for our survival, for it filters out most of the ultraviolet (UV) radiation from the Sun and prevents it from causing harmful chemical reactions in our cells, such as burns and damage to DNA and RNA (which may lead to cell death or cancer). In the absence of an ozone layer on the young Earth, UV radiation penetrated freely to the ground. But instead of being harmful, it provided the energy for triggering numerous chemical reactions that were important for prebiotic evolution, as discussed in the following sections.

7.2.4 The Next Step

Very likely, the first outgassings on the primitive Earth began long before the planet was fully formed and they continued with great intensity for hundreds of millions of years thereafter. Only when the initial burst of earthquake activity, volcanism, and meteorite impacts had abated, approximately 4.0 to 3.5 billion years ago, did the outgassing slow down. Geologic evidence indicates that by then the oceans covered large areas of our planet, and we may assume that the atmosphere had acquired a substantial fraction of its present content of N_2 . Most of the outgassed CO_2 had been washed out of the atmosphere and locked up in sedimentary rocks, but not all of it. Some of the CO_2 together with other gases that had been discharged in small amounts, such as H_2 , CH_4 , NH_3 , and H_2S , embarked upon a rather different and much more interesting evolutionary course. They reacted chemically with each other to form molecular structures of ever-increasing complexity.

7.3 SYNTHESIS OF MONOMERS

There are good reasons to believe that conditions on the surface of the young Earth were quite suitable for the occurrence of a great variety of chemical reactions and the production of many kinds of molecules. Volcanic outgasings supplied ample amounts of atomic and molecular raw materials. The temperature was low, but not so low as to freeze water everywhere. Lightning, UV radiation from the Sun, geothermal heat from volcanoes and hot springs, and meteorite impacts supplied plenty of energy for driving the reactions. At the same time, the ocean and ground offered protection from too much UV radiation and heat that might have destroyed the molecular products.

Among the molecular raw materials on the surface of the young Earth, H_2O , N_2 , and CO_2 were the most abundant. They offered a nearly inexhaustible supply of hydrogen, carbon, nitrogen, and oxygen. Hence, organic molecules must have been among the dominant products of the chemical reactions. This is the case in interstellar clouds, in which these elements are also abundant and in which organic molecules are manufactured copiously. The same was true of the Sun's protoplanetary disk, as manifested by the presence of organic molecules in meteorites.

Just how readily organic molecules form under conditions similar to those on the early Earth was first demonstrated experimentally by two American chemists, Stanley L. Miller and Harold C. Urey, in the early 1950s, using a glass apparatus. They filled the lower part of the apparatus, which included a small flask, with water to simulate the juvenile ocean. They pumped a gaseous mixture of CH_4 , NH_3 , and H_2 into the upper part to represent the primitive atmosphere. Then they boiled the water in the small flask to produce steam and to drive the gases in a closed circuit through the apparatus. At the same time, they generated electric sparks in the larger upper flask to simulate lightning in the primitive atmosphere and to provide a source of energy for chemical reactions. Below the large flask, they cooled the circulating gases so that the water condensed and returned as droplets to the lower part of the apparatus, thus completing the circuit. After they had run the experiment for a day or two, the water in the lower part of the apparatus turned pink and later it became red. This indicated, and careful analysis confirmed, that organic molecules (such as hydrogen cyanide, formaldehyde, sugars, amino acids, bases) had been formed by the electric sparks and were trapped in the liquid water.

When Miller and Urey first carried out their experiment, it was

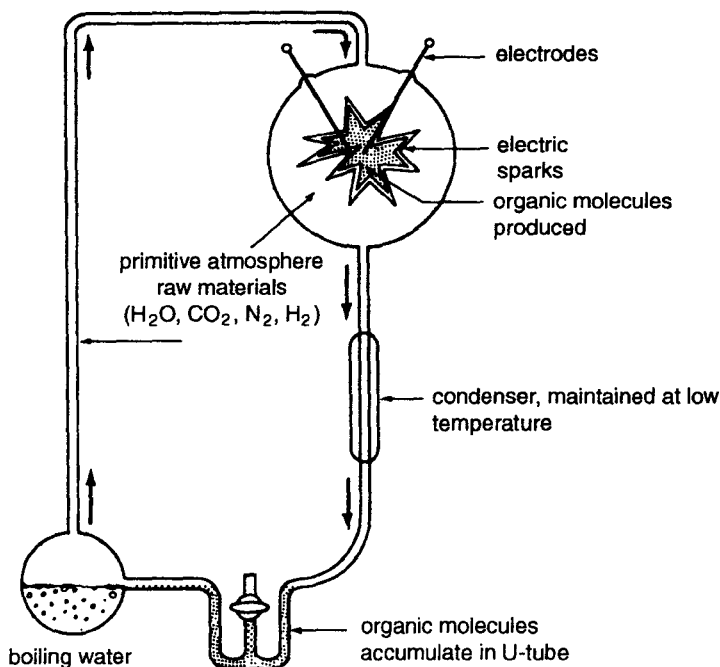


Figure 7.3 Apparatus used in Miller-Urey experiments.

commonly thought that hydrogen-rich molecules had been the major constituents of the Earth's primitive atmosphere. Hence, they used the gases CH_4 and NH_3 instead of CO_2 and N_2 . Since then, the experiment has been repeated many times with various combinations of CH_4 , C_2H_4 (ethylene), C_2H_6 (ethane), CO_2 , CO , NH_3 , N_2 , H_2S , H_2 , and H_2O and with different sources of energy, from intense heat (from about 1100 to 1600 K) to UV radiation and electric sparks. Some experimenters added sand, clay, or lava rocks to simulate the catalytic effects the ground of the young Earth might have had on the chemical reactions. All of the experiments yielded rich mixtures of organic molecules.

Apparently, the main requirement for synthesizing organic molecules is the presence of hydrogen, carbon, nitrogen, and oxygen in some molecular form or other. The details -whether the atomic raw materials are supplied as H_2O , CO_2 , N_2 , and H_2 , as H_2O , CH_4 , NH_3 , and H_2 , or in some other form -matter little. Nor does it matter much what kind of energy source is used, as long as it is sufficiently intense to drive the chemical reactions. The chief limitations are that oxygen not be present as O_2 and that the newly formed molecules do not remain

exposed to the energy sources for too long. If they are, the same energy source that creates them also destroys them. Protection from too much energy is achieved by dissolving the molecular products in liquid water (that is, in the juvenile ocean) or by allowing them to attach themselves to the surfaces of grains of sand, clay, mud, or lava rocks.

Note, however, that the Miller-Urey experiments do not yield many organic molecules of prebiotic importance if the only raw materials are H_2O , CO_2 , and N_2 . To enhance the production of organic molecules, H_2 , CH_4 , NH_3 , or other hydrogen-rich molecules need to be present among the starting materials, at least in small amounts (without them, the chemical reactions are dominated by the reactivity of oxygen). This is particularly true of the production of hydrogen cyanide (HCN), which is a precursor molecule of many of the more complex organic molecules that are believed to have played significant roles in prebiotic chemistry.

7.3.1 Synthesis of Amino

Acids, Sugars, and Bases

How are the simple starting materials converted into organic molecules in experiments simulating primitive Earth environments? Much attention has been paid recently to answering this question. Here are a few examples of these chemical reactions.

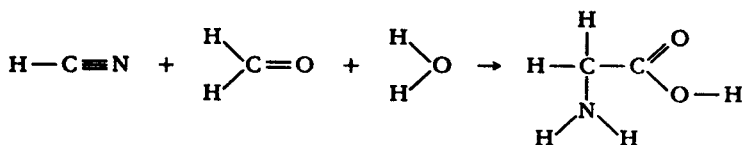
When molecules such as H_2O , CO_2 , N_2 , and H_2 are exposed to electric sparks, intense heat, or UV radiation, their bonds are broken and, temporarily, free atoms (H , C , N , O) and molecular fragments (for example, OH and CO) result. Almost instantaneously, new bonds reform between the atoms and the molecular fragments, but often in combinations that are different from the original ones. This breaking and reforming of chemical bonds constitutes the mechanism of chemical reactions by which new kinds of molecules are created. Such chemical reactions take place in statistically predictable ways and according to well-known physical laws. Given identical starting conditions, the molecules produced will, on the average, always turn out the same.

Two of the most common molecules that result during the initial stages of a Miller-Urey experiment are hydrogen cyanide (HCN) and formaldehyde (H_2CO). Both of them are important intermediates in the formation of still more complex organic molecules, in particular of amino acids. For instance, reactions among HCN , H_2CO , and water yield the amino acid glycine. The reactions involve several steps, but they may be summarized by this chemical equation:

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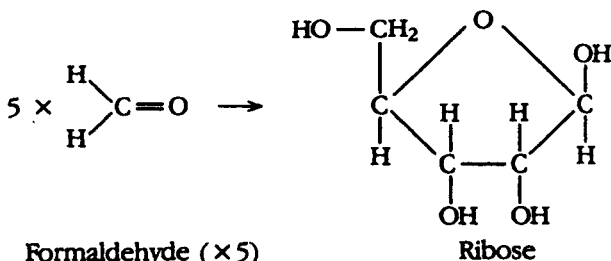


Hydrogen cyanide Formaldehyde Water Glycine

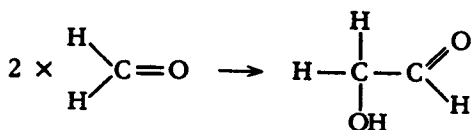
Reactions involving more complex aldehydes than formaldehyde yield more complex amino acids. The nature of the end product depends somewhat on the sources of energy used and on the presence of certain atoms among the starting materials. If electric sparks are used, glycine, alanine, leucine, serine, threonine, asparagine, and other relatively simple amino acids result. If the energy source is heat of about 1600 K, some of the products are amino acids with ring structures, such as phenylalanine and tyrosine. If UV radiation is used and H_2S is present in the gas mixture, small amounts of sulfur-containing amino acids are formed.

In addition to being precursor molecules for the making of amino acids, formaldehyde and hydrogen cyanide may also be the starting materials for the synthesis of other organic molecules. For instance, reactions among five molecules of formaldehyde, in the presence of calcium carbonate, produce a complex mixture of end products, including the five-carbon sugar *ribose*:

As before, this formula merely summarizes the input material and the final product of the reaction. The actual reaction sequence is much more complex, involving several steps. In the first step, two formaldehydes react to form glycolaldehyde:

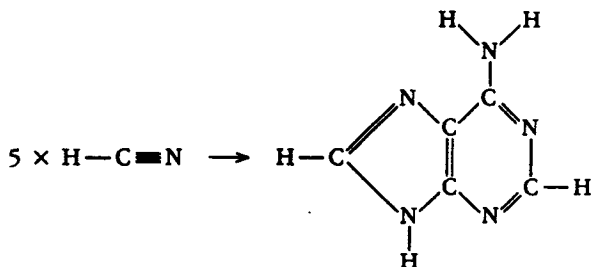


Glycolaldehyde may be either the precursor of ribose, if three more formaldehydes are added (as in the reaction shown above), or the precursor of the amino acid serine, if HCN and H_2O are added. In either case, the reactions are *autocatalytic*. At first very little happens. Then suddenly, after several hours, ribose and serine are

Formaldehyde ($\times 2$)

Glycolaldehyde

produced. Apparently, molecules of glycolaldehyde slowly form during the induction period. Once they exist, they act as catalysts for the production of more glycolaldehyde and then the reactions to ribose and serine run their course rather rapidly. Six-carbon sugars, such as glucose and fructose, are formed by similar reaction pathways.

Hydrogen cyanide ($\times 5$)

Adenine

Not all organic molecules of biological interest are as readily produced as the amino acids and sugars. For example, the syntheses of the bases of DNA and RNA call for rather high concentrations of starting molecules, which on the early Earth probably occurred only under unusual circumstances. *Adenine*, which is the easiest base to make (perhaps that is why it is the most common base found in organisms, being present in RNA, DNA, and ATP), requires the reaction of five molecules of hydrogen cyanide.

Again, this formula is merely a summary. In reality, several sequential reactions must occur, and the presence of UV radiation and ammonia are helpful. *Guanine*, the other double-ringed base, is very similar to adenine except that it possesses an oxygen atom and its side groups are slightly different. Its synthesis starts out identically to that of adenine, but in the final steps of the reaction sequence, water and cyanogen ($\text{N}\equiv\text{C}-\text{C}\equiv\text{N}$) or urea ($\text{H}_2\text{N}-\text{C}-\text{NH}_2$) need to be present.



7.3.2 L- and D-Amino Acids

These have been just a few examples of the numerous chemical

reactions that occur in Miller-Urey type and other kinds of experiments simulating prebiotic terrestrial conditions. Many of the molecules produced are identical to those found commonly in present-day life. Others are rather novel and have unusual structures. For instance, many of the amino acids have two configurations, one being the mirror image of the other. In one configuration, the side chain points to the left as one looks from the carboxyl group to the amino group (for details, see the Proteins section in the Introduction to Part Two). In the other, the side chain points to the *right*.

The two kinds of amino acids are known as L- and D-amino acids, where L and D stand for *levo* and *dextro* meaning "left" and "right." Both kinds are found in meteorites, and it is reasonable to assume that they were manufactured by prebiotic terrestrial chemistry as well. In contrast, with few exceptions today's organisms produce only L-amino acids. Somehow, during the origin of life, L-amino acids were selected over D-amino acids. Once that had happened, enzyme-aided biochemical reactions kept manufacturing chiefly the L-types. Was this selection an accident? Was it due to certain conditions on the early Earth, such as the particular molecular structures of clays and rocks, that may have acted as the first catalysts in prebiotic chemical reactions? Or do L-amino acids have some inherent advantage in the chemistry of life, perhaps due to some intrinsic bias in physical laws that is not yet understood? A number of answers have been suggested, but none of them is convincing. We don't really know.

In addition to L- and D-amino acids, other molecular variations that do not occur in present-day life show up in Miller-Urey experiments and, presumably, were also synthesized on the prebiotic Earth. An example is valine, which is produced in three different versions or *isomers* - valine, norvaline, and isovaline. All three of these isomers have the same chemical formula, $C_5H_{11}O_2N$, and they are amino acids, but their side chains are put together differently.

We have seen that varying the conditions - the starting materials, energy sources, and catalysts - of the Miller-Urey experiment and others simulating early-Earth conditions produces a great variety of organic molecules. Some of the molecules form readily under almost any kind of condition as, for example, many of the amino acids. Others, like the bases, require rather special conditions and are more difficult to make (though some of them are found in meteorites).

On the young Earth, environmental conditions must have spanned an enormous range. In the rivers, lakes, and oceans water was amply

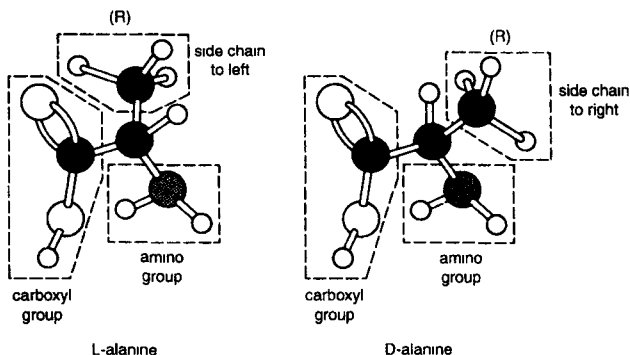


Figure 7.4 The two configurations of alanine.

available, while land parched by the Sun was characterized by dryness. The raw materials varied from molecules without hydrogen, like CO_2 and N_2 , to local concentrations of H_2 , CH_4 , C_2H_6 , NH_3 , H_2S , and other hydrogen-rich molecules. In the northern and southern latitudes the temperature was well below freezing, while near thermal pools it was in the hundreds of degrees, and near lava flows it was well above 1000 K. Interplanetary debris ranging in size from microscopic dust to bodies kilometers across bombarded the Earth, bringing in new materials and locally heating the atmosphere and ground. The Sun's UV radiation penetrated freely to the ground. Primordial storms produced lightning and released concentrated forms of heat. Rocks and mineral grains of various sizes, textures, and compositions offered additional raw materials, besides those discharged by outgassing, and were available to serve as catalysts for many of the reactions.

From day to night, season to season, and year to year, the early terrestrial conditions fluctuated. We may expect that in the course of thousands to many millions of years a broad range of chemical reactions occurred and that organic molecules were produced in great abundance and in many different forms. Even molecules that are very difficult to make were produced occasionally. The American biochemist, George Wald, put it as follows: "Given so much time, the 'impossible' becomes possible, the possible probable, and the probable virtually certain. . . Time is in fact the hero of the plot."

Once the molecules were formed, they were not easily destroyed as they would be today by bacteria and molecular oxygen. The oceans, rivers, and lakes offered protection from LN radiation and excessive heat as did sand, clay, and mud. Thus, organic molecules accumulated until, as Haldane suggested, the primordial ocean reached the

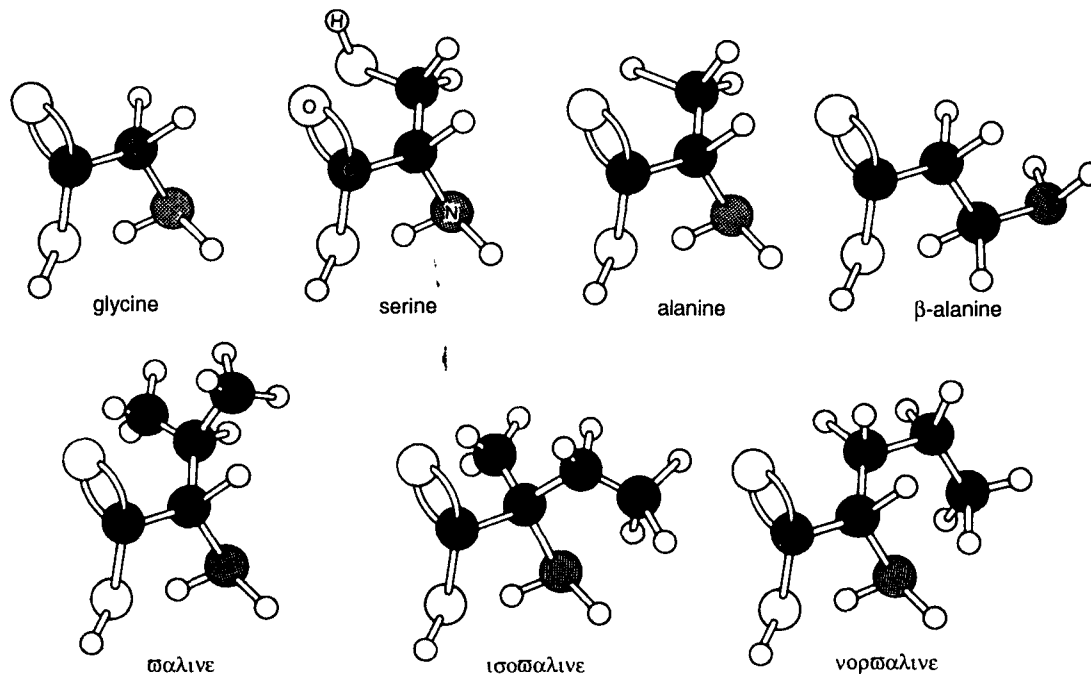


Figure 7.5 Examples of amino acids produced on the early Earth. Many of the amino acids existed in several isomeric forms and, with the exception of glycine, they occurred as both L- and D-types.

consistency of dilute soup. We do not know exactly how concentrated the soup became. That depended on the rate of synthesis of organic molecules as well as on the rate of their destruction. It also depended on the volume of the ancient ocean. However, we may be certain that at least locally, such as in shallow marine basins, tide pools, and lakes, its concentration was sufficiently great for the next phase in chemical evolution to get started -the assembly of simple organic molecules (the monomers) into polymers.

7.4 SYNTHESIS OF POLYMERS

The prebiotic synthesis of simple organic molecules was only the beginning of a complex and multifaceted evolution of chemical reactions. As amino acids, sugars, bases, and other molecules accumulated and their concentrations in the primordial soup and in the clay, sand, and mud of the young Earth increased, further chemical reactions linked them together into larger molecular structures, namely polymers. Amino acids were joined into peptide chains; ribose, bases, and phosphates were combined into nucleotides; and nucleotides were connected into strands of RNA and, possibly, other kinds of nucleic acids. These are just a few examples of polymers that probably were constructed from monomers by prebiotic chemistry. Many early polymers were identical or similar to those found in nature today, but others were quite different, just as some of the monomers were different. For example, it is very likely that nucleotides were bonded together into short RNA and DNA polymers by both the so-called 3'-5' linkage, which is found in contemporary cells, and by the 2'-5' linkage.

The chemical reactions that produced polymers from monomers on the early Earth were probably the same dehydration reactions that take place in today's organisms (though initially they were not catalyzed by enzymes): A hydrogen atom was removed from one monomer and an OH fragment from another, and then a chemical bond was formed between the two monomers. The H and OH were combined into a molecule of H_2O , which was released into the environment.

In today's organisms the assembly of monomers into polymers takes place in the interior of cells. There the monomers are well concentrated, so that collisions and, hence, reactions among them are frequent; the energy required for tearing the Hs and OHs from the monomers is amply supplied by ATPs; and enzymes are present to help remove the water molecules and speed up the formation of the bonds.

On the prebiotic Earth conditions for making polymers were

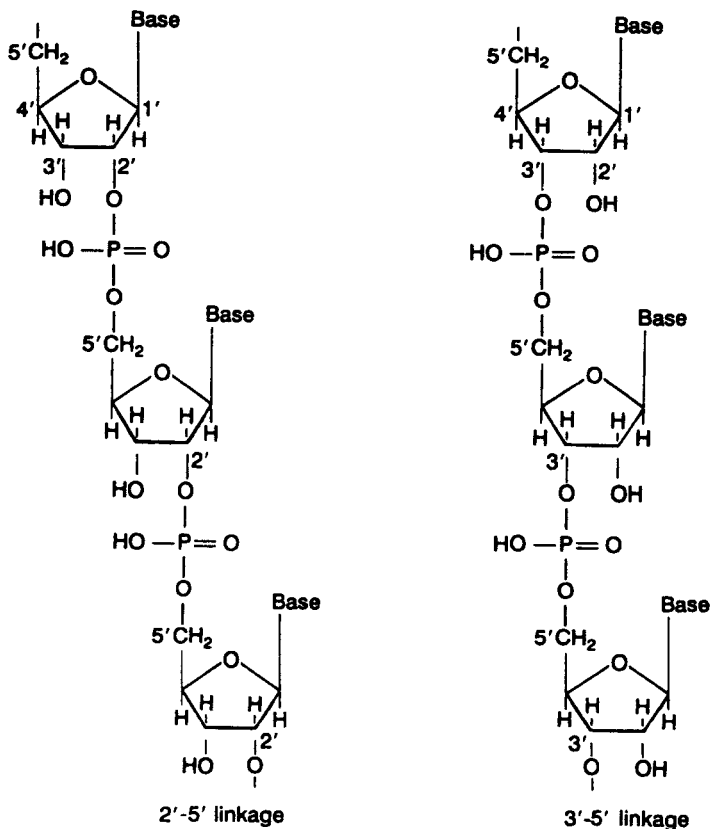


Figure 7.6 Chemically possible RNA "backbone" structures. The backbone structure of RNA, consisting of ribose sugars linked by phosphates, can exist in two forms: 2'-5' linkages and 3'-5' linkages. This notation means that the second carbon (as labeled in the diagram) of one ribose is linked by a phosphate to the fifth carbon of the next ribose, or the third and the fifth carbon atoms are thus linked. Very likely, both kinds of backbone structures formed in the primordial soup.

considerably less favorable. Because cells did not yet exist, reactions took place in the environment at large. There the concentrations of monomers were generally low compared to the interiors of present-day cells; energy was available, but most of it not in forms best suited for driving the dehydration reactions; and enzymes were lacking. In the oceans and lakes the absence of enzymes presented a particularly serious problem. Without enzymes it was difficult to form bonds by removing water molecules from monomers and expelling them into surroundings that already consisted largely of water. The reactions were much more likely to run in the opposite direction, namely in the direction of taking

water molecules from the surroundings, adding them to polymers, and splitting their bonds (a process called *hydrolysis*).

7.4.1 Concentration Mechanisms

With all of these obstacles, how were the first polymers assembled? We may assume that in local areas conditions were occasionally suitable for polymerization. For instance, evaporation of water in shallow lakes and ponds during dry spells might have satisfied the requirement of concentrating the monomers by leaving most of the organic molecules behind on the muddy bottoms. The heat near hot springs or from freshly expelled lava flowing into bodies of water would have had similar effects. The periodic rising and falling of the water level in tide pools would have regularly concentrated the molecules, especially in hot, dry climates. Still another concentration mechanism would have been the freezing of lakes and ponds during winter. As water froze to ice, organic molecules would have become concentrated in the remaining liquid water. (The same method was used by American pioneers in the making of applejack. A barrel of cider was put outside during the freezing weather and left standing until most of the water in the cider was solid ice, leaving the liquid alcohol concentrated as applejack in a small volume near the center of the barrel. The colder the temperature, the higher was the proof and, hence, the alcohol concentration of the applejack.)

The concentration mechanisms just discussed were all a result of changes in the environment. Concentrations may also have been accomplished by the organic molecules themselves. For instance, in the laboratory when amino acids are put in water that is then heated to above the boiling point, peptides form and cluster spontaneously into spherical, membrane-enclosed structures called *proteinoid microspheres*. These structures are about 1 μm across and, during their self-assembly, concentrate in their interiors many of the organic molecules present in their vicinity. Another concentration mechanism occurs when certain combinations of organic polymers, such as peptides, carbohydrates, and nucleic acids, are mixed together in water. They spontaneously organize themselves into clusters that are marked off from their environment by structured layers of water molecules. These clusters are called *coacervates*. They have sizes of up to 500 μm and, like the proteinoid microspheres, concentrate organic molecules in their interiors. Both proteinoid microspheres and coacervates may well have played significant roles in concentrating organic molecules in the primordial soup of our planet.

7.4.2 Energy Sources

The second requirement for assembling polymers - the availability of energy was probably at first satisfied by lightning, heat, and UV radiation, even though these sources of energy are in general not very efficient in linking monomers together. As the number and diversity of monomers increased, the chemical energy stored in the bonds of some of them became available for dehydration reactions as well. Hydrogen cyanide, cyanogen, and other nitrogen-containing molecules similar to cyanogen were probably most effective in driving the reactions. Very likely, chains of phosphate molecules, the *polyphosphates*, were also important energy sources. In fact, polyphosphates may have been the precursors of ATP, which consists of a triphosphate chain attached to an adenine-ribose trunk. Polyphosphates must have been quite abundant on the surface of the early Earth. They would have formed readily by mild heating of minerals containing phosphate, particularly during dry periods when the minerals became concentrated (along with other materials, such as organic molecules) on the bottoms of lakes and ponds.

7.4.3 Catalysts

The third requirement for making polymers is the presence of catalysts for removing water molecules from the monomers and speeding up the reactions. Until enzymes evolved, the most obvious catalysts were the surfaces of mineral grains. Many clays consist of very thin sheets of silicates, that are separated from each other by molecules of water. The water layers would have given the organic monomers easy access to the silicate surfaces, where they could have become attached and been ready to undergo dehydration reactions with newly arriving monomers. Polyphosphates would have been amply available in the minerals as sources of energy. Experiments indicate that peptide chains as long as one hundred amino acids form in the presence of certain days. The assumption that the surfaces of mineral grains acted as catalysts on the prebiotic Earth is supported by evidence from astrophysics. It appears that many of the molecules (including organic molecules) that are observed in the dark gas and dust clouds of spiral and irregular galaxies are also assembled on the surfaces of dust grains.

Experiments carried out with proteinoid microspheres suggest that they and other accidentally produced protein-like structures might have acted as catalysts as well. However, until the development of genetic information, the sequences in which amino acids were assembled into peptides and protein-like structures remained largely a matter of chance.

Therefore, whatever catalytic properties such protein-like structures may have possessed lacked the specificity and efficiency that characterize enzymes in contemporary cells.

This section described some of the processes by which organic monomers are thought to have been assembled into polymers on the early Earth. However, at present scientists are far from understanding the full range of chemical reactions that took place then. No doubt, processes other than those described played important roles in the assembly of polymers as well. Our knowledge is limited because it is very difficult to duplicate in the laboratory the early terrestrial environment. As noted in the previous section, there are a great many variables to consider: an enormous variety of chemically possible precursor molecules, a broad range of likely concentration mechanisms, fluctuations in temperature of the environment from below freezing to well above 1000 K, numerous sources of energy, and the effects of many different kinds of inorganic materials from clays to rocks, sand, mud, lava, and sediments. Furthermore, experiments of prebiotic chemical evolution must be conducted in closed apparatuses in order to avoid contamination by microorganisms. In contrast, chemical evolution on the early Earth occurred in an open and ever-changing environment consisting of the atmosphere, water, and land. Above all, experiments are limited by the factor of time. Prebiotic chemical evolution took place over thousands to many millions of years, a time span that cannot be duplicated in the laboratory.

7.5 ORIGIN OF THE CELL

The last two sections described the enormous variety of organic molecules that are believed to have been produced during our planet's early evolution. Some of these molecules were probably similar or identical to those found in life today, while others were quite different. Many survived for long times in sheltered niches, while others were soon broken down into simpler components by the same sources of energy that created them. The simple components were then reassembled into new, more complex molecules. Initially, these chemical reactions occurred in a random, helter-skelter fashion and depended only on the prevailing physical conditions: the concentration of atomic and molecular raw materials, the available sources of energy for driving the reactions, and, possibly, the presence of catalysts such as clays, rocks, certain ions, and accidentally produced small protein-like molecules.

Eventually, an *organizing mechanism* or, as Manfred Eigen calls

it, an “organizing principle” emerged among the reactions. This organizing mechanism included information and the means of translating that information into chemical function. The emergence of this organizing mechanism was the beginning of a chemical evolution that, in the course of time, led to life. Today, such an organizing mechanism is present in the cells of all organisms, including those of our bodies. It consists of *genetic information* and *a biochemical machinery* that translates the information into *enzymes*, which, in turn, make the entire biochemical machinery run.

Before going on to the theory of how the organizing mechanism of life might have arisen, let us review how this mechanism works in today’s cells (figure 6.8; for additional details, see the Introduction to Part Two). The components of the organizing mechanism are the following:

1. *DNA molecules* store *genetic information* by a code consisting of triplets of bases, called *codons*. The codons are arranged sequentially and partitioned into specific units, the *genes*, each of which codes for a particular protein molecule.
2. *Messenger RNAs (mRNA)* carry the information of a given gene from DNA to the protein assembly plants, the *ribosomes*.
3. *Transfer RNAs (tRNA)* carry *amino acids* to the ribosomes. For every one of the twenty amino acids found in nature, there exists one or more specific tRNA to which that amino acid becomes attached. Each of the tRNAs is characterized by a distinguishing triplet of bases, called an *anticodon*.
4. *Ribosomal RNAs (rRNA)* are essential components of ribosomes, the protein assembly machineries of the cells.
5. *Enzymes* are protein molecules that act as catalysts and are crucial for the smooth functioning of all biochemical reactions, including those of the organizing mechanism. (They function on the molecular level much as tools and machines function in a workshop.)

The organizing mechanism works as follows:

1. The information of a gene is *transcribed* (copied) from DNA onto mRNA.
2. The mRNA moves to a ribosome, enters and threads its way through it, thus *delivering* the genetic information.
3. As codon after codon of the mRNA pass through the ribosome, tRNAs with matching anticodons bind to the codons and deliver

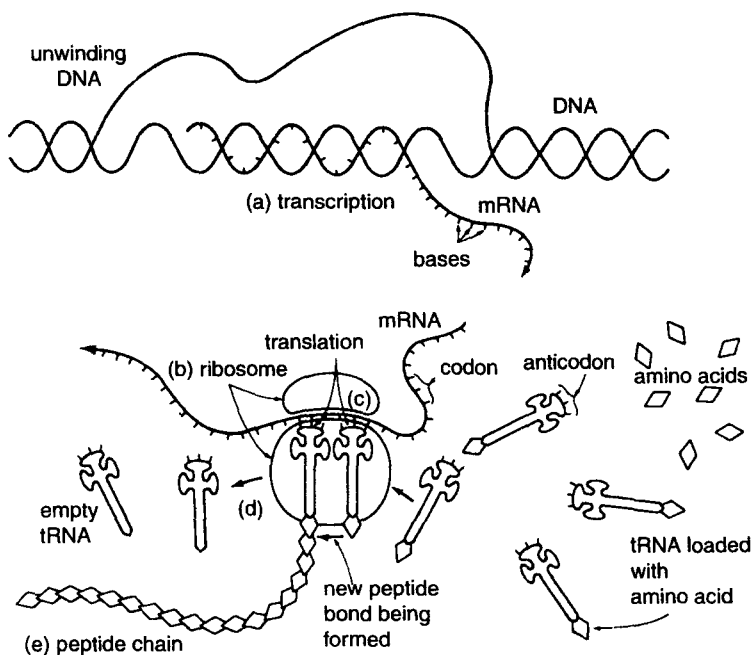


Figure 7.7 Protein synthesis. The information for the synthesis of proteins in modern cells is carried by molecules of DNA. The information consists of a sequence of bases, three of which constitute a codon, the basic unit of genetic information. Protein synthesis begins with the unwinding of a double-stranded DNA molecule and the transcription of information onto a molecule of messenger RNA.

their amino acids, which become linked together by peptide bonds. The now free tRNAs are then released. The genetic information carried by the mRNAs -as a sequence of codons -is thus *translated into a peptide chain*.

4. As each peptide chain is assembled, it starts folding back and forth and assumes a unique shape, depending on its sequence of amino acids. The result is a functional protein - a structural protein, a regulatory or transport protein, or an enzyme protein.

In summary, the organizing mechanism of life directs the synthesis of proteins. The proteins-in particular, the enzymes-make the chemical reactions in an organism work, including the chemical reactions of the organizing mechanism itself.

7.5.1 Origin of the Organizing Mechanism

The next question is, "How did the organizing mechanism of life

have its start among the disorder and randomness of the chemical reactions on the early Earth?" Note that the organizing mechanism in contemporary life is based on the presence of both *informational* and *functional* components. The informational components are DNA and RNA molecules. The functional components are RNA molecules and proteins (enzymes). Interestingly, the RNAs have dual tasks: they carry information and they perform functions. The DNAs and proteins have single tasks each: the DNAs carry information and the proteins perform functions.

There are a number of reasons why in today's cells the RNAs, DNAs, and proteins have these particular features. RNA molecules are usually singlestranded, consisting of a linear sequence of monomers (the nucleotides, which contain the bases A, G, C, and U). The sequential arrangement of monomers allows RNA molecules to carry information, similar to the way DNA molecules carry information. Furthermore, the single-strandedness allows RNAs to fold into three-dimensional shapes suitable for carrying out functions such as bringing amino acids to the ribosomes. Some RNAs are also capable of catalyzing certain biochemical reactions and thus act like enzymes, as was recently discovered by a number of researchers. Such catalytic RNAs act either alone or as part of RNA protein complexes. Finally, short strands of RNA possess autocatalytic properties (see below). The three-dimensional folding of RNA has the additional advantage in that some of the shapes make the molecule resistant to destruction or alteration by hydrolysis and certain other chemical reactions.

In contrast, DNA molecules and proteins, the other components of the organizing mechanism in contemporary cells, do not have the RNAs' dual quality. The DNAs are generally double-stranded and, hence, are much less able than RNAs to fold into specific three-dimensional shapes that would allow them to carry out functions. They are mainly used for storing and reproducing genetic information. We may assume, therefore, that DNA was initially not part of the organizing mechanism but was introduced later on. Proteins are made of peptide chains and resemble RNA in that they also fold into a great variety of shapes, which gives them their excellent functional qualities. However, peptides are not capable of making faithful replicas of themselves and, hence, are not suitable as information carriers.

It is unlikely that all of the components of life's organizing mechanism—RNAs, DNAs, and proteins (enzymes)—came into existence at once in the primordial soup. They must have evolved gradually.

from primitive and inefficient precursors that were barely distinguishable from accidentally formed molecules. Here it is tempting to ask the old chicken and egg question, as biologists have since the 1930s: "Which came first, the informational or the functional components?" Experimental evidence gathered by Manfred Eigen and his coworkers, the American biochemist Leslie E. Orgel, and others suggests that neither came first. The informational and functional components of the organizing mechanism originated and evolved together. The start of this evolution is presumably to be sought among molecules of RNA because, of all the components of the organizing mechanism in contemporary cells, they alone carry information and perform functions.

7.5.2 RNA Quasi-Species

How did RNA molecules become the first component of life's organizing mechanism? A crucial laboratory experiment for finding an answer was carried out by Leslie Orgel. He demonstrated that short strands of RNA, such as U—U—U— ...—U or C—C—C—...—C (poly-U and poly-C strands) are capable of making complementary copies of themselves in solutions containing activated nucleotides (that is, nucleotides with triphosphate chains). For example, poly-C strands form poly-G strands (recall that G is the complementary base of C), and they do so with a fairly high degree of fidelity, meaning that very few errors (the substitution of A, U, or C for G, in this example) are introduced. An important feature of this experiment is that the RNA strands have autocatalytic qualities and their replication proceeds without enzymes. This adds realism because, initially, enzymes would either not have been available in the primordial soup or, if present, would have been of poor and nonspecific quality. If zinc ions are added to the solution, much longer strands (up to 40 nucleotides in length) are copied and with greater fidelity than without those ions. The zinc ions act as inorganic catalysts, which, very probably, were available in the soup. Interestingly, today's RNA polymerases (enzymes that catalyze the formation of RNA) all contain zinc ions, which led Eigen to ask, "Has nature perhaps 'remembered' how replication started?"

Orgel's experiment demonstrated that strands of RNA are capable of selfreplication: They provide both the information and the function for making complementary copies of themselves. This result, as well as the dual roles RNA plays in contemporary cells, suggests that the first components in the evolution of life's organizing mechanism were indeed molecules of RNA or, at least, molecules similar to RNA.

We may imagine that life's organizing mechanism got its start when short strands of RNA—perhaps only two or three nucleotides long—were randomly assembled in the primordial soup. The original RNAs then began to replicate themselves by processes such as those in Orgel's experiment. In the absence of well-developed enzymes, errors were commonly made during replication, despite the RNAs' short lengths. Hence, the replicated copies differed frequently in minor and major ways from the original ones with regard to sequences of bases and lengths. *Variation*—the first requirement of Darwinian evolution—was thus introduced right from the beginning into the pool of RNAs in the primordial soup.

Along with the creation of variation among the RNAs, *natural selection*—the second requirement of Darwinian evolution—got its start as well. The different kinds of RNA were not all equally successful in replicating themselves, avoiding errors during replication, and surviving. For example, RNAs with an abundance of Cs and Gs probably

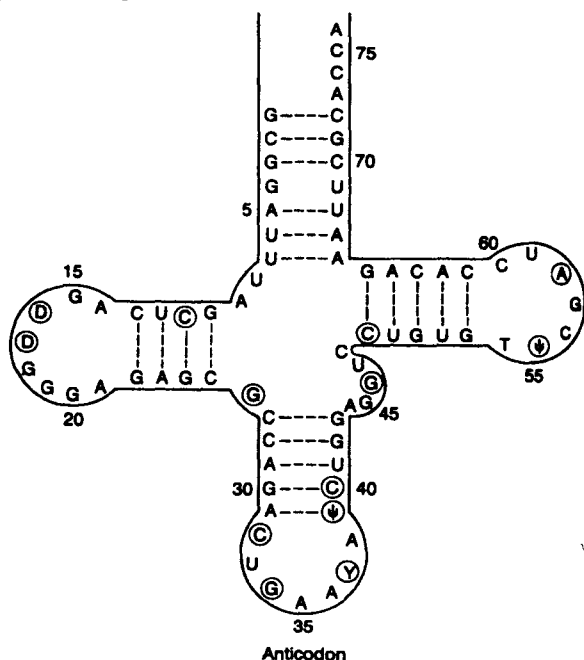


Figure 7.8 Two-dimensional representation of the nucleotide sequence (76 bases) of yeast tRNA (coded for phenylalanine). It illustrates the clover leaf shape that is typical of all tRNAs. The letters A, G, C, and U stand for the normal bases. The circled letters stand for unusual bases, all of which differ from the normal bases in only minor ways. Dashed lines indicate H-bonding between the bases.

tended to replicate themselves more faithfully than those with mostly Us and As; and RNAs with certain kinds of three-dimensional folding were more resistant to being broken up by water molecules and other chemically reactive compounds. It probably was also at this early stage that the particular bonding between nucleotides that we find universally in nature today won out over other, competing bonds.

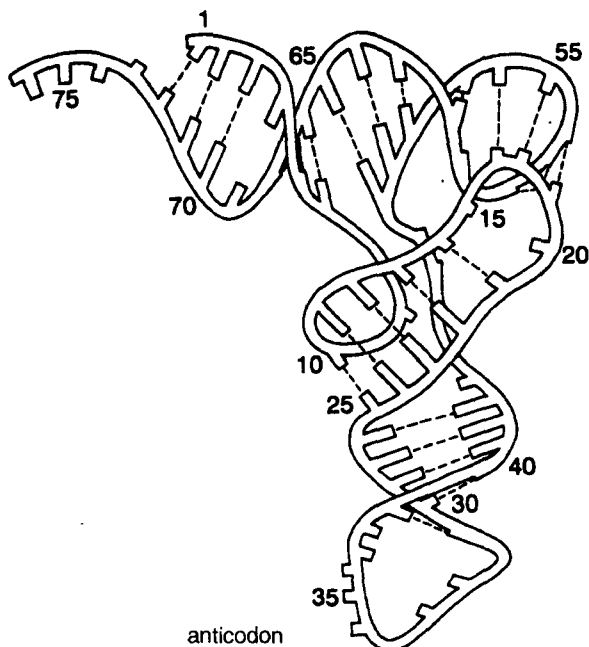


Figure 7.9 Model of the three-dimensional folding of yeast tRNA. The ribosephosphate backbone is drawn as a continuous structure; positions of the bases are shown as bars; and the H-bonds between the bases are indicated by dashed lines.

In the course of time, the most successful RNA strands accumulated and gave rise to so-called *quasi-species* of RNA with variations in characteristics among their member RNAs, analogous to the variations observed today among the members of species of organisms. The quasi-species of RNA differed from one environment to another; and, as the environments changed, the quasi-species either changed also and adapted or they disappeared. Thus Darwinian evolution, based on variation and natural selection, appeared in the primordial soup long before life as we know it today had come into existence.

Very likely, the chemical reactions by which the earliest strands of RNA replicated themselves were aided by inorganic catalysts such

as clays, lava, rocks, a sand, and ions of zinc and other elements. Accidentally produced protein structures such as Fox's proteinoid microspheres might have played a role as ' well.

In the absence of specific enzymes that catalyzed replication, Eigen estimates that the early RNAs could at most have been about 50 to 100 nucleotides long, which is comparable to the lengths of today's tRNAs. Had they been much longer, the number of errors introduced during replication would have been so great that within a few generations their particular base sequences would have become changed beyond the range of viability.

7.5.3 RNA Hypercycles

As long as the strands of RNA consisted of no more than 100 nucleotides, they could do little else than help in their own replication. They were not long enough to store information for the assembly of enzyme catalysts. However, enzymes were needed to allow the organizing mechanism to progress further. They were needed for the faithful replication of longer RNAs, and the longer RNAs were needed to store the information for the assembly of the enzymes. Furthermore, specialized functional RNAs were needed, analogous to today's tRNAs (and possibly rRNAs), to help in the translation of the information into enzyme structure.

According to theoretical studies by Eigen and his coworkers, the evolution toward longer strands of RNA and enzymes required the development of cooperative couplings between different RNA quasi-species. A simple example of such couplings involves three quasispecies. The RNAs of one of the quasi-species carry information for the assembly of enzymes (albeit, very primitive ones) and the RNAs of the other two quasi-species function as tRNAs. With the aid of the tRNAs, the information of the first quasi-species is translated into primitive enzymes. The enzymes, in turn, aid in the replication of the RNAs of all three quasi-species. Such cooperative couplings are called *hypercycles*.

In reality, hypercycles in the primordial soup probably were never as simple as the one just described. We may assume that many more quasi-species of RNAs and kinds of enzymes than those indicated in the example were required, even for inefficient hypercycles. Hypercycles probably evolved from single RNA quasispecies that consisted of large numbers of RNAs with the usual variations with regard to length and base sequence. At first the participating tRNAs, helping in the assembly of peptides according to information carried by the longer RNAs, were quite inefficient. Likewise, the peptide chains, which functioned as

enzymes, were quite inefficient also. They were probably not much more effective in this task than many of the accidentally produced peptide chains and other inorganic catalysts. In the course of time, however, the lengths of some of the participating RNAs increased, and their information content -coding for the assembly of enzymes -grew more distinct. Other RNAs remained short (50 to 100 nucleotides) and took on the role of today's tRNAs. The enzymes thus produced became more specific and efficient in their tasks. Slowly the original RNA quasi-species evolved into a number of distinct RNA quasi-species, with each quasi-species specializing in a particular task: to store information for the assembly of specific enzymes or to function as tRNAs. Thus the different RNA quasi-species, along with their enzymes, became firmly coupled and dependent on each other for survival. They evolved into hypercycles.

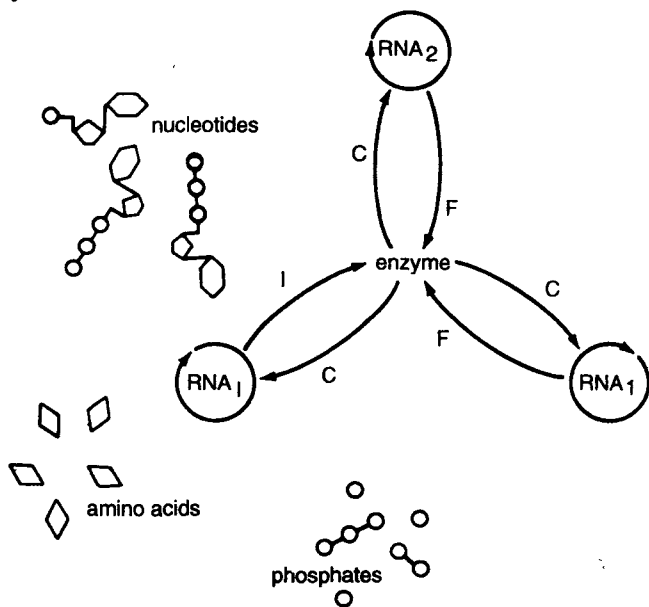


Figure 7.9 Diagrammatic representation of a hypercycle, consisting of three distinct RNA quasi-species (labeled RNA₁, RNA₁, and RNA₂) and enzymes.

The longest RNAs of hypercyclic quasi-species were conceivably up to several thousand nucleotide units in length, as suggested by the gene lengths of certain contemporary viruses. That was roughly a ten-to-fiftyfold increase over the lengths of the RNA strands of the earlier, independent RNA quasi-species. This increase was possible because the RNAs of the hypercycles were replicated with the aid of specific

enzymes, which greatly improved copying fidelity and efficiency. We may assume that at this stage of prebiotic evolution the modern triplet code for storing genetic information came into existence as well. The code was needed to translate accurately the information carried by RNAs into enzyme structure. However, at present it is not clear whether the triplet code started out that way or was preceded by singlet or doublet codes. Singlet or doublet codes would have made the initial stages of the development of the organizing mechanism easier, but it is difficult to see how the switch from a singlet or a doublet code to a triplet code could have come about, because such a switch would have invalidated much of the previously accumulated information. Nevertheless, some researchers believe that the first code was singlet or doublet and that somehow, at a later stage during the origin of life, it became converted into the triplet code that is in universal use today.

Prebiotic evolution progressed from independent RNA quasi-species to hypercycles not only because the latter produced their own enzymes. It also progressed in this direction because, through the enzymes, the participating RNA quasi-species became dependent on each other and were forced to cooperate for the common good. They could not afford to outcompete and to eliminate each other. To survive, *they* had to cooperate. This cooperation greatly increased their reproduction rate and made them the dominant quasispecies in the soup.

The advantages of hypercyclic cooperation may be illustrated by an example from economics. Let us imagine a primitive human society in which every family is self-sufficient and competes with other families for available raw materials. Each family grows its own food, makes its own tools, builds its own shelters, and defends itself against competitors. Such an economy may be compared with the earlier, noncooperating RNA quasi-species, which competed with each other for the available raw materials. A much more efficient and productive economy results when the families begin to join into cooperative units, with each family specializing in specific tasks. Some families are farmers and grow food. Others are blacksmiths, butchers, bakers, or carpenters and specialize in the making of tools, slaughtering, baking, and building. Such a cooperative economy will easily outproduce and outcompete the simpler one in which each family fends for itself. The same was true of the hypercycles of coupled and cooperating RNA quasi-species, in which the different kinds of participating RNAs had specialized informational and functional tasks and in which they cooperated for the common good.

Another example of hypercycles is our present-day ecosystem, with its many interdependent and cooperative linkages among animals, plants, and microorganisms. Without the support from microorganisms, animals and plants could not exist. Likewise, many microorganisms depend on plants and animals for their nutrients. Furthermore, all animals depend (directly or indirectly) on plants for food and oxygen. In analogy we may regard the RNA quasi-species that were linked into cooperating hypercycles in the primordial soup as our planet's first ecosystem.

7.5.4 Protocells

The arrival of hypercycles of coupled and cooperating RNA quasi-species constituted an important step forward in the development of life's organizing mechanism. However, there still remained a serious obstacle. There still existed no decisive feedback from the enzymes to the information content of the RNAs. Natural selection did not favor RNAs because they carried information for superior enzymes; it favored RNAs that were the most stable and reproduced most rapidly under the prevailing conditions, regardless of whether they also carried useful genetic information or not. Even RNAs that carried no information at all and performed no functional tasks benefited from the enzymes produced by other RNAs.

This feedback problem resulted from the fact that up to now all chemical reactions had taken place in the open environment of the primordial soup. As long as this remained the case, the obstacle could not be overcome. Only by enclosing the hypercycles with membranes and forming protocells did evolution progress further. Competition could then occur between the protocells, which forced natural selection to focus on the fitness of the protocells' entire enzymedriven biochemical machineries. Cells that survived possessed, by definition, the most advantageous enzymes, and (because the cells survived) their genes were passed on to the next generation. Thus, the feedback from enzyme to gene or from phenotype to genotype -that is characteristic of all contemporary life became established.

Besides solving the feedback problem, membrane enclosure had other advantages. It kept the enzymes produced by the hypercycles close to the RNAs and prevented their diffusion into the environment. It also created the possibility of concentrating biochemical raw materials-such as amino acids, nucleotides, and phosphates -by the development of selective transport mechanisms into the cells' interior.

At present no one knows just how the evolution from hypercycles to protocells came about. It was a step that probably required the

parallel evolution of numerous major and interconnected biochemical capabilities: the evolution of suitable membranes; selective transport of the right kinds of raw materials through the membranes into the cell interiors; elimination of waste products; extraction of energy from energy-rich molecules; replication of genetic information with high fidelity; and efficient translation of the genetic information into enzymes and other kinds of proteins.

Though we do not know how these capabilities arose, it is clear that to function reasonably efficiently protocells required many more enzymes than were produced by the earlier hypercycles. Furthermore, these enzymes needed to be constructed with an ever higher specificity for catalyzing particular chemical reactions. Eventually, the information required for assembling the enzymes became so great that it could not have been stored and passed on from generation to generation if strands of RNA had remained the only information carriers. Too many errors would have been made during the replication of RNA, despite the assistance by enzymes, and any one of the errors might have been fatal to the cells. The only way out was the development of an error suppression mechanism.

The error suppression mechanism that evolved depended on the introduction of a double-stranded information carrier. That information carrier was DNA. As noted before, the two strands of DNA are complementary-the base adenine (A) of one strand is always bonded to thymine (T) on the other strand, and cytosine (C) is always bonded to guanine (G). Hence, the two strands carry identical information. The presence of the same information twice permitted the development of a "proofreading" mechanism during replication. While a copy is made of one of the strands of DNA, it is checked by proofreading enzymes against the other, complementary strand. Any errors detected are corrected.

This *error suppression* mechanism operates in all contemporary cells. It must also have evolved, at least in primitive form, in the protocells. It allowed their DNAs to store genetic information that extended over hundreds of thousands to millions of nucleotides, which probably was enough for the protocells' genetic requirements. The DNAs were replicated sufficiently faithfully that errors - that is, mutations - did not, in general, accumulate to harmful levels. The occasional errors that did occur contributed to the genetic variations of the protocell species and, hence, to their evolution.

Very likely, species of protocells arose separately on numerous

occasions in the primordial soup. Nevertheless, the universality today of the structures of proteins and nucleic acids, the triplet code, the use of ATP, and the workings of the organizing mechanism suggest that competition and natural selection eliminated all but one of the early species of cells. The surviving species became the ancestors of all subsequent terrestrial life -from bacteria to protists, fungi, plants, and animals.

As discussed in the following chapter, there are good reasons to believe that the earliest cells were prokaryotes and that they reproduced by binary cell division, similar to the reproduction of contemporary bacteria. Furthermore, they probably derived their energy by fermenting energy-rich molecules, such as sugars, that they found in the soup.

No one knows how much time elapsed before chemical evolution made the transition to the first species of cells. It may have been a million years or less. Or it may have been many hundreds of millions of years. All we know, from the fossil record, is that by about 3.5 billion years ago single-celled life inhabited shallow areas of the sea. There are also indications that these cells were capable of photosynthesis and, hence, had already evolved considerably beyond the state of the earliest fermenters. For lack of more specific information, let us assume then, somewhat arbitrarily, that protocells arose approximately 4 billion years ago. This is a round figure that will suffice for discussions in the next chapter, even though it may be off by a few hundred million years one way or the other.

With the arrival of protocells in the primordial soup, the fundamental components of life as we know them today had come into existence, at least in rudimentary form: the organizing mechanism, consisting of DNAs, RNAs, and enzymes; the triplet code for writing genetic information; the means of selective absorption of raw materials and of expulsion of waste products across the cells' enclosing membranes; the extraction of energy from energy-rich molecules; and the capacity for cell reproduction and passing genetic information on to the next generation.

The driving mechanism in the development of the protocells was Darwinian evolution. According to the theory presented here, this evolution began with the self-replication of short strands of RNA among the otherwise random chemical reactions in the primordial soup and progressed step-by-step to RNA quasi-species, hypercycles, and membrane enclosure of some of the hypercycles. Remember that the theory is based on many assumptions and contains many gaps. Some of

the assumptions may turn out to be wrong. In fact, researchers in a number of laboratories today are developing and testing alternative theories, some of which rely on RNA and RNA like molecules as the first replicator (see Trachtman 1984) and other that don't (see Cairns-Smith 1985 and Dyson 1985). Clearly, investigation of the origin of life is at present characterized by great uncertainties and enormous intellectual challenges. Nevertheless it is an exceptionally exciting field of scientific research, dealing as it does with one of nature's most fundamental secrets.

8

Commercial Microbes

Today microorganisms and their enzymes are the basis of industries grossing billions of dollars annually. Industrial processes based on microbial action are of several general types. Microorganisms may be cultivated:

- A. In food products (e.g., fermented vegetable products and dairy products) for the purpose of *producing certain flavours, consistencies and nutritive values* in the products;
- B. In media where they decompose (ferment) various substrates (usually carbohydrates), *the products of the fermentation* (various alcohols, organic solvents, lactic, acetic and citric acids) being recovered, purified and sold;
- C. In flavoured nutritive solution, notably fruit juices and extracts of grains that are fermented, the entire culture fluid (after clarification and processing) then becoming *beverages such as beer and wine*;
- D. In contact with a specific substance such as a sex hormone (the chemical group of *steroids*) so that a single, specific enzyme of the microorganisms brings about a *specific transformation of the substrate molecule* into a molecule of another desired substance;
- E. In media so that the *enzymes* or other substances that the organism *synthesize* (amylase, protease, antibiotic) may be collected, purified and sold for commercial or medical use.

In addition to these uses, *organisms themselves* (principally yeasts, some algae) are sometimes cultivated *for use as food*. Such food products are generally used as feed for poultry and livestock. This is not as yet a large industry in the United States, where other foods are plentiful.

However, in view of enormously increasing population, the possibilities are being seriously considered and studied experimentally. As mentioned previously cultivation of certain eucaryotic algae in waste materials as a source of oxygen and food in space and prolonged submarine travel is also under experimentation.

Microorganisms are sometimes used for *special industrial purposes* such as removal of certain sulfur compounds from petroleum, and for vitamin assay. These have highly specialized applications

The *uncontrolled actions* of fortuitous *mixtures of microorganisms* in processes such as retting of flax, preparing hides for leather, and coffee-bean hulling are time-honoured practices. They are now being replaced in more advanced industries by processes in which purified cultures or enzymes of the *effective* species in the mixtures are used under carefully controlled conditions. Since the older processes are not scientifically designed they are not discussed further here.

8.1 DEVELOPING AN INDUSTRIAL PROCESS

In developing an industrial process based upon the action of microorganisms many details must be given consideration. Important among these are the type of culture needed, cultural conditions and the adaptability of the organism to large-scale production.

8.1.1 Purity and Mature of Cultures

It must be ascertained whether absolutely pure cultures must be used, or whether the mere predominance of one organism is sufficient. This may be a deciding factor, as the cost of preparing and maintaining pure cultures and sterile apparatus throughout a process is relatively high.

8.1.2 Cultural Conditions

The organism must be able to grow well in the medium to be used and under the conditions of the process. This necessitates exact studies and careful control of *optimum* conditions of aerobiosis or anaerobiosis, temperature, nutrition and pH. Appropriate adjustments of the process and apparatus must be made to provide the necessary conditions.

8.1.3 Productive Mutants

The organisms selected must be such as will produce the desired substance(s) or results in the medium under the conditions furnished, in amounts sufficient to yield a profit. Some firms have "pet" strains

of microorganisms that excel in producing certain products, such as butyl alcohol, certain antibiotics or itaconic acid, that they have "developed" (selected mutants) for these purpose. It has been found possible to induce industrially valuable mutations in microorganisms by ultraviolet radiations. Where sexual processes are known to occur, the breeding of yeasts and molds for similar purposes is analogous to breeding of farm animals for special purposes.

8.1.4 Medium or Raw Material

The substrate or medium should support luxuriant growth of the organism to be used and it must be available constantly at costs compatible with profit. Expensive handling machinery may be needed for some substrates.

An important item is the possible necessity of a preliminary treatment such as liming of very acid yeast slops, distillery wastes, molasses and whey. Some substrates, such as sawdust or fiber, may need preliminary "digestion" with hot acid or alkali to hydrolyze them to fermentable substances. This all adds to the expense and time.

8.1.5 Nature of the Process

The more complicated and exacting the system of cultural details and preliminary heatings, dilutions and digestions, as well as the type of machinery (cracking stills, tanks, pumps) to handle the end- and by-products and the final wastes, the greater will be the cost and therefore the less the commercial practicability of any process. Any time-consuming aging or ripening process eats into profits. Sometimes, very desirable end or by-products may be found in commercial fermentations, yet the cost of their recovery may be prohibitive.

8.1.6 Preliminary Experimentation

The microbiologist working with 10-ml. test-tube cultures may find many valuable things. When attempts are made to reproduce the test-tube experiments on a 100,000-gallon factory scale, however, the laboratory discoveries often fail to yield the promised result. Any process developed in the experimental laboratory must next prove its worth in the factory. A small-sized model, or pilot plant, is usually tried after the preliminary laboratory work. All may depend on such a seemingly far-removed detail as international relations. These may affect the cost or importation of some raw product essential to the process under investigation. Then the industrialist turns to home resources, goes to Washington, or *employs a resourceful microbiologist!*

The whole matter is a complex of microbiology, chemistry,

engineering and economics. Many chemical and microbio-logical processes in use at present are patented and secret, and specific strains of bacteria, yeasts and molds, which are zealously guarded, are often carefully developed in the labor-atories of manufacturing concerns. As a result of continuous and intensive industrial research, methods change or are superseded frequently.

8.2 TYPES OF FERMENTATION PROCESSES

Industrial fermentation processes may be divided into two main types: (1) batch fermentation and (2) continuous process. There are various combinations and modifications of these.

8.2.1 Batch Fermentation

A tank or fermentor is filled with the prepared *mash* (material to be fermented, e.g., diluted molasses, comminuted potatoes, digested corn cobs). The proper adjustments of pH, temperature, nutritive supplements and so on are made. In a pure-culture process, the mash is steam-sterilized, the entire fermentation tank sometimes being the autoclave. The inoculum, a *pure culture*, is added from a separate pure-culture apparatus. The fermentation proceeds. Some pressure may to be maintained within the tank to prevent inward leakage or conamination and sometimes to maintain increased tension of special gasses. After the proper time, the contents of the fermentor are drawn off for further processing, the fermentor is cleaned, and the process begins over again. Each fermentation is a discontinuous process divided into *batches*.

8.2.2 The continuous-growth process

In continuous-growth processes, the substrate is fed into a container continuously at a fixed rate. The cells grow (or enzymes act) continuoulsy as the material passes through the apparatus. The organisms and process are said to be in a *steady state* or condition of *homeostasis*. The product or fully fermented mash is drawn of continuously. The engin-eering arrangements may be complex, permitting aeration, cooling or heating, adjustment of pH or addition of nutrients continuously during the process. There must also be means of controlling rate of growth, phase of growth curve, and removal of dead organisms. The culture must remain pure and must not undergo any variation.

One may conceive of such a process as taking place in a long pipe (actually it may be a rotating conical tank or series of connected

tanks). At one end the prepared mash enters. It at once encounters the growing organisms. These act on the substrate as it flows through the system. At the stage at which the valuable product of the fermentation is at its maximum concentration, the fluid is drawn into receiving vessels for further processing (e.g., distillation). The animal alimentary tract may be thought of as a natural, continuous-growth process.

8.2.3 Submerged Aerobic Cultures

Many industrial processes, casually called "fermen-tations," are carried on by strictly aerobic microorganisms: for example, production of penicillin by *Penicillium notatum*, a strictly aerobic mold. In older aerobic processes it was necessary to furnish large surfaces of culture media exposed to air. The limitations of space, difficulties from contamination, and expense of hand labour can well be imagined, though little expense for power equipment was necessary. Now it is common commercial practice to carry on such "fermentations" in closed tanks with *submerged cultures*. Aerobic conditions are maintained by constant agitation of the contents of the tank with an *impeller* and constant *aeration* by forcing sterilized air through a porous *diffuser*. The flow of air through the tank removes gases such as ammonia and carbon dioxide. In each sort of process very careful adjustments of O-R potentials, mechanical agitation, ratio of dissolved oxygen to other ingredients in the medium, and pH are mechanical agitation, ratio of dissolved oxygen to other ingredients in the medium, and pH are necessary. This is one of the many fascinating and potentially very lucrative fields for research in industrial microbiology.

8.3 INDUSTRIAL ETHYL ALCOHOL MANUFACTURE

Much industrial ethyl alcohol is now made from by-products of *cracking* petroleum to make gasoline. However, the manufacture of ethyl alcohol from fermentation by yeasts is still an important industry. It serves to illustrate industrial fermentation processes in general. Crude molasses is often used as mash. It generally requires only to be diluted and the pH adjusted (usually with sulfuric acid) to 4.5. This pH is favourable to the yeast and unfavourable to many bacteria. A source of nitrogen such as ammonium sulfate or ammonium phosphate is usually added. The final solution is a richly nutrient carbohydrate culture medium or *mash*.

This is rather heavily inoculated with an aciduric and alcohol-resistant strain of yeast, the variety depending on the conditions under

which the fermentation is to proceed and the exact end products desired. A good strain of *Saccharomyces cerevisiae* is commonly used. The inoculum comes from a large tank of carefully maintained pure culture in the laboratory. At present stainless steel continuous-culture apparatus is available for maintaining constantly large amounts (many gallons) of pure cultures of inoculum.

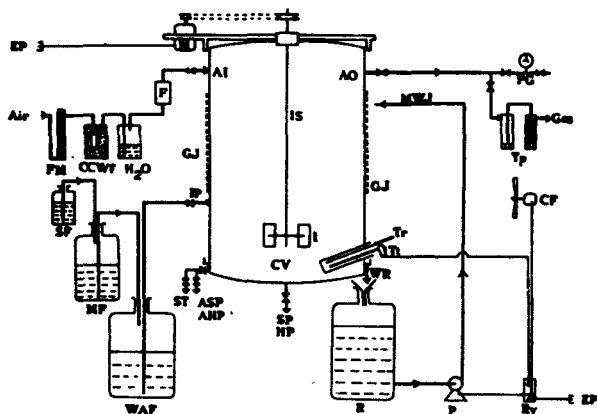


Figure 8.1 An example of an experimental laboratory pilot plant devised to obtain dense growths of *Brucella* sp. for immunization of cattle. The basic principles of submerged aerated growth are well illustrated, as in a typical pilot plant setup. At left of the main tank (CV) are apparatuses for admitting sterilized air, and medium seeded with pure culture. At right of the tank are devices for controlling temperature of the culture tank. The various parts are: AI, air inlet; AO, air outlet; AHP, alternate harvesting point; ASP, alternate sampling point; CCWF, part of air filter; CF, colling fan; CV, culture vessel; EP, electric plug; Of, Seitz filter; FM, flow meter; FJ, gauze jacket; HP, harvesting point; I, impeller; IS, impeller shaft; IP, medium inlet point; M, motor; MF, flow meter; GJ, gauze jacket; HP, harvesting point; I, impeller; IS, impeller shaft; IP, medium inlet point; M, motor; MF, medium flask; MWJ, multiple water jets; P, pump; PG, pressure gauge; R, reservoir; RY, relay; SF, seed flask; SP, sampling point; ST, steam inlet point; Tp, trap; Tr, thermometer; Tt, thermostat; WAF, water and antifoam flask; WR, water return.

The maintenance of purity of the inoculum is a responsibility of the microbiologist, and woe betide him if some sporeformer, *Lactobacillus*, wild yeast or bacteriophage gets in and ruins 100,000 gallons of mash! The mash and all of the machinery are generally sterilized before the inoculation and then cooled. The microbiologist is kept busy at every stage of the process, making cultural and microscopic examinations making cultural and microscopic examinations of the water, mash and apparatus to detect and eliminate contamination.

In the batch process, much used for this purpose, fermentation in enormous tanks is allowed to continue for about 48 hours at a carefully

controlled temperature of about 25°C. until the yeast stops growing because of the concentration of alcohol and other products. Aeration with filtered air is used at first to promote rapid growth, but anaerobiosis is soon established to promote fermentation and alcohol accumulation, and prevent its oxidation to carbon dioxide and water.

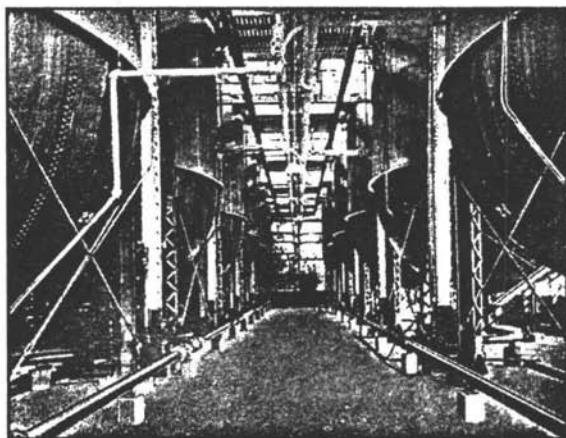


Figure 8.2 Lower level of 50,000 gallon fermentation tanks.

The fermented mash contains the crude alcohol or *high wine*, as it is called. This is usually a mixture of ethyl alcohol and a small amount of glycerol with *fusel oil*. The last contains amyl, isoamyl, propyl, butyl and other alcohols with acetic, butyric and other acids, as well as various esters. The high wine is driven off from the mash or *beer* by heat, and further purified by fractional distillation, which is a problem in chemical engineering.

The chemical reactions involved in the fermentation are complex; the principal stages follow the Embden-Meyerhof scheme. The overall reaction in the production of alcohol from glucose is:



The chief constituents of fusel oil are probably derived from the action of the yeasts on amino acids in the mash. The large amounts of carbon dioxide evolved are purified and compressed in tanks or made into solid carbon dioxide. Part of this may be used for cooling the fermentation vats.

8.4 ALCOHOLIC BEVERAGE INDUSTRIES

8.4.1 Whiskey

In principle the production of alcoholic distilled beverages in

similar to the production of industrial ethyl alcohol. Refinements are introduced in beverage production with respect to flavour, aroma, colour and sanitation that are not necessary in the making of industrial alcohol.

There are four general types of distilled liquor: brandy, from fermented fruit juices; rum, from fermented molasses; whiskey, from fermented mash made with single types of grains; neutral spirits, from fermented mash of mixed grains. In making whiskey and neutral spirits the grain, mixed with water, is autoclaved, colled, diluted, and 1 per cent barley *malt* (aqueous extract of sprouted barely) is added to hydrolyze the starchy and proteins of the grain. The "mashing," or hydrolysis, proceeds in a special tank at about 65°C. for about 30 minutes. The mash is then pumped to the fermentation tanks. Here, as in beer-making, it is heavily inoculated with a starter of selected yeast, which has been cultivated in a mash previously made somewhat acid (pH 4.0) with lactobacilli. Fermentation is complete in about 72 hours, as in industrial alcohol production. The mash is then removed to the distillery and the ethanol, with various by-products, is recovered.

8.4.2 Beer

This time-honoured and popular beverage is one of the class of malt liquor: stout, porter, ale and others. In preparing beer, grain, usually barely, is kept moist for two or three days to induce sprouting, or *malting*. Amylase enzymes that are released in the malt grains during the sprouting process hydrolyze the starches of the grains to simple sugars, mainly maltose and dextrins. Malting (Ger. *malz* = to soften) is necessary since brewer's yeast does not produce amylase and therefore cannot directly attack the starch of the grain. At the same time proteases in the malt grains convert proteins in the grains and flour to soluble nitrogenous foods.

The sprouts are removed mechanically and the *malt grains* are dried. They are later crushed and soaked, or *mashed*, in warm water. The aqueous extract of these malt grains and flour, prepared at just the time when there are maximum amounts of maltose, dextrins and protein derivatives, constitutes a rich nutrient medium. It is called *beer wort*. The beer wort is now drained off and heated to kill contaminating microorganisms. Hops are added for additional antibacterial (stabilizing) effect, colour, flavour and aroma.

After cooling, a large inoculum of pure culture of *Sacch-aromyces*

cerevisiae (brewer's yeast or "*barm*") is added to the wort. This is called "*pitching*." Rival brewers maintain very special strains of yeast for the process. The inoculated wort is aerated at first to stimulate *rapid growth* of yeast; anaerobic conditions prevail later on to favour *fermentation*, when carbon dioxide and 3 to 6 per cent ethanol are produced. After fermentation is complete, the beer is clarified ("chillproofed"), and pasteurized and otherwise processed and aged (lagered). Unless scrupulous care is taken, many contaminants (*Pediococcus*, *Lactobacillus*) will grow vigorously in beer wort, producing buttery flavour, turbidity and "off" flavours. It was in the study of such spoilages, or "diseases," of beer and wines that Pasteur first became famous and developed *pasteurization* to prevent them. He was one of the first industrial microbiologists.

8.4.3 Wine

The term wine is broadly used to include any properly fermented juice of ripe fruits, or extracts of certain vegetable products such as dandelions and palm shoots. The juices or extracts contain glucose and fructose in concentrations of from 12 to 30 per cent. Fermentation of these sugars by various species of yeasts produces carbon dioxide and ethanol up to concentrations of 7 to 15 per cent, the alcoholic content depending on the kind of juice and yeast involved and the conditions of fermentation. In Europe the fermentation is produced mainly by wild yeast, i.e., those brought to the fruits (largely by insects) from soil or other fruits. Yeasts similar to the species called *Saccharomyces ellipsoideus* are common in such wines.

Although other organisms are usually present, the yeasts soon predominate in the fermenting juice under suitable conditions. Tartaric, malic and other acids, as well as tannin and other substances, including added sulfur dioxide in commercial wine, tend to inhibit growth of many undesirable organisms in the juices.

Even though practices may differ in different wineries, basically they are similar. Commonly in modern American commercial practice, sterilized fruit juices are inoculated with a pure culture of a desirable species of yeast. The preparation and maintenance of the yeast inocula are the special tasks of the microbiologist.

The inoculated juice is, as in beer-making, at first aerated to promote active and pre-emptive growth of yeast. Were this to continue, only carbon dioxide and water and massive growth of yeast cells would

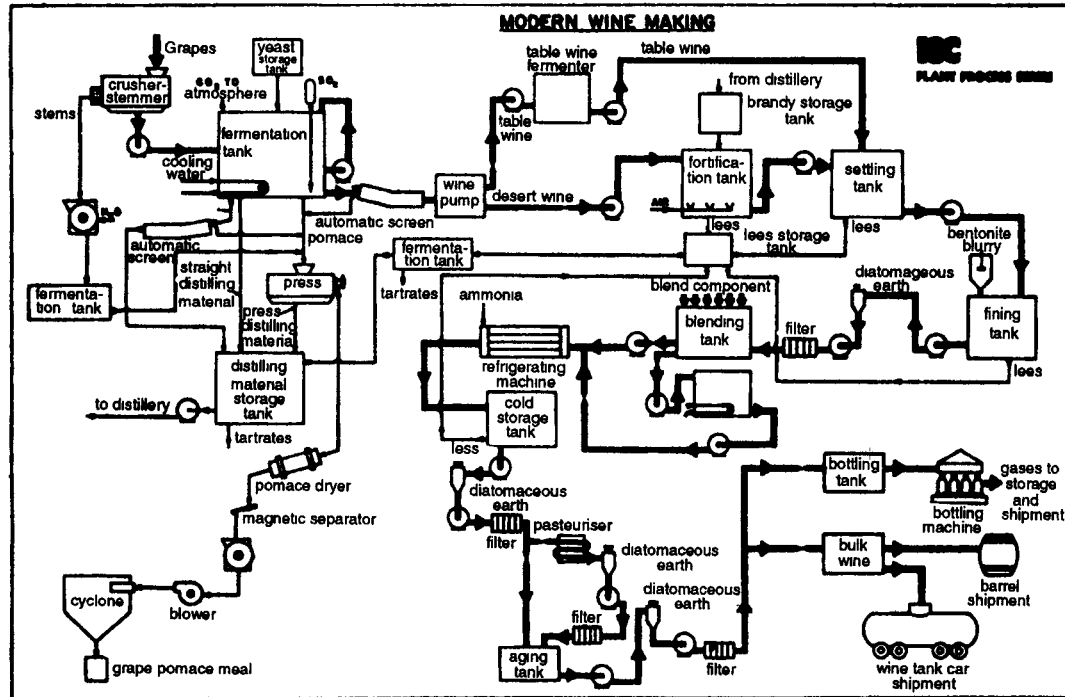


Figure 8.3 Modern winemaking. At upper left is shown the beginning of the process, with crushing and stemming of the grapes. The juice passes to a fermentation tank, where SO_2 and yeast are added. (White wines are made with juice only; the juice with skins and seeds is made into red wines.)

result. As soon as a good growth of yeast has occurred, the aeration is stopped and the fermentation proceeds anaerobically, so that ethanol, in concentrations of from 7 to 15 per cent (vol.) is produced. The new wine is placed in large casks to settle, clarify and age.

Spoilage by alcohol-oxidizing species of *Acetobacter*, molds and other *aerobic* microorganisms may occur if conditions are not anaerobic and the reaction not sufficiently acid.

8.5 PRODUCTION OF BUTANOL

The production of butanol is outlined as an example of an industrial fermentation based on a species of bacterium.

As is true of industrial ethanol production, much butanol is now derived as a by-product of petroleum "cracking." However, the biological process is still used to some extent and illustrates important microbiological principles. There are numerous species of *Clostridium* that ferment carbohydrates with the production of butyl alcohol and other materials of value in drugs, paints, synthetic rubber, explosives and plastics. Some species produce isopropyl alcohol and acetone as well. Important among these organisms are *Cl. acetobutylicum* and *Cl. felsineum*. The name of another species suggests its potentialities as an industrial agent: *Clostridium amylosaccharo butylpropylicum*!

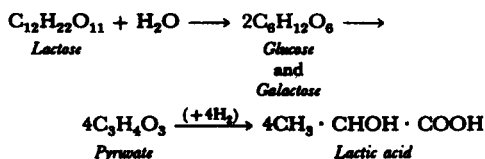
The successive reactions in the production of butanol and various side-products from glucose are as follows:

Many wastes are rich in fermentable carbohydrates, e.g., cannery refuse. Complete sterilization of all apparatus is essential. Conditions cannot be kept as acid (and antibacterial) as they are in yeast fermentations because *Clostridium* has its optimum pH near 7.2. Particularly troublesome contaminants are species of *Lactobacillus*. An organism called *B. volutans*, a gram-positive, nonsporeforming rod (possibly a species of *Lactobacillus*?), is also especially dangerous.

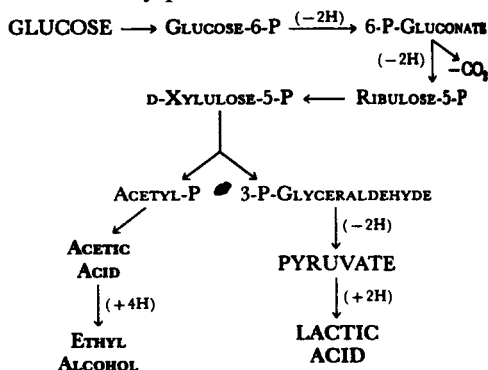
Fermentation proceeds anaerobically for about three days. Normally butyl alcohol, acetone and ethyl alcohol, with carbon dioxide and hydrogen in large amounts, predominate when *Cl. acetobutylicum* acts in a mash rich in glucose. Other substances may occur in smaller amounts. Riboflavin (a vitamin of the B complex) is a valuable constituent of the residue after distillation of the fermented mash. Butyl and isopropyl (rubbing) alcohols are important among the volatile fermentation products of a related species, *Cl. butylicum*.

Lactic acid is commonly produced from lactose or glucose by species of homo fermentative lactic acid bacteria, notably *Streptococcus*

lactis or *Lactobacillus delbrueckii*:

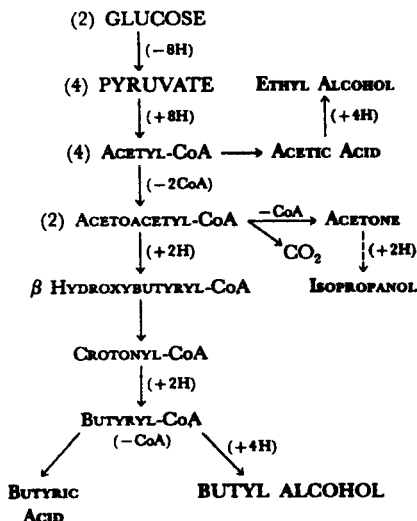


Heterofermentative species of lactobacilli like *L. buchneri* produce lactic acid and several by-products:



8.6 PRODUCTION OF VINEGAR

Acetic acid is almost entirely responsible for the sour taste of vinegar. Indeed, a slightly sweetened, three per cent, aqueous solution



of acetic acid makes a reasonable substitute for vinegar. The occurrence of vinegar in fermented fruit juices was known to the ancients, although they had knowledge of its cause. The bacteria involved were called *Mycoderma aceti* in 1862 by Pasteur.

The acid of natural vinegar is derived from alcohol by the oxidative action of bacteria of the Family Pseudomonadaceae (Genus *Acetobacter*). Pleasant flavours of natural vinegar are given by traces of various esters like ethyl acetate, and by alcohol, sugars, glycerin and volatile oils produced in small amounts by microbial action. Flavours are also derived from the fermented fruit juice, malt, or other alcoholic liquor (wine, beer, hard cider) from which the vinegar was made.

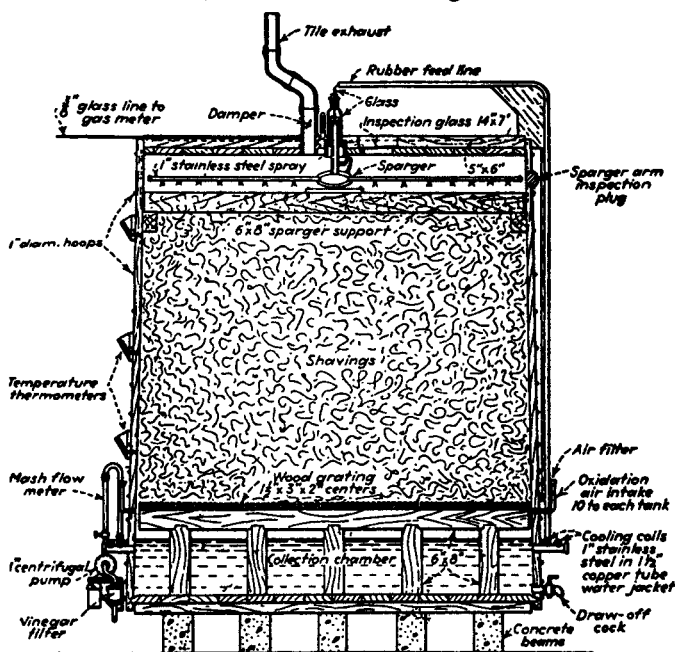


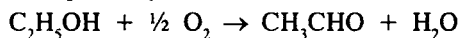
Figure 8.4 Cross section of the Frings vinegar generator. The alcoholic liquor is sprayed over the shavings by the rotating stainless steel spray (sparger) near the top. Note the thermometers, cooling coils and air intakes.

In commercial vinegar-making by biological methods, preliminary fermentation of fruit carried out by means of *Saccharomyces cerevisiae* (brewers' yeast). The *Acetobacter* then utilize the alcohol as a source of energy, oxidizing it to acetic acid in the presence of air. They utilize other substances in the fermented liquor as foods. The alcoholic liquor trickles over the surface of aerated shavings, coke, gravel or

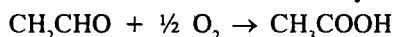
other finely divided material inoculated with *Acetobacter*. Such an arrangement is called a *two-phase continuous process*; one phase is the down tricking alcoholic liquor, the other phase is the column of coke or other material covered with growth of *Acetobacter*.

8.6.1 Genus *Acetobacter*

These are nonspore forming, polar or peritrichous flagellate, gram negative rods about 0.5 by 8.0 μ , although species vary in size. Branching involution forms and large swollen cells frequently occur, especially in *mother-of-vinegar*, the gummy or slimy growth phase of the organisms sometimes seen in natural vinegar or sour cider. Various species are found in souring fruits and vegetables. A species of historical interest is *Acetobacter* (*Mycoderma*) *aceti*, originally used by Pasteur to demonstrate the biological nature of vinegar formation. In practice, several species of *Acetobacter* usually act jointly. The alcoholic and acidic nature of the process suppresses most contaminants. The overall reactions probably are as follows:



Alcohol Acetaldehyde



Acetic acid

In a generator, the rapid oxidation of alcohol by the organisms produces so much heat that careful control of the internal temperature by cooling coils is necessary.

8.7 FOODS FROM WASTES

In the paper-pulp industry, wood chips are cooked for 6 to 18 hours at 60°C. in solutions of calcium bisulfite with free sulfur dioxide. The waste *sulfite liquor*, after the cooking process and removal of the wood fibers for paper, contains much available wood sugar (largely *xylose*) and other extrac-tives. These form a good nutrient for asporogenous yeast or *Torula*.

The nutrients in such a medium may be turned into masses of yeast by adjustment of pH to about 5.0, removal of SO_2 , aeration, addition of nitrogen and phosphorus as $(\text{NH}_4)_2\text{HPO}_4$ and NH_4OH , and inoculation with *Torulopsis utilis*. *Aerobic* growth is induced in aerated vats so that alcohol is not produced. The separation, drying and pressing of the resulting yeast growth are mechanical details. Yields of up to 50 per cent of the total reducing sugar consumed, in terms of dry torula, are obtainable. (At present, attempts are being made to cultivate yeasts on petroleum wastes.) The yeast cells are rich in proteins, fats and vitamins.

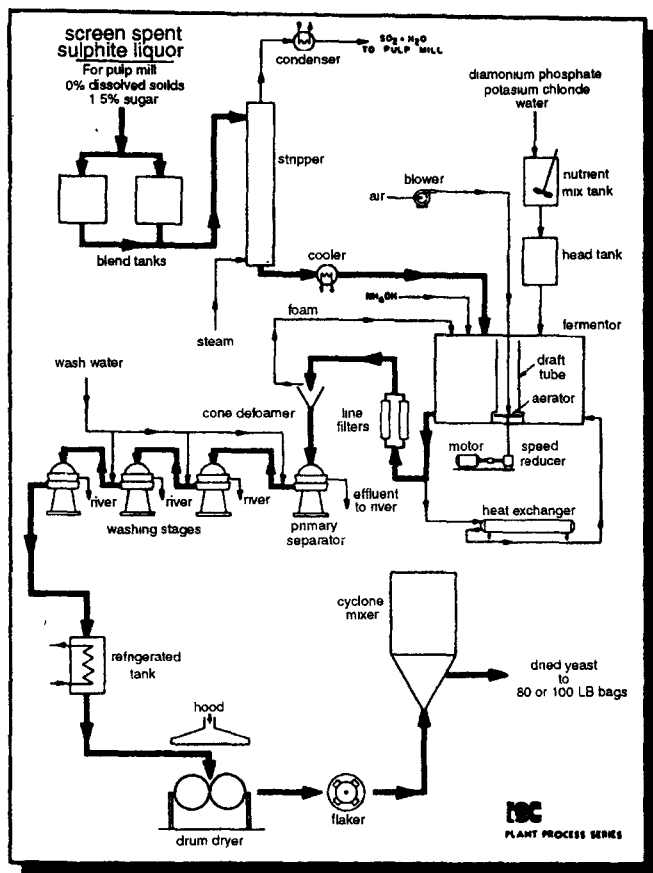


Figure 8.5 Paper-mill waste to stock-feed. the blended pulp-mill liquors are heated in a "stripper" to drive off SO_2 and H_2O , cooled and piped to the fermentation tank. Nitrogen and phosphorus are added as ammonium phosphate and as NH_4OH . The yeast culture in the fermentor is aerated to promote maximum growth. The yeast culture is then filtered and passed through a series of centrifuges and other washing and concentrating devices. As "cream" it is finally cooled, dried and packed for shipment.

These are fed to stock or poultry and thus turned into meat and dairy products. Surely the transformation of a knotty old pine slab into a succulent pork chop or a fried egg is modern magic!

8.7.1 Amino Acid Production

Not only may yeasts serve as foods themselves, but some species, especially *Saccharomyces cerevisiae* and *Torula utilis*, while growing can synthesize large amounts of various amino acid, e.g., L-lysine. This is an expensive amino acid widely used to "fortify" many familiar

foodstuffs. It is requisite that the growth medium for the yeast contain L-adipic acid or its derivatives. The yeasts use the adipic acid derivatives as *precursors* (i.e., as the molecular raw material) for their synthesis of L-lysine.

8.7.2 Hydrocarbons for Protein

Various hydrocarbon (petroleum) wastes are metabolizable by certain yeasts, eucaryotic fungi and also by some bacteria, e.g., *Bacillus* spp., especially thermophilic species. One difficulty (or possibly a source of profit?) is the production of large amounts of heat by bio-oxidation of hydrocarbons. The proteins produced in the process are of high nutritive value and the method is potentially profitable.

8.8 STEROID TRANSFORMATIONS

In ancient legend sorcerers, by means of their wands, changed beautiful princesses into graceful swans. Today's wizard changes one substance into another, but the sorcerer, is now the microbiologist and the sorcerer's wand is replaced by much more potent microorganisms, e.g., *Penicillium*; *Rhizopus*, *Streptomyces*. An outstanding example of the use of microorganisms to change one substance into another is the transformation of the steroids. Steroids are physiologically active compounds of complex structure (hormones) and are represented by cholesterol, ergosterol or vitamin D, sex hormones such as testosterone and progesterone, and the adrenal steroids such as corticosterone and its derivative, cortisone. Microbial transformations of these compounds differ basically from industrial fermentations previously described. In industrial fermentation the alcohol or other product results from the action of numerous enzyme systems in the overall metabolism of a substrate, such as the sugar in molasses. The same product might be made with any of several different microorganisms that ferment saccharose or glucose. In steroid transformation one particular form of molecule is changed into another by the action of a single, specific enzyme. The requisite enzyme may be present in only a single species of microorganisms. Many steroid derivatives thus obtained are of immense value in the treatment of various disease conditions or in the development of other hormones. On a commercial scale, many are at present available only through the action of certain specific microorganisms.

A single example will illustrate the type and importance of these transformations. Corticosterone, an important hormone from the cortex of the adrenal gland of mammals, was originally obtainable only from animals. It had a wide use in the treatment of shock and other prostrating conditions. A still more valuable derivative was made by chemical, and

later by microbiological, transformation of the corticosterone molecule. One of these alterations was the introduction by microbial action of an -OH group into the 11 position in the corticosterone molecule. The resulting compound is the now familiar cortisone, widely used in treating arthritis and many other inflammatory conditions.

The sex hormones, testosterone, estradiol and progesterone, are closely related in molecular structure to corticosterone and cortisone. They differ only in the nature and location of attached side groups. especially -OH and -CO. CH₂OH. These groups may be added or withdrawn or shifted about on a practical scale only through the action of certain specific microorganisms. Some of these transformations are indicated. The resulting compounds are often of much greater value than the natural hormone or steroid from which they are derived.

Similar transformations can be brought about in other kinds of molecules, e.g., various alkaloids.

8.9 ENZYMES OF MICROORGANISMS IN INDUSTRY

Knowledge that many of the essential chemical changes that occur in microbiological processes are entirely enzymic led to attempts to separate and concentrate the purified enzymes themselves on a commercial scale. The production of microbial enzymes for industrial use is a considerable industry in itself. The enzymes are derived mainly from molds, yeasts and bacteria already familiar to us. A few of the more widely used organisms, their enzymes and their uses, are listed.

8.9.1 Mold-bran Process

For obtaining enzymes from *Aspergillus* and *Penicillium*, the *mold-bran* process is often used. To provide an extensive aerated surface, flaky or fibrous material, commonly wheat bran, is moistened with a nutrient medium of composition and pH appropriate to the mold being cultivated and the enzyme desired. The nutrient bran is sterilized, spread out in shallow trays and inoculated with the mold conidia. The trays are incubated in carefully air-conditioned cabinets. After sufficient growth the moldy bran is thoroughly extracted with water or other solvent to remove the enzyme. This fluid may be filtered, centrifuged, concentrated and the enzyme precipitated and dried for sale.

For bacterial enzymes the desired species of *Bacillus* is generally cultivated on the surface of broad, shallow layers of liquid medium. This is often prepared from inexpensive cannery or dairy refuse (e.g., whey) rich in organic matter. After incubation the bacteria are removed

by filtration or centrifugation, and the enzyme is extracted and processed as indicated above.

The submerged culture process may be used in enzyme production by molds or bacteria, in much the same manner as in antibiotic production.

8.9.2 Gibberellin (Gibberellic Acid)

This sensationally effective plant-growth stimulant, now available in every garden-supply house, was discovered by Kurosawa, in Japan in 1926. Analogous to penicillin, it is a waste product of a mold, *Fusarium moniliforme*, or *Gibberella fujikuroi*, and is a mixture of gibberellins. In nature the mold grows in young rice plants and causes the "overgrowth disease," *bakanae*. A pest in rice paddies, the mold and its *gibberellin* are welcomed by agriculturalists and gardeners. Gibberellin is produced on a commercial scale by submerged aerated growth in media and by methods similar to those used in producing penicillin.

8.10 MICROBIOLOGICAL ASSAY

Microbiological assay is a highly specialized application of the fact that certain organisms lack certain specific synthetic powers, i.e., are auxotrophs. *Lactobacillus plantarum*, for example, is unable to synthesize nicotinic acid ("niacin"). We may furnish the organism with a medium that is complete and satisfactory in all other respects but if niacin is lacking, absolutely no growth occurs. (Humans are no better off; with out niacin they die of pellagra.) If a minute amount (say, 0.01 microgram) per milliliter of niacin is added to the medium for *L. plantarum*, some growth will occur. More growth will occur in the presence of more of the missing factor. Up to the point of satiation or acidification, growth bears a linear relationship to the amount of the specific growth factor added.

For example, to assay the nicotinic acid content of fresh green beans, we prepare a medium for *L. plantarum* that is complete in all respects except niacin. This we omit. We now prepare two series (A and B) of 10 sterile tubes each. Each tube receives 10 ml. of the niacin deficient medium. To each tube in series A we add known and graded amounts of pure niacin. To each tube in series B we add graded amounts of bean extract, niacin content unknown. All tubes are now inoculated with carefully washed (niacin-free!) cells of *Lactobacillus plantarum*. Accurate, photometric measurements are then made of the growths (turbidities) obtained in the cultures. If the medium contains

glucose, titrations of acidity instead of turbidity may be used as a measure of growth. By comparing growths in series A and B it is possible to estimate closely the concentration of niacin in the green beans. This method of estimation of growth factors is spoken of as *microbiological assay*.

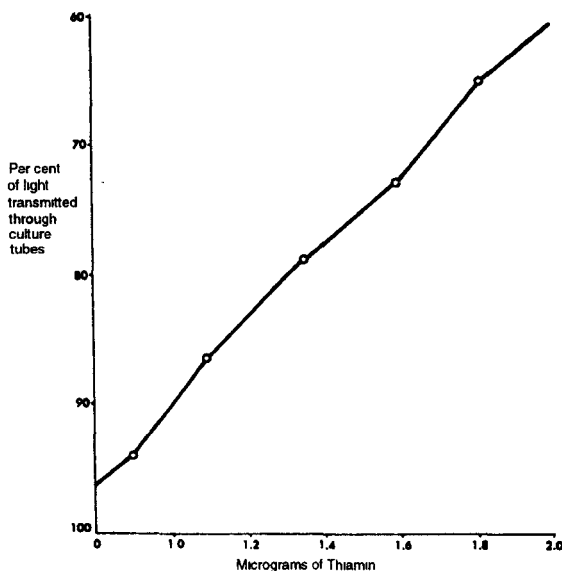


Figure 8.6 Representative curve obtained with a series of "standard" tubes for microbiological assay of thiamin with *Lactobacillus casei*. A similar form of curve would be obtained in any assay by this method, such as that described for niacin (*L. plantarum*) in the text. Slight deviations from the theoretical straight line are due to slight technical errors. Growth was measured photoelectrically: increasing growth (turbidity) reduced the amount of light transmitted through the culture tubes.

Although the basic principle of all microbiological assays is the same, there are other methods of measuring the growth (or other physiological) response. These affect the cultural methods used. A commonly used procedure is the measurement of carbon dioxide produced by fermentation of sugar in the test medium. Yeast is routinely used in the microbiological assay of *thiamine* (vitamin B₆) assays, the mold *Neurospora* is the test organism. After sufficient incubation the culture is steamed and the entire mycelium of *Neurospora* is removed from the culture medium, dried and weighed. Dry weight is directly proportional to concentration of pyridoxine in the sample of material being assayed. Another assay procedure depends on the spheroplast-producing power of the assayed substance.

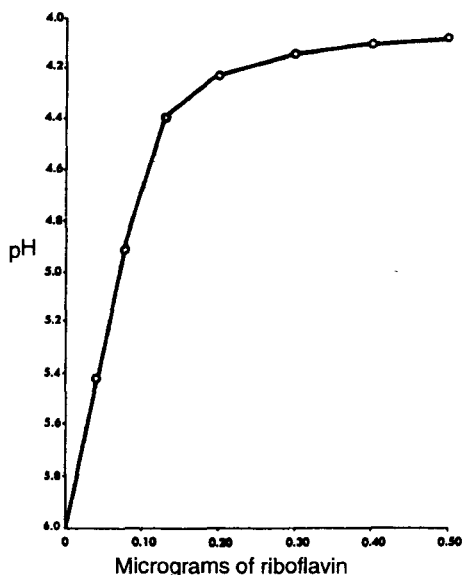


Figure 8.7 Representative curve obtained in the assay of a food substance for riboflavin content by measurement of pH. This is the "standard" curve used as the basis for measurement of the "unknowns." Note the linear relationship between growth of the test organism (*Lactobacillus arabinosus*) as measured by pH, and amount of riboflavin in the first part of the curve. In the later parts of the curve acidity inhibits unlimited response of the organism to the larger amounts of riboflavin.

Certain organisms lend themselves very well to such assay procedures. *Lactobacillus casei* and *L. arabinosus* are easy to cultivate, relatively hardy, harmless and wholly dependent upon several growth-factors including various amino acids, riboflavin, biotin, pantothenic acid and nicotinic acid. Other organisms may be used for assay of other organisms may because for assay or other substances, for example, *Streptococcus lactis* for folic acid. Ultraviolet induced, synthetically deficient auxotrophs of molds, yeasts and bacteria are extremely valuable in assay work.

Even though the basic principle of microbiological assay is easily understood, the technological details are often exceedingly complex and filled with pitfalls. Many obscure factors affect the test organisms, and they may also undergo mutation and other injuries may be held to a minimum by storage of the stock cultures in containers with liquid nitrogen at -196°C . (-321°F .). Temperature, pH and presence or absence of air may be of critical importance. For example, under *aerobic* conditions, *Lactobacillus lactis* will die before it will grow

without vitamin B₁₂! *Anaerobically*, it sneers at vitamin B₁₂! There are many other examples. We may smile, but knowledge of this and many other peculiarities is essential to successful assay procedures.

8.11 INDUSTRIAL SPOILAGE

In contrast with the useful activities of bacteria, a word may be said of their destructive action. Several causes of industrial spoilage (e.g., "diseases" of fermentations) have been mentioned in this chapter. Species of *Micrococcus*, *Alkaligenes*, *Flavobacterium*, *Serratia*, *Clostridium*, coliform organisms, yeasts and molds are common causes of spoilage.

Each type of product is attacked by certain species of microorganisms that can metabolize the substance especially well. For example, spoilage of cellulosic products such as lumber; telephone poles; paper; sisal, jute and flax fibers; tobacco and cotton is brought about by cellulose decomposers such as molds, various species of *Clostridium*, *Cellulomonas*, *Cytophaga* and many other such organisms of the soil. Fermentable substances such as syrups and beverages are attacked by yeasts, lactobacilli, organisms of the coli-aerogenes group and various environmental bacteria including the Genus *Clostridium*. Spoilage of proteins such as meats, fish, milk and so on results from the action of proteolytic species such as *Pseudomonas*, *Bacillus*, *Proteus*, *Micrococcus*, *Clostridium* and many others. Petroleum hydrocarbons are attacked by certain soil bacteria, as already mentioned, and rubber insulation of vital communication wires is attacked by bacteria and eucaryotic fungi.

Lactobacilli and *Leuconostoc* species have already been noted as particular villains in the acid-food, fermentation and distillery industries. Species of both can ruin fruit or vegetable juice or various industrial mashes (beer and wine) during processing. They produce a buttermilk flavour. Pasteur found *Lactobacillus* and *Leuconostoc* causing "diseases" of beers and wines, they are just as active today. Lactobacilli also discolouration (oxidized porphyrins) of cured hams and sausages.

The slimy dextran- or levan-forming species, such as *Leuconostoc mesenteroides* and *L. dextranicum* and some lactobacilli and micrococci, produce slimy and ropy conditions in a great variety of human endeavors: sugar refineries, pickle brines, dairy products, ham-curing cellars, and the like. These organisms prefer acidified products such as partly fermented foods, mashes and citrus juice. Examples of several of these types of spoilage have been given in discussions of the various products.

Development of undesirable flavours in fatty products such as butter, especially rancidity, is due in great part to the formation of butyric acid as a result of lipolysis. It is caused by species of *Aspergillus* and other molds, *Pseudomonas* species and streptococci related to *Str. liquefaciens*. These difficulties do not arise when clean equipment, clean milk and proper precautions to avoid contamination are used.

Proteolytic organisms such as *Str. liquefaciens* are responsible for undesirable bitter flavours and early spoilage of cheeses and other protein products. Gas production is usually caused by coli forms and *Clostridium*; putrefaction or digestion by *Clostridium*, *Pseudomonas* and *Bacillus*. Such conditions result mainly from dirty milk or other food equipment, or careless handling.

Included among other sabotage activities of bacteria are corrosion of the inside of structural aluminum alloys used in fuel tanks of jet-fuel aircraft. Pitting and scaling of the metal occurs beneath heavy, slimy growths of hydrocarbon-utilizing bacteria such as species of *Pseudomonas* and *Desulfovibrio*. Some species of molds are also involved. Bacteria of the Gen-era *Mycobacterium* and *Nocardia*, among others, are also implicated in the deterioration of bituminous products, including asphalt highways and asphalt coatings and pipe-linings. The microorganisms seem to utilize the high-viscosity hydro-carbons and resins in asphalt.

Prevention of spoilage

This, in each instance, is a problem that can be solved only by careful examination of the process involved to find: (a) the nature of the organisms(s) involved; (b) where the contamination is getting in, and (c) then devising means of excluding it. It is impossible to lay down a blanket rule for industrial spoilage in general. Everything depends on maintaining conditions unfavourable to, or excluding by asepsis, organisms that can grow on or in the particular product involved. This may involve complete steam sterilization of fermentation equipment (tanks, pipes, pumps); drying; refrigerations; aeration; the use of inhibitory salt, sugar or acid concentrations; radiation with ultraviolet light; exposure to sunlight; treatment with substances such as creosote, sodium benzoate and the like. In some processes specific antibiotics may be used, as penicillin and tetracyclines in alcoholic fermentation of molasses.

9

Decomposers

Perennial woody plants are the predominant vegetation on earth. Forests form the climax vegetation of all parts of the world except where temperature and moisture extremes limit plant growth. Forests also contain the greatest biomass varying from 500 Mg 10^4m^{-2} in tropical rain forests to 100-300 Mg 10^4m^{-2} in northern temperate coniferous forests. Perennial woody parts above ground make up about three-quarters of this biomass. Woody tissues thus provide the bulk source of organic carbon for decomposer heterotrophs. Fungi are the major group of organisms responsible for wood decay and a number of groups of fungi are solely wood-inhabitants. They exist entirely on the components of wood. In this chapter, a detailed consideration of these fungi will further illustrate the versatility of fungi as saprotrophs.

9.1 THE STRUCTURE AND COMPONENTS OF WOOD

Technically, wood is the xylem cylinder inside the bark of trees. In many trees it consists of an outer, light coloured sapwood and an inner, darker heartwood. The bulk of the wood consists of dead and empty lignified vessels and/or tracheids and fibres but it also contains xylem parenchyma. Much of the parenchyma in the sapwood remains alive and unlignified and acts as a food store, mainly for starch but soluble sugars, proteins, peptides, and amino acids, lipids, nucleic acids and vitamins, such as thiamine, are also present. Once the tree is dead, these afford substrates for a wide variety of fungi but are all relatively minor and ephemeral components.

Wood consists of three major components - 40-60% cellulose, 10-30% hemicelluloses and 15-30% lignin. Although the biological decomposition of lignin is of critical importance in the continuous cycling of carbon, its degradation is incompletely understood. This can

be attributed to many facts; not least of these are our lack of understanding of its precise chemical structure, the diversity of its structure in different woods, our inability to produce a pure form of lignin for cultural studies and a suitable assay for lignin degradation, the availability of a very potent lignin degrader, and the general cellular and chemical complexity of wood.

Lignin, in addition to making up about one-quarter of the dry mass of wood, is undoubtedly the structurally most complex of all the polymers and the most resistant of all to microbial decomposition. It is a three dimensionally branched aromatic polymer, formed by the oxidative polymerization of three different building blocks, not just one as in cellulose. The building blocks are the phenyl propanes: coumaryl, coniferyl and sinapyl alcohol. The lignin of different plants may contain different proportions of the three building blocks. Conifer lignin consists of mostly coniferyl alcohol, with small amounts of coumaryl alcohol and minor amounts of sinapyl alcohol. In angiosperm lignin there are approximately equal amounts of coniferyl and sinapyl alcohol and minor amounts of coumaryl alcohol. These phenyl propane units are built up into a branched polymer by covalent bonding involving three major linkage types. By far the commonest, making up 40-60% of the total, and most important linkage type is the arylglycerol-(3-aryl ether type. Phenylcoumaran structures form 10-20% of the linkage types and biphenyl structures another 10-25%. Thus in lignin there are three functional monomers, varying in proportions in the various lignins, and three major linkage types, but also other minor ones. There is no regular repeating unit as there is in starch or cellulose, *nor are there bonds which are easily hydrolysed. Because of this* structural complexity, decomposition must necessarily differ from that of most natural polymers where there is usually straight cleavage, often by hydrolysis, to produce the monomers. There is also the possibility of microbial enzymes bringing about a variety of limited changes to the intact molecule and only partially degrading it to substances which pass with little further change into humic materials. It appears that only the so-called whiterot fungi can completely decompose lignin to carbon dioxide and water. Lignin imparts rigidity and resistance to mechanical stress in woody plants and also resistance to microbial attack. Nevertheless it is degraded in natural environments but degradation is a very slow process.

9.2 WHITE, BROWN AND SOFT ROTS

As with other decomposing substrates, the form in which wood is presented to micro-organisms and the environment in which it occurs

have a major influence on the path that degradation takes. The degradation of wood in the form of trunks and large branches of trees above the soil may be very different from that of the woody tissues of leaves and small roots in the soil. This may be quite different again from logs submerged in the sea.

The fungi which cause the decay of large masses of wood, such as tree trunks above ground, have been most thoroughly investigated because such wood is the natural material utilized in greatest quantity by man and any fungi which attack it are of potential economic importance. Three types of wood decay have been recognized - white, brown and soft rots. In white rots, the wall polysaccharides, such as cellulose and hemicelluloses, are attacked more or less simultaneously with the lignin and the wood becomes markedly paler and fibrous as the pigmented amorphous lignin is removed.

There is a general progressive thinning of the secondary cell walls of the -xylem outwards from the cell cavity, the enzymes responsible acting in the near vicinity of the hyphae. Decomposition occurs uniformly in the region of attack. Fungi causing such rots preferentially attack hardwoods and simultaneously decompose all the components of the lignified cell walls. This type of rot has sometimes been called simultaneous rot and the term white rot used in a more restricted sense for rots in which the lignin is removed much more rapidly than the carbohydrates. The cellulose microfibrils in this latter case are unmasked and the cellulose utilized later. Brown-rot fungi preferentially attack softwoods. In these rots the wall polysaccharides are principally utilized. Very little, if any, of the lignin is used, although it may be altered structurally as, for instance, by the removal of methoxyl groups. With decay the wood becomes darker brown. There is no thinning of the walls. The enzymes responsible diffuse away from the hypha and act on the entire cell wall, often at some distance from the hyphae. The structural polymers are removed, leaving a framework of lignin to maintain the general cell shape so that there is little apparent damage until the cell walls collapse. Decomposition occurs in irregular patches in the attacked wood. This leads to the cubically cracked appearance of brown-rotted wood. It also crumbles readily to a powder when rubbed between the fingers. In both white and brown rots, the hyphae grow and branch in the cell cavities and penetrate the walls mechanically via pits or the surfaces in general, by coupling penetration with enzymic erosion, producing bore holes somewhat wider than the hyphae. In both, they penetrate deeply into the wood. Soft rots, on the

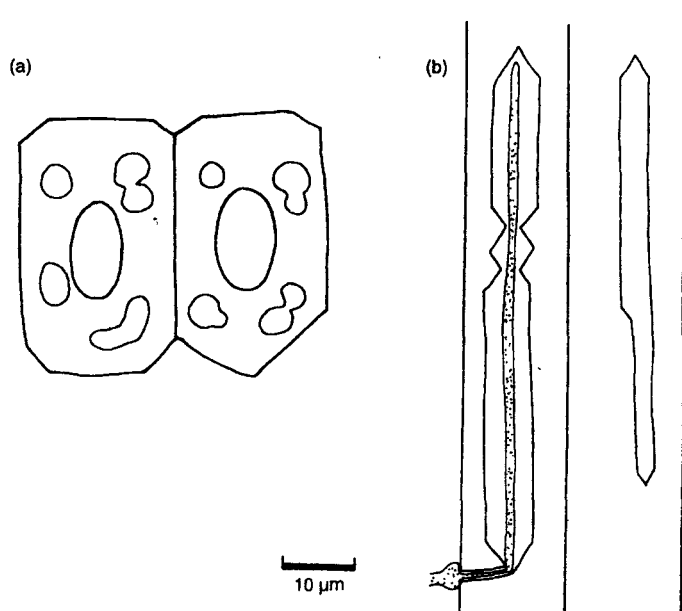


Figure 9.1 Diagram of (a) transverse and (b) longitudinal sections of tracheids of *Pinus* with soft rot cavities in the secondary walls.

other hand, are more conspicuous near the surface, advancing inwards after destroying the outer layers of the wood. They occur only in wood of unusually high moisture content, such as water-logged river and marine timbers. The soft-rot fungi again principally utilize the cellulose and the hemicelluloses of the walls, but their hyphae penetrate and grow within the secondary cell walls; here they enzymatically create chains of typically rhomboidal or elongated cylindrical cavities, with conically tapering ends. Decomposition is restricted to the immediate neighbourhood of the hyphae.

Whereas soft rots are caused by Ascomycotina, such as species of *Chaetomium* and *Ceratocystis*, and anamorphic states such as *Alternaria* and *Phialophora*, white and brown rots are caused mainly by Basidiomycotina. Two good examples of these are *Coriolus versicolor* and *Piptoporus betulinus* respectively. The former is one of the commonest polypores and is found on a great variety of hardwoods whereas the latter is a facultative wound parasite restricted to birch (*Betula* spp.). *Coriolus* can degrade over 90% of the lignin in wood. Several hundred species in the Hymenomycete Agaricales, but more so in the Aphyllophorales, an order almost entirely confined to wood,

cause white rots. But apart from these, only a very few Ascomycotina, including *Xylaria polymorpha* and *Ustilina deusta*, can cause a white rot. Somewhat fewer Basidiomycotina cause brown rots. Thus even in the fungi, the ability to degrade lignin completely is limited to the relative few.

9.3 LIGNIN DEGRADATION

It is still not at all clear how these fungi act on lignin, in spite of the fact that many studies have been made on the effects of white-rot fungi on lignin model compounds consisting of two phenyl propane units, such as the dilignol pinoresinol, or extracted lignin in liquid culture and the changes that occur in wood as it rots. Extracted lignins have been of little use in laboratory studies simply because of the physical and chemical changes brought about in its structure on extraction. Two extracted lignins, Kraft lignin and lignosulphate, have been widely studied because they are produced in such vast amounts as waste products of the paper industry. The compound which has been most widely used is a synthetic lignin designated DHP (dehydrogenative polymerizate). Like lignin, it is produced by condensation and it is chemically very similar but has a much lower molecular weight. ^{14}C labelled DHP is usually used. The amount of $^{14}\text{CO}_2$ evolved is the most sensitive measure of ligninolytic activity.

9.3.1 Role of Extracellular Phenolases

When cultured on agar containing phenolics, such as gallic or tannic acid, most - over 90% - of the white-rot fungi produce extracellular phenolases, such as laccase, peroxidase and tyrosinase, which oxidize these acids; a brown coloured diffusion ring appears around the colony margin. These catalyse the removal of electrons from phenols. They have long been considered as being involved in lignin degradation because lignin is a phenolic and so a substrate for these and lignin degradation is certainly an oxidation. Lignin is resistant to decomposition in anaerobic conditions. Also white-rot fungi produce these enzymes but the closely related brown-rot fungi, which do not decompose lignin, do not. Thus there is the apparent correlation between the ability to degrade lignin and the production of extracellular phenolases. The ability to degrade implies the formation of smaller compounds, yet it is usually considered that these oxidases act by coupling and polymerization to form compounds of higher molecular weight. There is, however, some scant evidence from experiments using white-rot fungi and model compounds that these enzymes can bring about limited depolymerization. It has also been shown that the continued action of these enzymes on wood itself leads

to some degradation of the lignin. It is doubtful, however, whether they play any significant part in lignin degradation. In any case they can only be part of the enzyme complex which attacks lignin. It has been suggested that they have an indirect role in polymerizing and so detoxifying any toxic phenolics released during degradation - that is to suggest that they act after the monomers have been cleaved off. Simple phenolics are often toxic to fungal growth and they may have an important function in coupling these. This would be comparable to depside and depsidone formation from monocarboxylic acids in lichens.

9.3.2 Cleavage of Major Linkage Groups

One obvious step towards decomposition would be to cleave any of the major linkage groups between the phenyl propane units to release the $C_6 C_3$ monomers. *Coriolus versicolor* and a number of other white-rot fungi can cleave lignin models bonded by the arylglycerol-13-aryl ether bond. Although oxidative cleavage of the β -ether linkage occurs, there is again no convincing evidence that any great part of the lignin molecule is cleaved by white-rot fungi to produce the single monomers. There is also evidence that the monomers may be attacked while still bonded in the polymer, not by breaking the bonds between them but by directly attacking the aromatic rings, by either ring cleavage and/or demethylation of the methoxyl groups to hydroxyl ones. Demethylation may also be coupled with side chain oxidation. These are oxidized by the loss of two carbon atoms and the formation of new carboxyl groups. Both these, the formation of -OH and -COOH groups, would lead to increased solubility. Support for demethylation and side chain oxidation comes from two sources. Lignin degraded by white-rot fungi contains less carbon, slightly less hydrogen, fewer methoxyl groups but more oxygen and carboxyl and hydroxyl groups. Culture filtrates from white-rot fungi grown on extracted lignin contain small amounts of vanillin, vanillic acid and syringaldehyde. There is also slightly less vanillin in rotted wood I compared with sound wood. This is taken to indicate that some phenyl propane units, either in the lignin or after cleavage, have had their side chains oxidized with the loss of two carbon atoms. Alternatively, it has been argued that vanillin and vanillic acid are attached as side groups along the main polymer and are released on hydrolysis.

9.3.3 A hypothetical scheme for lignin degradation

Although white-rot fungi unquestionably can use lignin as a sole carbon source and completely decompose it, we are by no means certain as to how lignin is degraded. A number of very hypothetical schemes

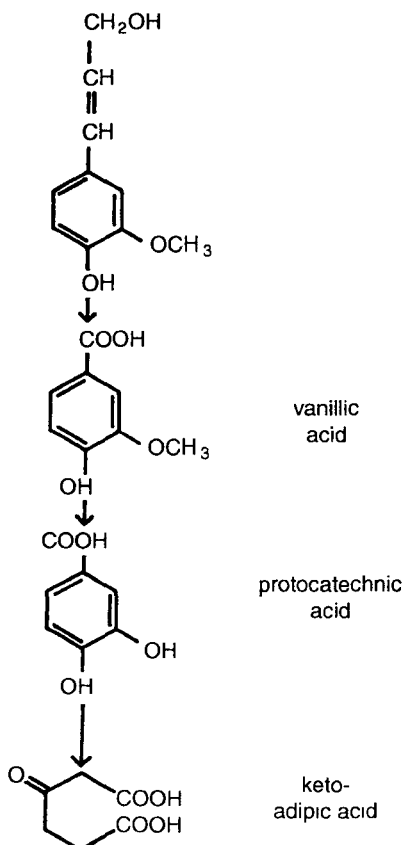


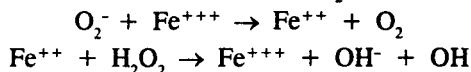
Figure 9.2 Schema for lignin degradation.

have been put forward. These usually assume initial cleavage of the arylglycerol- β -aryl ether bonds between the monomers. This is followed by oxidative cleavage of the side chain with the loss of two carbon atoms and the formation of a carboxyl group to give vanillic acid. Vanillic acid is demethylated to protocatechuic acid. Ring cleavage then occurs to keto-adipic acid and this is used in the tricarboxylic acid cycle. It is most likely that these reactions occur simultaneously over the surface of the polymer with the oxidative cleavage of the side chains being centrally significant for fragmentation of the polymer.

9.3.4 Role of Agents Other Than Enzymes

Evidence is accumulating that agents other than enzymes, such as the hydroxyl radical (OH), may be involved in lignin degradation. In cultures of *Phanerochaete chrysosporium*, one of the most widely used

white-rot fungi, hydroxyl dependent formation of ethylene coincides with ligninolytic activity. The radical is probably formed from hydrogen peroxide in the so-called Haber-Weiss reaction which is catalysed by iron and requires the superoxide radical (O_2^-)



The dependence of ligninolytic activity on the radical is verified by the fact that specific OH quenchers, such as mannitol, inhibit it. Wood rotting fungi produce sufficient hydrogen peroxide, by the action of a variety of oxidases, from the components of wood. Such oxidases are synthesized most rapidly when readily available carbon and nitrogen containing nutrients are low, when ligninolytic activity is at its peak. Sufficient amounts of Fe^{++} are also present in wood.

Cultural conditions are critical for ligninolytic activity. For example, to convert some 40% of DHP to carbon dioxide and water with *P. chrysosporium*, the culture must be maintained in the stationary phase at pH 4-5, with very low levels of metabolizable carbon compounds, low available nitrogen and high oxygen concentrations. The oxygen supply is a very critical variable as can be shown from the fact that incubation in pure oxygen increases lignin degradation 10-fold over incubation in air. The rate of degradation also increases if very thin mycelial mats are used rather than thick ones, diffusive supply being important. Cultures have also to be starved of carbon sources, such as glucose, sucrose and cellobiose, as well as nitrogen sources, such as ammonia, if high rates are to be maintained. Kirk and Fenn thus argue that lignin degradation is a strictly secondary metabolic function in that the products, as opposed to primary metabolites such as amino acids and simple sugars, are not essential for growth. But the process of degradation itself is of a selective value to the fungi. It gives such fungi a competitive ecological advantage in providing access to the cellulose and hemicelluloses masked by the lignin. White-rot fungi clearly produce a very elaborate and complex ligninolytic system, in part enzymatic and in part associated with the hydroxyl radical, to be able to degrade lignin completely to carbon dioxide and water. Brown-rot fungi lack the complete system. The most that many of these can do is to bring about a limited attack on the lignin molecule and cause such effects as demethylation.

9.3.5 Physical Barrier to Cellulase

Both the brown-rot and soft-rot fungi decompose the carbohydrates,

especially the cellulose of the wood. In the soft rots, the characteristic cavities are caused by decay being restricted to the immediate neighbourhood of the hyphae. The diffusion of their cellulase is definitely restricted. This situation contrasts markedly with the brown-rot fungi where the cellulase diffuses freely into the walls, hydrolysing the cellulose throughout and leaving a skeleton of predominantly lignin. It has often been suggested that the cellulolytic enzymes of the two groups differ in size and shape and that the lower diffusibility of the cellulase produced by the soft-rot fungi indicates larger molecular dimensions but this is not so. The cellulases produced by the two groups have similar dimensions and properties. Many actively cellulolytic fungi may be restricted in their ability to utilize cellulose in wood by virtue of the intimate nature of the association between the cellulose and the lignin. A particularly good example is *Chaetomium globosum* which rapidly degrades cotton and filter paper cellulose completely, but only attacks wood of high moisture content and merely produces soft rot cavities in the cell walls. Lignin appears to act as a physical barrier that prevents the cellulase from reaching sufficient glycosidic bonds in the cellulose to permit any large scale hydrolysis. Thus the accessibility of the cellulose to the degrading enzymes is a most important factor. The evidence for this comes from a number of sources. Increased accessibility can be achieved by breaking down the wood to much finer particles before adding cellulase. This exposes a larger surface area of the cellulose free of its association with lignin. For example, in experiments using sawdust and ball-milled sawdust, increased hydrolysis occurred in the latter when cellulase was added. It thus appears that brown-rot fungi possess some system - a pre-cellulolytic phase - which enables the cellulase to get at cellulose in wood. In the cell walls of wood, the cellulose microfibrils are encrusted with and surrounded by lignin and hemicelluloses. One suggestion that has been made is that brown-rot fungi produce enzymes which the soft-rot fungi lack. Some of these degrade the hemicelluloses and others disrupt the links between the cellulose and the lignin. There is also some evidence that one does not necessarily have to postulate enzymic dissociation of the lignin from the cellulose. Brown-rot fungi growing in wood, develop and maintain their own pH of between 2-4, whereas soft-rot fungi develop best in near neutral conditions. It may well be just that acidic conditions are necessary to disrupt the association between the lignin and the cellulose. If wood is treated initially with acid and, after removing the acid, soft-rot fungi allowed to attack it, the fungi bring about a greater loss than in untreated wood.

9.4 NATURAL RESISTANCE TO FUNGAL DECAY

9.4.1 Lignification

Lignification of the cell walls is obviously a very important factor that contributes to the natural resistance of wood to fungal decay. This is more important in soft rots. Softwoods are more resistant to these than hardwoods. This is usually attributed to the higher degree of lignification and the higher density of cell walls in conifers. It seems unlikely that mere abundance of lignin can account solely for the difference in resistance. The different proportions of the various phenyl propane units in the lignin, the degree of cross linkage with the cellulose and the nature of the hemicelluloses must also be important. Nevertheless lignification must act as some sort of physical barrier. Many very actively cellulolytic fungi and bacteria cannot attack wood because the lignin prevents their cellulase from reaching sufficient glycosidic bonds to permit hydrolysis on such a scale that they can grow on the proceeds.

9.4.2 Refractivity of Cellulose

Many other factors contribute to decay resistance. The cellulose in wood tends to have a higher degree of refractivity or crystallinity than in the cell walls of herbaceous plants. The microfibrils are more highly ordered and there are correspondingly less amorphous or more randomly organized areas. The higher the refractivity, the smaller the surface immediately accessible to the components of cellulase.

9.4.3 Nitrogen Content

In addition to being distinguished by its high lignin content, wood can also be distinguished from other plant materials by its very low nitrogen content. This also increases its resistance to decay. Woody tissues contain 0.03-1.0% nitrogen as compared to 1.0-5.0% in herbaceous tissues. The carbon: nitrogen ratio in most woody tissues is thus high, in the order of 350-500:1 and may exceed 1000:1. For most fungi a substrate with such a high carbon:nitrogen ratio would be nitrogen deficient and growth limiting. Wood-decay fungi are unusual in that they can grow in such substrates. They metabolize large amounts of carbohydrates (and lignin in white rots) in the presence of very small amounts of nitrogen. The mycelium of most fungi, grown on nutrient media, contains about 5.0% nitrogen and has a carbon:nitrogen ratio of about 10:1. The nitrogen content of the medium may fall, under starvation conditions, to around 1.0% before growth stops. The

white-rot fungus *Coriolus versicolor* is unusual in that on high carbon:nitrogen containing media, the total nitrogen in the mycelium may fall as low as 0.2% before growth rapidly declines. The ability to grow under such conditions suggests a greater efficiency in its nitrogen metabolism. This may be achieved in a number of ways. Mycelial nitrogen may be re-used either by internal translocation from old to young hyphae or by autolysis and re-use. Extracellular lytic enzymes may be secreted which break down the old hyphal walls making the constituents, especially the nitrogen in the chitin, available for re-assimilation. Preferential allocation of available nitrogen to nucleic acids and enzymes may occur. For example, when growth of *C. versicolor* on media containing low and high carbon:nitrogen ratios was compared, the total nitrogen, expressed as percentage dry mass of the mycelium, fell from 4.4 to 0.2- but the percentage nitrogen in nucleic acids rose from 4 to 25 and the amount of cellulase produced per unit of mycelium was comparable in each. In fungi in general, cellulolysis diminishes with increase in the carbon:nitrogen ratio. White-rot fungi are unique in being able to produce cellulase at a carbon:nitrogen ratio of 2000:1 whereas in most other fungi this ability is negligible at a ratio of about 200:1.

9.4.4 Moisture Content

Wood-decay fungi have higher moisture requirements for growth than fungi which attack most other plant materials. Their growth rate is very sensitive to changes in the water activity (a_w) of the medium. Whereas cotton is susceptible to fungal attack when it has a moisture content of more than 10% on a dry mass basis and cereal grains more than 13 %, wood decay can be initiated only at moisture levels of about 26-32%. In standing trees and freshly felled timber, most of the cell cavities in the wood are water-filled. Such wood may have a moisture content of well over 100%. Such completely saturated wood is quite immune to, fungal attack, presumably because the oxygen tension is too low to support active hyphal growth and the carbon dioxide content raised considerably above atmospheric levels. Many wood-decay fungi are very tolerant of high carbon dioxide concentrations. Whereas litter-inhabiting Basidiomycotina may be inhibited from growth by a partial pressure of 10 kPa, wood-decay species still grow at 30 kPa and some, including *Piptoporus betulinus*, still grow at 70 kPa. It appears that air equivalent to something more than 20% by volume of the wood is necessary for actual decay to take place. The existence of intact wooden galleys, submerged since Roman

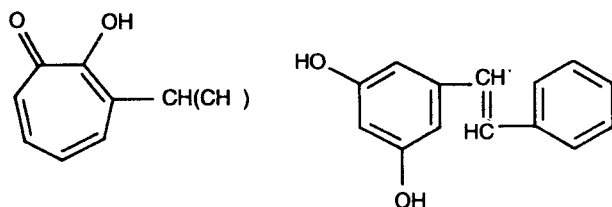
times, is adequate proof that completely waterlogged wood does not decay. The point at which all the free water has disappeared, but the cell walls are still fully saturated, is known as the fibre saturation point. For most woods this is around 26-32% (c.0.3 g g⁻¹, equivalent to an a_w of 0.97). Wooddecay fungi begin to grow at around this level and make optimum growth at about 40%. Tresner and Hayes tested just over 100 species of Basidiomycotina and found that 94 were unable to grow at an a_w of 0.97 and below and only one species grew down to 0.94. Other evidence suggests that the lower limit for growth of wood-decay fungi is about 0.97, with the linear growth rate reduced to about half normal even at 0.989.

Worked wood that has been thoroughly air-seasoned contains 15-18% moisture, which is far too low to support any fungal growth. Requirements for such high moisture contents, and thus water activities, obviously contribute to the resistance of wood to decay. Dry rot, caused by *Serpula lacrimans*, is an exception. Wood with a moisture content as low as 20-24% becomes liable to attack by *S. lacrimans*. Furthermore, as a brown-rot fungus, it produces metabolic water during cellulose degradation which considerably raises the moisture content of the wood on which it is growing. Once established on a small pocket of damp wood it can continue to colonize dry wood in this way. The exact relationship between water activity and wood decay is difficult to obtain because all, like *S. lacrimans*, degrade the cellulose in the cell walls. The complete degradation of 1.0 g cellulose liberates 0.56 g metabolic water. This is sufficient to alter the a_w of the wood significantly.

9.4.5 Toxic Substances

All these factors contribute to decay resistance but the principal sources of such resistance in wood are toxic substances deposited during the formation of the heartwood. These are synthesized in the senescing parenchyma cells and diffuse out into the walls of the adjacent xylem elements. The distribution of decay resistance within a tree has been correlated with both the distribution and the nature of these toxic substances. They have been studied most in Gymnosperms. Most are phenolics. They fall into four main chemical groups, terpenoids, tropolones, flavonoids and stilbenes. Of these the thujaplicins (tropolones) are the most inhibitory. They all provide protection from decay for many years but with time they may become lost by leaching or become inactivated. In spite of their toxicity, it is well-known that several fungi are able to destroy the heartwood, even in living trees, and also

timber impregnated with similar phenols, such as pentachlorophenol and 2,4-dinitrophenol, which are used to protect less durable timbers



a tropolone (thujaplicin)

a stilbene (pinosylvin)

Figure 9.3 Structure of two toxic chemicals extracted from gymnosperm wood.

and the sapwood of conifers. Such heartwood rotters are not insensitive to these toxins. They use their phenolases to oxidize them and polymerize the products to non-toxic melanins. Tannins are very common in the heartwood of Angiosperms and they play a similar role in decay resistance there. They inhibit fungal phenolases but decreased toxicity of the heartwood occurs with time by auto-oxidative polymerization of the tannins.

Sapwood is ordinarily very susceptible to decay but the resistance of different heartwoods is very variable. Trees with very resistant heartwoods include many oaks, cedars and the redwoods and those with non-resistant or only slightly resistant heartwoods include alders, beech, elms and poplars. A durable heartwood may be of survival value to the tree itself. Cedars live 2000 years or more whereas any of those in the slightly resistant category rarely live as long as 500 years.

9.5 OTHER WOOD-INHABITING FUNGI

Other fungi which inhabit wood occur chiefly in the sapwood where they obtain their food supply from the contents of the dead xylem parenchyma cells. These are the so-called moulds and stain fungi.

Mould fungi are mainly conidial Ascomycotina. They discolour the wood by producing pigmented conidia on the surface. Their hyphae accumulate within the ray parenchyma cells but may also be present in the cell cavities of most of the surface xylem elements, spreading from cell to cell via pits. This causes shallow discolouration and surface staining of the wood. For instance, surface blue-stain occurs most frequently on sawn timbers and on any wood surface exposed to the rain. It is caused by the surface growth of common airborne fungi, but especially *Cladosporium spp.*, with dark brown hyphae and coloured conidia. Such staining is easily removed during planing treatments.

9.5.1 Blue-stain Fungi

Typical blue-staining is caused by pigmented hyphae that grow in the wood, whereas other stains, such as brown ones, are caused by chromogenic substances actually excreted by the hyphae into the wood. At least one fungal stain has been used commercially. The mycelium of the Ascomycete *Chlorosplenium aeruginascens* permeates the dead wood of oak and beech on the woodland floor and colours it a brilliant green. Such 'green oak' has been used for inlays and decoratively as Tunbridge Ware. The wood is unaltered in texture and resists decay.

Blue-stain fungi are common in coniferous sapwood, especially pines, but are also found in hardwoods. They are non-cellulolytic 'sugar' fungi, in that they utilize only the more readily assimilable carbon compounds, such as sugars and starches, which occur in the ray parenchyma cells of freshly killed wood. They do no structural damage to the wood as they move across it mainly through the pits. Blue-stain is thus not the first stage of a form of rot, but its occurrence does indicate that the wood has been kept moist and exposed to conditions favourable to the development of decay fungi. Although the structural properties of the wood are unaltered, blue staining of coniferous sapwood is responsible for large financial losses to the timber producer. The mere discolouration of the wood makes architects disinclined to use it and it is less acceptable to the manufacturers of packing cases and paper. In vigorously growing pine trees, the moisture content of the sapwood is too high to permit growth of blue-stain fungi. The low oxygen tension again appears to be the major limiting factor in the growth of these fungi in wood with a very high moisture content. In nature they may colonize standing pine trees which have been killed either by root-rot caused by *Heterobasidion annosum*, or some other disease, or by suppression. They are much more common on felled pine logs and will soon appear on these if they are left on the forest floor for any length of time. However they are rapidly replaced by wood-decay fungi. Both death and felling cause the wood to dry out progressively and such wood will support the growth of blue-stain fungi unless the moisture content falls below about 27%.

Blue-stain fungi are Ascomycotina, mainly of the genus *Ceratocystis*, most of which have both perithecial and conidial states. The majority present their spores for dispersal in the form of stalked spore drops, the spores, in this case, being insect dispersed. The perithecia of *Ceratocystis* have a swollen base and a very long, slender neck, some often 1 mm or more in length. The ascospores are not violently

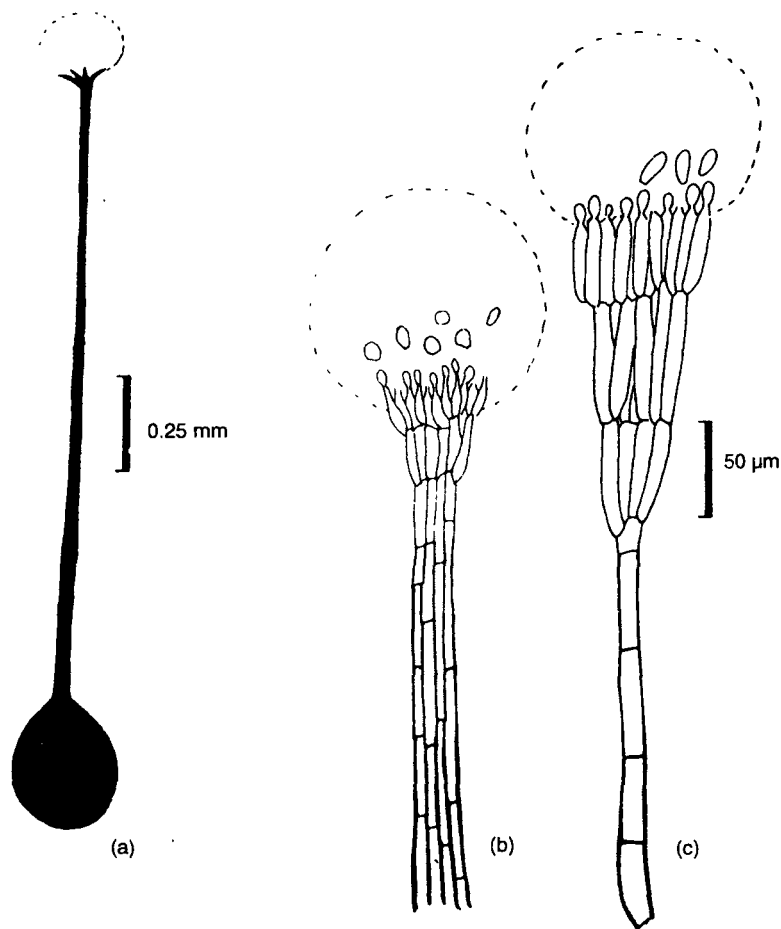


Figure 9.4 Stalked spore drops of blue-stain fungi. (a) *Ceratocystis*. (b) *Graphium*. (c) *Leptographium*.

discharged but the asci break down within the ascocarp and the ascospores are forced up the neck; they are extruded in a mucilaginous drop at the apex where they are held in place by a fringe of hair-like hyphae lining a pore. A variety of conidial states are produced. The *Graphium* state has a thick sheath of dark hyphae forming the stalk. The component hyphae branch at their tips and produce masses of sticky conidia, whereas, in the stalked spore drop of the *Leptographium* state, the stalk is a deeply pigmented and very wide single hypha which branches profusely at the apex.

Blue-staining is usually associated with attack by bark beetles. Species of *Hylastes*, *Myelophilus* and others introduce spores in making their brood chambers at the interface of sapwood and bark. The spores germinate and grow radially and longitudinally in the sapwood forming wedges of bluestained timber and then produce their conidia projecting into the brood chambers. These adhere to the young beetles as they emerge and are dispersed to other logs as they in turn make brood chambers.

Blue-staining becomes a problem where felled pine logs are left in piles on the forest floor for 2-3 months before being removed to timber depots. There are a number of ways of treating the problem, such as the use of insecticides and fungicides, but the most successful method of control, widely used in Europe, is to remove the bark immediately on felling. This not only prevents beetle attack but assists rapid drying out to moisture contents below those which will support fungal growth.

Depletion of the food reserves of the xylem parenchyma cells during ageing is one nutritional factor that tends to limit the susceptibility of sapwood to staining fungi. During ageing and the transition from sapwood to heartwood the parenchyma cells gradually die and become depleted of reserves, especially starch, and become less capable of supporting growth of blue-stain fungi. Similarly, during air-seasoning of wood, the parenchyma cells continue to respire reducing their food reserves and thus moulds and stain fungi are less common on seasoned than on unseasoned timber.

From the above, wood-inhabiting fungi can be conveniently divided into those which can live only on the cell contents, such as the moulds and the stain fungi, and those which in addition can degrade or partially degrade the cell walls, such as the white-, brown- and soft-rot fungi. Many are saprotrophs and can colonize only when the host tree has died or has been killed. Some such as *Piptoporus betulinus* and *Ceratocystis ulmi* are wound parasites, winning entry at sites where the xylem is exposed. Still others, such as *Heterobasidion annosum* and *Armillaria mellea*, are necrotrophic parasites. They invade and kill the living root tissues and then degrade the cell walls of the xylem.

9.5.2 Dutch elm disease

Ceratocystis ulmi causes Dutch elm disease; it is spread in a similar manner to the blue-stain fungi by bark beetles, especially *Scolytus scolytus* and *S. multistriatus*. The beetles bore and breed within the bark of weakened, dying and dead elms, including those which are

suffering from the disease. In infected trees, the fungus grows within the breeding galleries and produces either stalked spore drops of the *Graphium* state or rather smaller droplets of its *Cephalosporium* state. Perithecia are less easily found but in damp conditions develop on the surfaces of wood chips or partially immersed in fissures in the bark. The young adults emerge in May to October and the sticky conidia may adhere to their bodies. They fly immediately to feed on young, healthy, elm twigs and in doing so may introduce the conidia into the xylem in wounds made as they feed. Beetles thus spread the fungus from branch to branch and tree to tree. The fungus enters the xylem and grows in a yeast-like form. It can be carried up in the xylem in the transpiration stream as such or as conidia. Infected trees soon show signs of wilting and yellowing or drying out of the foliage. Fungitoxins may be involved but part of the symptoms may be explained by the occlusion of the xylem of the current year's growth by gums and tyloses.

9.6 ENVIRONMENTAL FACTORS

The decomposition of wood under natural conditions is an exceedingly protracted process. Whereas leaves of the majority of northern temperate deciduous trees may decompose in one, two or three years, a tree trunk under the same conditions may take a decade or even two to do so. The low level of available nitrogen may be the overriding factor contributing to its slow rate of decay. The addition of organic nitrogen to wood blocks inoculated with various Basidiomycotina has been shown to increase their decay rate by over 60%. Other minerals, especially phosphorus and potassium, may also be limiting. The relatively high demand for such mineral nutrients combined with their relatively low availability places a limitation on the amount of fungal mycelium such a substrate can produce. Fluctuations, both diurnal and annual, in temperature and moisture content must also be important. However, in aseptic laboratory experiments decomposition of wood by a single species of decay fungus may be relatively rapid. The white-rot fungi *Lenzites betulina*, *Coriolus hirsutus* and *C. versicolor*, inoculated onto small blocks (20 mm³) of birch wood kept in sterile moist soil at 22°C, caused more than a 75% loss in dry mass in three months. *Piptoporus betulinus* and a number of other brown-rot fungi caused mass losses of 50-70% over the same period. These are substantial losses, the more so when it is borne in mind that birch wood contains some 20% lignin which is not available to brown-rot fungi. These facts may be contrasted with the observation that *P. betulinus*, which had killed birch trees in East

Anglia, was still producing basidiocarps on these at least five years after they had fallen. The time period from infection to falling was not known but it may have been at least another five years. Even if the variable temperature and moisture regimes are taken into account, the decomposition of one of the least durable woods is very much slower in nature than in laboratory tests.

Most wood-decay fungi are mesophiles in terms of their temperature requirements, although some come into the category of cold-tolerant ones. The optimum temperature for the growth of most lies between 25 and 30°C. *P. betulinus* has an optimum at 25°C and its growth falls off very rapidly above and ceases at 30°C. Its minimum temperature for growth, which it must often experience in the field, lies between 7 and 9°C. For many others, the minimum lies below freezing point but decay at such temperatures would be very slow. The geographical distribution of a number of species is related to their temperature requirements. *Serpula lacrimans* has a low maximum of 25-26°C. It is absent from the tropics and other parts of the world with high summer temperatures.

In bulk wood, temperature is probably a more important variable than moisture content. With reference again to *P. betulinus*, it is able to decompose birch wood with a moisture content within the range 35-100% on a dry mass basis, although near maximum decomposition rates only occurred between 60 and 120% in laboratory experiments. Birch logs, stored outside in Central Sweden, had a moisture content of 85-91 % on felling and after three years the moisture content was still 51-67070. Fluctuations did occur, with some drying in the summer and some water uptake in the winter, but over the whole period the moisture content was somewhere near the optimum for decay. However, if the bark peels off the position is quite different. In summer, rapid drying out may occur to moisture contents below those which will support growth and equally rapid soaking will occur in rain. A thick, highly suberized outer bark is not only a structural deterrent to fungi, but because of its high content of tannins, phenols and the like, also a chemical one. However, given this, if it remains intact after death of the tree, it helps to maintain a more equitable moisture regime within the wood and this will favour any decay fungi.

9.7 SPECIFICITY OF WOOD-INHABITING FUNGI

The habitats of wood-inhabiting fungi vary from minute twigs, small and large branches to the most massive of tree trunks and stumps

and from minute rootlets to major roots and include such man-made habitats as fencing posts, house timbers, sawdust and chip piles. Any one of these substrates is particularly complex in more ways than one. The trunk of any one tree will have varying proportions of bark, sapwood and heartwood along its length. These proportions will differ from those in the trunk of another species. The wood from different tree species differs structurally as can be seen by contrasting ring-porous with diffuse-porous types. Further marked differences occur between softwoods and hardwoods. Over and above these differences as already indicated the composition of the lignin varies in different wood. This complexity and heterogeneity make it difficult to generalize about the decomposition process. A number of successional studies have been made on woody substrates but these have to be interpreted with caution. A succession can be defined as the appearance of different fungi in sequence on the same part of the substrate. The fact that one fungus appears on one part of a log at one time and another fungus on another part, even an adjacent part, at another time does not necessarily prove a succession. Their habitat niches may be quite different, one growing on the sapwood and one on the heartwood or the latter may be colonizing a part of the heartwood not colonized by the former. For example, basidiocarps of *Daedaleopsis confragosa* or of *Hypholoma fasciculare* may appear on a birch trunk which has, for a number of years, supported basidiocarps of *Piptoporus betulinus*, but the mycelia of these would almost certainly be growing on parts of the wood not colonized by *P. betulinus*. The latter is specific to birches and is a wound parasite gaining entry where a branch has been fractured. Infected trees are usually killed by the fungus and the trunks of these often break off remarkably cleanly and transversely at a height of about 3 m in high winds. The structural polysaccharides in the walls are rapidly and completely removed leaving a cellular framework of amorphous lignin which has insufficient tensile strength to withstand the bending strains incurred. The fungus then continues to grow and to produce its characteristic kidney- or hoof-shaped basidiocarps on the fallen and standing parts of the tree. As with many woody substrates, *P. betulinus* is the primary and sole colonizer. It may completely permeate the wood of the whole trunk and persist there, virtually in pure culture, for several years, by which time the wood is in a very late stage of decay and extremely friable. In such a state the wood is unlikely to be capable of supporting fungi such as *D. confragosa* and *H. fasciculare*. They would not succeed *P. betulinus* but would be growing on parts not colonized by it.

Wood-decay fungi exhibit all degrees of specificity. Considering the whiterot and the brown-rot fungi as two groups, there are many more of the former than the latter. Those of the white-rot group primarily attack hardwoods and those of the brown-rot group softwoods. Some of these may be restricted to a single host genus. *P. betulinus* is a good example. *Fistulina hepatica* which causes a serious decay of the heartwood of oaks is another. The causes of such marked specificity are obscure. Other fungi may be restricted to the wood of a relatively small number of trees. *Polyporus squamosus* is, like *P. betulinus*, a wound parasite, in this case of elm in particular but it is often found on other trees, such as ash and sycamore. It causes a white-rot of the heartwood and may persist for a number of years on fallen trees which it has killed or which have been wind-blown, as a consequence of the rot. On elm trunks *P. squamosus* is replaced, but only in a temporal sense, by a number of other wood-decay fungi. Two in particular, *Auricularia mesenterica* and *Pleurotus cornucopiae* are rarely found on other wood. Basidiocarps of the former soon appear on any felled elms and production of these continues for up to eight years. Spatially it utilizes the bark and surface layers of the sapwood so it does not succeed *P. squamosus*. Basidiocarps of *P. cornucopiae* appear on elm trunks only some 3-10 years after they have fallen. The fungus then persists until the wood is well-decayed. Its mycelium appears to be confined to the sapwood not utilized by *P. squamosus* and again it does not actually succeed the latter. Whereas *P. cornucopiae* is most common on fallen elm trunks, *Flammulina velutipes* is most often found on standing dead elms, especially those killed by *Ceratocystis ulmi* and which have lost their bark.

Still other fungi, such as *Coriolus versicolor* and *Stereum hirsutum*, are much less discriminating and grow on a wide range of hardwoods. The former is entirely saprotrophic and is one of the commonest fungi found on fallen twigs, branches, trunks and dead stumps of hardwoods where it produces the most rapid of white rots but, like *Stereum*, is confined to the sapwood. It can actually replace, and therefore succeed, other established and less aggressive white-rot fungi. *Xylaria hypoxylon* and *Daldinia concentrica*, two Ascomycotina, produce black lines, zone lines, in the sapwood of ash delimiting areas which they have colonized. The hyphae of *C. versicolor* will penetrate these and grow on to replace them. Other fungi show a preference for coniferous wood. *Heterobasidion annosum*, *Paxillus atrotomentosus* and *Tricholomopsis rutilans* are characteristic of conifer stumps, *Hirschporus abietinus* and *Stereum sanguinolentum* of coniferous twigs and branches and

Auriscalpium vulgare of pine cones. These are all Basidiomycotina but similar examples can be found in the Ascomycotina. For example, *Daldinia concentrica* is very common on ash but is occasionally found on other hosts, especially beech and, birch. *D. vernicosa* occurs on gorse, especially bushes which have been burnt and subsequently weathered. *Ustulina deusta* causes a white-rot of lime and beech, whereas *Xylaria polymorpha* and *X. hypoxylon* are very common on a wide variety of dead hardwoods.

Each tree species thus may have, within limits, its own particular wooddecay fungi. A number of these may enter as necrotrophic parasites at wounds above ground or along roots below ground and then persist as active saprotrophs after death. They would thus have a competitive advantage over purely saprotrophic fungi in being established first. As parasites they may only be able to overcome the host resistance of one or a few species of trees. This might account for some of the specificity noted. It may well be that the different naturally occurring tannins, terpenoids, etc. present in the different heartwoods further help to determine specificity. Only fungi which, can tolerate or degrade these are able to become established.

9.8 ECOLOGICAL STUDIES ON DECAYING WOOD

Numerous ecological studies have been made of fungi colonizing specific woody substrates including wounded living tree trunks and fallen dead ones, tree trunks after insect attack, fire-killed trees, branches and slash on the ground, tree stumps, fence posts, beech cupules, etc.. Changes with time in the fungal communities on these have been recorded and described, accurately or inaccurately, as successions. As might be expected, the sequences of fungi observed on these show considerable variation depending upon the species of wood, the type of substrate and, in addition, the environment in which decomposition is occurring. But fungi are not the only organisms found in decaying wood. A very wide variety of invertebrates and bacteria also occur and they, too, may play an important role in its decomposition.

Swift recognized three stages in the decay process - the pioneer colonization stage, the major decomposition stage and the incorporation stage, in which the products of decay are incorporated into the soil.

9.8.1 Pioneer Colonization Stage

Patterns of colonization may vary. In some cases, the Basidiomycotina which are going to dominate the decomposition stage

are the primary and sole colonizers. In others, their colonization is preceded or accompanied by a variety of decay or non-decay fungi or bacteria. This may be illustrated with some specific examples. *Heterobasidion annosum* is a white-rot fungus causing butt- and root-rot of conifers. It may colonize via roots or the surfaces of freshly cut stumps. Infection of a healthy living root almost invariably occurs as a result of mycelial transfer from another infected root coming into contact with it. From the root the fungus grows up to the base of the stem and colonizes and kills the cambium, thus effectively girdling and so killing the tree. It then progressively rots the roots and the stem base. Rapid desiccation of the wood after death usually prevents extensive spread up the stem. In this case it is the sole colonizer. Alternatively it may colonize the surfaces of freshly cut stumps via its air-borne basidiospores. These stump surfaces are highly selective substrates and are initially colonized by a relatively small number but, nevertheless, a variety of fungi. These include, in addition to *H. annosum*, non-cellulolytic blue-stain fungi utilizing the contents of the parenchyma cells, cellulolytic fungi, such as *Phialophora* and *Trichoderma* spp., utilizing cell contents and any easily accessible cellulose, and other wood-decay fungi such as the white-rot fungus *Peniophora gigantea*. This is a much more competitive situation; whether or not it emerges as the major decomposer will depend upon a multiplicity of factors, including its ability to compete with these for the more readily available nutrients which are necessary if it is to become established. Similar patterns of colonization can be seen in the initiation of decay in trunks following wounding, such as by the branches breaking off in high winds. Again, in some cases the only fungi to colonize are the wood-decay Basidiomycotina which later become the dominant decomposers. This applies to most species of *Stereum*. They invade only freshly exposed tissues and are inhibited by the presence of other pioneer micro-fungi and bacteria. In other cases, such as with *Phellinus igniarius* invading wounds on poplars and other hardwoods, prior colonization by bacteria and micro-fungi such as the stain, mould and soft-rot fungi generally occurs and may even be a prerequisite if it is to attack and cause a progressive rot of the heartwood.

Insects, especially members of the Ipidae and Scolytidae, may attack living trees and introduce bacteria, yeasts, blue-stain or ambrosia fungi below the protective bark. The combined activities of the insects and the fungi may weaken or kill the tree. The wood-decay Basidiomycotina then follow. The attack of *Scolytus scolytus* on elms

introducing *Ceratocystis ulmi*, followed by *Flammulina velutipes*, is a case in point.

9.8.2 Decomposition Phase

The decomposition phase is dominated by the white- and brown-rot fungi but wood-boring beetles (Coleoptera) and wood-eating termites (Isoptera) may also contribute to decay. In many woody substrates only one fungus may be involved in the decomposition phase; examples of *Piptoporus betulinus* on birch, *Heterobasidion annosum* on pines and *Coriolus versicolor* on hardwoods in general, have already been given. In others, a number, but usually a very limited number, of fungi are involved. Each of these occupies discrete volumes of wood which are often clearly demarcated from each other by distinct dark zone lines. These colonies may intricately interlock but their mycelia do not intermix. They remain isolated by zone lines into virtually pure cultures. This balanced state may persist for a number of years, but, depending upon the relative competitive ability of adjacent mycelia, there may be eventually some replacement of one fungus by another, or aggressive saprotrophs, such as *Hypholoma fasciculare*, *Phallus impudicus* and *Phlebia merismoides*, may colonize from the surrounding litter and replace them. For example, both *C. versicolor* and *Stereum hirsutum* are susceptible to replacement by any of these three in hardwood trunks and branches and *Heterobasidium annosum* by *Peniophora gigantea* in pine stumps. *H. annosum* is particularly sensitive to hyphal interference caused by the latter and this may be one factor involved in its replacement.

9.8.2.1 The role of animals in degradation

Many wood-boring beetles and their larvae and termites are wood feeders depending upon microbial symbionts in their guts to semi-digest the wood. Some feed on sound wood, others on decaying and well-rotted wood. In the latter case, the fungi growing in the wood may be an important component of their food. Many termites are polyphagous. When *Kalotermes flavicollis* is fed on wood, it decomposes 94-95% of the cellulose, 60-70% of the hemicelluloses and 3-40% of the lignin. Thus large populations of these wood-boring beetles would almost certainly contribute substantially to wood decay. Another important aspect of their degradative activity is the comminution of the wood as they attack it.

As the white- and brown-rot fungi exploit the wood, it softens and becomes friable and as such is more attractive to animals as a food source, as somewhere to live and as a breeding ground. Their access

to it, if it is bulky, may be dependent upon the prior activity of wood-boring animals, such as the beetles. Their bore holes afford ports of entry for a very great variety of animals generally common in litter and soil. These include micro-arthropods, such as Acari and Collembola, and macro-arthropods, such as Diptera and Isopoda, as well as Oligochaetes, such as Enchytraeid and Lumbricid worms. Many of these, such as the Mycetophilid dipterous larvae feed mainly on the mycelia of the fungi. They all accelerate the process of comminution and carry spores from the surrounding litter and soil into the wood and thus inoculate it with common soil fungi. Many Zygomycete Mucorales appear for the first time on the decaying wood, along with a variety of conidial fungi including species of *Penicillium*, *Scytalidium* and *Trichoderma*. These now have a quite wide choice of substrates on which to grow. They may utilize the partly degraded wood, the dead hyphae of the wood-decay fungi, dead fauna or their faecal remains. Some may live as commensals sharing the hydrolytic products of the enzyme systems of the major decomposers. This is the incorporation stage. As the wood becomes more extensively decayed, the activity of the wood-decay fungi may decline and they are eventually replaced by such soil-inhabiting fungi. With time, the wood disintegrates and as it does so it is incorporated into the soil.

9.8.2.2 Cycling of mineral nutrients

Wood decay is important in regulating the cycling of mineral nutrients in the woodland ecosystem and contributes to the process of soil development there. Over the period of fungal decay, virtually all the important minerals, but in particular nitrogen and phosphorus, become immobilized in an organic form in the fungal hyphae and their reproductive structures, such as basidiocarps. Although the mineral content is low per unit volume, the sheer volume of decomposing wood means that it forms a very substantial part of the total minerals in the woodland ecosystem. Comminution of the decaying wood by the animal invaders leads to a release of some minerals. The small particulate form of the frass or faecal materials means that they are more effectively leached. As with the fungi, the animals themselves act as a further reservoir of plant nutrients in a considerably more concentrated form than in the wood itself. Their wanderings, after feeding, lead to some redistribution of minerals but by far the more major redistribution and export from the wood occurs when adult stages emerge from the broods reared in and on the decaying wood. But this is essentially only a redistribution. The adults eventually die and their

tissues are mineralized elsewhere in the ecosystem, while any fungal remains are mineralized *in situ*.

9.9 DECOMPOSITION AND HUMUS IN THE SOIL

The white- and brown-rot fungi are, more often than not, associated with relatively large masses of wood, such as the dead tree trunk and decaying stump. This may be because only these contain enough energy resources for these fungi to amass sufficient to produce their relatively massive and conspicuous basidiocarps. Vast quantities of lignin are incorporated into the soil in the vascular network of the leaves, fine rootlets and so on. These are very different substrates for fungi and other micro-organisms and they are in a vastly different environment. The substrate is richer in terms of associated readily available carbon and nitrogen sources, as tissues other than the highly lignified xylem are present in relatively larger proportions than in bulk wood. It is also presented to a much more varied population of micro-organisms. As such it would support a more diverse micro-flora and any lignin decomposers would be competing for, not necessarily lignin, but other more generally assimilable components, which are necessary for establishment, and would also be exposed to antagonism by others. This situation is markedly different from the decaying tree trunk with its one or few decomposer fungi in isolation. Further large quantities of lignin may be introduced into the soil in the form of organic residues from wood decay, especially from brown rotted wood. The process of lignin degradation in the soil may be quite different from that occurring in a tree trunk. There is very little direct evidence that any Basidiomycotina degrade such lignin in the soil. This may be because we are ignorant of the facts. In studies on soil fungi, Basidiomycotina are only rarely recorded. They tend to be slower growing and so are easily overgrown on most widely used culture media. They are often very sensitive to antagonism by others and so suppressed. Most do not produce spores or possess any other readily recognizable feature so could easily be overlooked. Nevertheless, they may be equally important as lignin decomposers in the 'soil itself as they are in the litter and decaying wood. The number of soil-inhabiting micro-organisms which have been reported as being able to utilize lignin is very small. These include a few aerobic, Gram-positive, non-sporing, rod-shaped bacteria, in the genera *Bacillus* and *Flavobacterium*, and a few conidial fungi, in genera such as *Humicola* and *Phialophora*. The evidence for their ability to utilize lignin has again been obtained from the use of extracted lignin

and lignin model compounds. These have been used incorporated in Kaolin pellets to enrich soil and fungi subsequently isolated from them and tested for their ability to utilize such compounds as vanillic acid and syringaldehyde. The ability to grow on and utilize these should not be taken as an ability to utilize lignin itself just as the ability to utilize carboxymethylcellulose is not taken as an ability to utilize native cellulose. They are partial degradation products and these fungi should be regarded as occupying a similar niche with regard to lignin as secondary sugar fungi do to cellulose. The latter do not possess the whole enzyme system necessary to hydrolyse cellulose. They lack the C_1 component but possess the C_x component and β -glucosidase so that they can utilize the hydrolytic products. Some also lack the C_x component as well. Similarly with lignin, the fact that a fungus lacks one

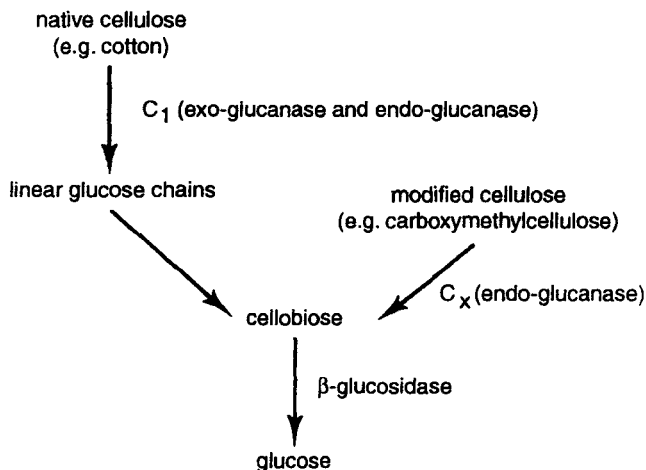


Figure 9.5 A schema for the degradation of cellulose.

component of the multi-enzyme system does not necessarily debar it from participating in lignin degradation. Fungi may co-operate, sometimes synergistically, in the degradation of both cellulose and lignin. For example, it has been shown in experimental systems that a mixture of the C_1 component from one fungus and the C_x component of another is as efficient at cellulolysis as when both components are derived from the same fungus. It has also been shown using lignin preparations in culture tests that, in many cases, when two wood-decay fungi are grown together in mixed culture, degradation is more pronounced than when both fungi are grown apart.

Lignin in the soil decomposes very slowly and its degradation there is more of a joint effort. There may well be a pooling of enzymes from

a variety of fungi and perhaps bacteria and actinomycetes - some enzymes capable of cleaving bonds between monomers, others of demethylation and still others of side chain oxidation and so on until the final products enter the respiratory pathways of one organism or another.

9.9.1 The Nature of Humus

With this breakdown there is a gradual accumulation of dark, amorphous, organic humus. The chemistry of humus has by no means been fully elucidated. It is very heterogeneous and can be separated into a number of molecular categories using extraction techniques. It forms a very dark solution in dilute NaOH and a black precipitate called humin. Acidification of the solution with HCl to between pH 1-2 precipitates out a fraction called humic acid, leaving fulvic acid. The humic acid fraction is the major molecular category and forms from between 50 to 80% of the soil humus. It usually contains about 5% nitrogen, mainly in the form of bound amino acids but also in amino sugars and heterocyclic purine or pyrimidine derivatives. The most favoured idea is that humic acid has a heterogeneous aromatic core with carbohydrates, peptides and proteins, phenolics and metals, attached peripherally. In some soils, especially under woodlands, the humic acid may originate from lignin residues, possibly the end products of the brown-rot fungi, which have been considerably modified by microbial action. Syringic and vanillic residues can often be detected in the degradation products of humic acid and the distribution of these residues is consistent with the composition of the lignin found in the vegetation above. Work with tracers has shown that as much as one third of the humic acid in the soil is derived from lignin and only about one twentieth from cellulose. Reductive cleavage of most humic acid fractions shows that they also contain units based on phloroglucinol. This suggests that seed plant flavonoids also contribute to humus. Flavonoids are phenolics with two aromatic rings and include pigments, such as anthocyanins. The phenolics are degraded to simple phenols which become polymerized into the humic acid fraction. But humus is not solely a product of degradation of the more resistant parts of seed plants. It is also in substantial part a product of microbial synthesis. When ^{14}C labelled glucose is added to the soil, 40-80% of the carbon is lost as carbon dioxide within a few days but, even after two years, about 5-10% is still present in the soil humus. Intracellular transformation of carbohydrates and other simple organic substances occurs to produce phenols, quinones and other aromatic substances. These are oxidatively polymerized and combined with peptides and other cell

constituents to form humic-like pigments, melanins, inside or outside the cell. These serve several functions. They may be deposited in the walls of hyphae, spores or ascocarps, to protect against excessive ultra-violet light or as a water-proofing to prevent water loss. Eventually, on the death of these structures and with time, they become variously transformed and incorporated into the humus fraction. The existence of significant amounts of amino sugars and non-protein amino acids, such as diaminopimelic acid, also suggests that residues of bacterial cell walls may form part of the humus.

9.9.2 Turnover of Humus in Soil

Humus is extremely resistant to microbial degradation but nevertheless there is a very slow turnover with the rate depending upon the soil type. A sample from a chernozem soil from the USA was ^{14}C dated as 990 ± 60 y old.

In other soils, humus is less stable e.g. humus from a coniferous forest soil in Sweden was dated as 370 ± 100 y. A number of fungi have been found to decompose humic acid in laboratory tests. Humic acid was extracted from a Canadian soil. It contained 26% of the total soil carbon and was dated as 785 ± 50 y old. It was supplied as the sole carbon and nitrogen source as a 0.2% solution to a number of microorganisms, isolated by direct plating of the soil onto humic acid containing media. Four bacteria, in the genera *Bacillus* and *Pseudomonas*, and two conidial fungi, *Penicillium frequentans* and *Aspergillus versicolor*, could utilize the humic acid as a sole carbon and nitrogen source but no actinomycetes could. *P. frequentans* made the best growth and it appeared to utilize the humic acid by initially reducing carboxylic groups to aldehydes and then alcohols. Salicylaldehyde and salicyl alcohol appeared in culture filtrates. A number of Basidiomycotina, including *Coriolus versicolor*, *Hypholoma fasciculare* and *Trametes suaveolens*, all active white-rot fungi, can also utilize humic acid and this ability is always associated with the reduction of carboxylic acids to alcohols. This suggests that one of the first steps in the degradative process is an aerobic reductive one. Subsequent steps have as yet to be elucidated. Very little can be concluded from such studies about the process of degradation in soils. Resistance to degradation may not be so much that it is not susceptible to microbial enzymes but that its multi-dimensional complex structure physically restricts the access of such enzymes.

9.10 FUNGAL DECOMPOSERS OF LEAVES

Leaves of all manner of types form suitable substrates for many

fungi. Long before any leaf falls, the complex process of its decay is initiated. The greater part of this process takes place above ground in the litter covering the soil surface. In this chapter some facets of this are discussed but particularly the epiphytic leaf surface or phylloplane micro-flora, the leaf surface as a habitat niche for fungi, colonization by the common primary saprotrophs as leaves senesce and die, the attributes which make these primary saprotrophs such widespread and successful colonizers, and some of the subsequent events occurring in the litter.

9.10.1 The Leaf As A Spore Trap

As any leaf unfolds it is a relatively clean sheet which immediately provides landing sites for air-borne particles such as bacteria, yeast cells and fungal spores but also pollen. Spore trapping by leaves is a natural phenomenon of nature. Spores may reach leaves in three main ways: wind-borne and deposited by impaction or by sedimentation under gravity; in falling rain drops; or in rain splash droplets. Air-borne spores are usually dry and often rough or spiny and readily detachable from their stalks, excellent examples being the urediospores of rust fungi. They are readily washed out of air by falling rain drops. Rain splashed spores tend to be wet or slimy and borne in a sticky liquid. Adaptations facilitating deposition are far less obvious than in spores of aquatic fungi. Amongst dry spores, a larger size, as seen in the powdery and downy mildews, favours impaction and sedimentation. Rain splashed spores tend to be smaller and spherical. But these are only generalizations and there are many anomalies. The most ubiquitous and by far the most numerous of the phylloplane fungi are members of the Sporobolomycetaceae, the shadow yeasts. They produce air-borne spores which in relative terms are quite minute.

Leaf surfaces are differential spore traps. Their efficiency as traps depends upon whether they are horizontal or vertical, wet or dry, hairy or glabrous, glossy or mat, waxy or non-waxy and so on. Not all spores that land become securely attached. Some are washed off by rain, blown off by wind or redistributed by dew. Some have a two phase dispersal system. For example, the large sporangia of pathogenic species of *Phytophthora* and some other Oomycete Peronosporales are wind-borne and normally impacted onto leaf surfaces. Under moist conditions the impacted sporangia may germinate directly by a germ tube or indirectly to produce motile zoospores which may swim about in moisture or be redispersed further to other leaves in rain splash droplets.

Virtually any spore which may become air-borne can be found on leaves. If leaf surfaces are washed and the washings plated out onto nutrient agar, numerous yeasts and filamentous conidial Ascomycotina, some Zygomycete Mucorales and the occasional Mastigomycotina and Basidiomycotina develop on the plates. But microscopic examination of stained leaf surface impressions or peels reveals the presence of not only these but also spores of many other Ascomycotina and Basidiomycotina, including those of agarics, polypores and Gasteromycetes. They just do not grow or grow too slowly on the culture medium used. These impressions of peels can be made by spraying leaves with cellulose acetate in amyl acetate or painting with nail varnish or molten 1 % agar, leaving to dry and then stripping off.

Many of these fungi and an even larger number of bacteria actively grow on the surface of the living leaf and have been called 'resident inhabitants' in contrast to 'casual inhabitants' which are unable to grow in such an environment because of the lack of essential nutrients, unfavourable physical factors, competition with or antagonism by others or some combination of these factors. This rather simple distinction can be extended by dividing the epiphytic fungi into three categories: non-pathogenic epiphytes; pathogens; and exochthonous or casual inhabitants. Exochthonous is a fitting, if somewhat clumsy, term as it is used for fungi found on or in a substrate which is not their habitual one.

9.11 PHYLLOPLANE INHABITANTS

Amongst the non-pathogenic epiphytes, two main groups, the phylloplane inhabitants and the common primary saprotrophs, can be recognized. The phylloplane inhabitants are able to complete their life cycle or a significant part of it on the living leaf without damaging it. *Sporobolomyces roseus* not only is a very good example of such a fungus but is virtually omnipresent, being found on leaves of grasses, dicotyledonous herbs, trees and shrubs, wherever they grow. Its cells multiply very quickly by budding when conditions are favourable, forming distinct yeast-like colonies on the leaves. Budded cells can be redistributed on an individual leaf or from leaf to leaf by rain splash and are similarly locally dispersed to leaves of other plants. It also reproduces by ballistospore formation. The ballistospores are very effectively wind dispersed. They are produced under high humidities at night, as are the budded cells, and constitute the major component of the air-spores at that time. Other members of the Sporobolomycetaceae are also common. Species of *Bullera* behave similarly, whereas members of the genera *Tilletiopsis* and *Itersonilia* produce a sparse mycelium from which

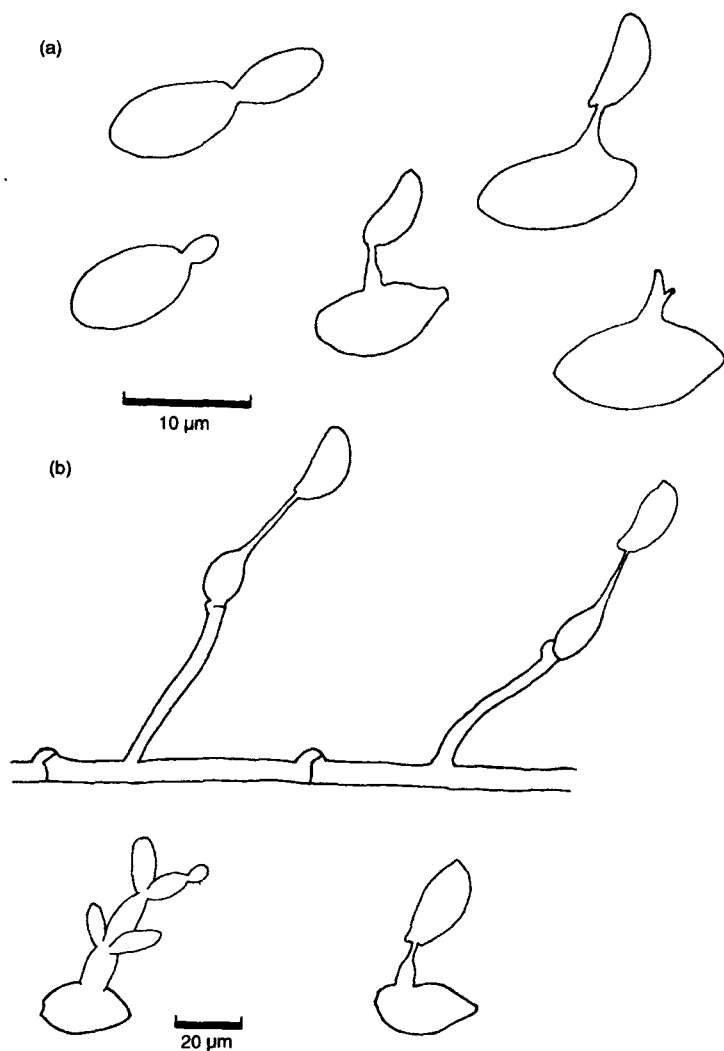


Figure 9.6 (a) Budding and ballistospore formation in *Sporobolomyces roseus*. (b) Ballistospore formation in *Mortierella perplexans*, ballistospores germinating by budding and ballistospore formation.

ballistospores arise. Other yeasts, especially members of the Cryptococcaceae, the non-sporing yeasts, exist in the budding phase. They all complete their life cycle in the phylloplane. Such fungi are often called 'shadow yeasts'. Their presence can be demonstrated by suspending leaves from the underside of a Petri dish lid for about 12 h over 2% malt extract agar. In such a humid and still atmosphere, ballistospores are produced and discharged. They fall vertically onto the agar below and start budding. Tiny colonies, pink in *S. roseus*, become visible after 2-3 days. These form a mirror image of the distribution of the cells of the leaf.

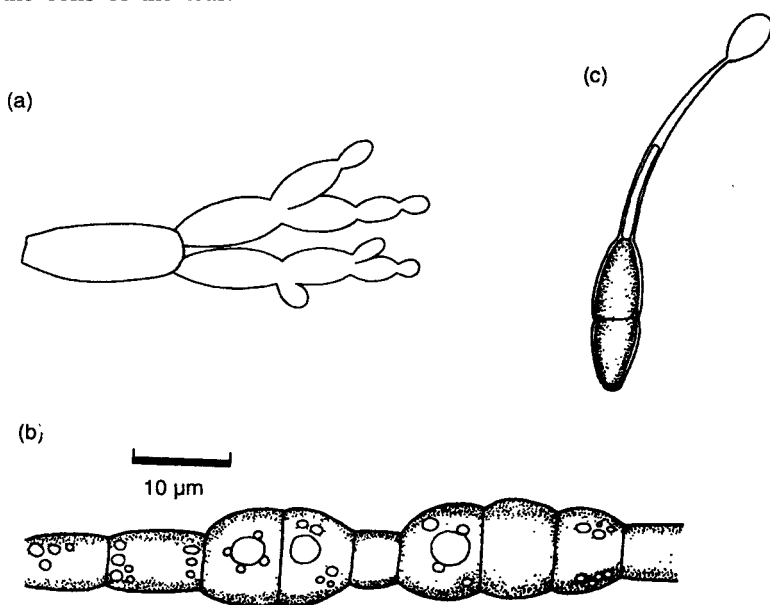


Figure 9.7 (a) Yeast-like budding by *Aureobasidium*; and (b) chlamydospores of *Aureobasidium* on a leaf surface. (c) Conidium of *Cladosporium* germinating to produce a secondary conidium.

Two conidial Ascomycotina also grow in the phylloplane. The conidia of *Aureobasidium pullulans* and several species of *Cladosporium* may germinate after impaction and develop into hyphae forming quite extensive colonies under favourable conditions. *Aureobasidium* more often grows by yeast-like budding with minimal hyphal growth. This is a modification of its normal cultural form. The budded cells may again be redistributed in moisture films or by rain splash droplets. The conidia of *Cladosporium* may germinate and produce secondary conidia from short germ-tubes rather than grow as hyphae. These conidia

are dry and become air-borne. So both reproduce rapidly and complete a significant part of their life cycle in the phylloplane. Both eventually produce ascocarps to complete their life cycle. These are found only in the spring on overwintered fallen leaves in temperate climates. Ascospores are discharged from these as new leaves unfold.

Aureobasidium and *Cladosporium* are also both very well-adapted to survive in this rigorous habitat. Their hyphal walls rapidly become thickened and melanized. This enables them not only to survive exposure to damaging ultra-violet light from the sun but may also help to prevent excessive desiccation and make them more resistant to bacterial lysis. *Aureobasidium* produces dark, thick-walled multicellular chlamydospores in chains or in clumps. *Cladosporium* produces more distinct microsclerotia, compact spheres of 10-100 cells with an outer layer of thick-walled cells with heavily melanized walls. Under favourable conditions, these produce clusters of conidiophores and abundant conidia which, like the ascospores, serve as a source of inoculum as new leaves unfold. In contrast *Sporobolomyces* does not appear to be able to withstand prolonged adverse conditions, such as low relative humidities. At relative humidities of 65% and below, it rapidly disappears but its population equally rapidly expands from reservoirs on more protected less exposed leaves, when favourable conditions return. In temperate climates with a combination of warm humid weather and aphid infestation producing honeydew on leaves, the so-called sooty moulds appear as black, soot-like coverings over leaves, especially of trees such as limes (*Tilia* spp.). These are the result of the profuse growth of *A. ureobasidium* and *Cladosporium* using the trisaccharide melezitose in the honeydew as a carbon-source, together with aphid faeces, sloughed off parts and dead remains. In w& tropical climates, such as in Amazonia, parts of Africa, Australasia and the Caribbean, true sooty moulds occur. These, like the perfect states of *Aureobasidium* and *Cladosporium*, are also Loculoascomycetes and again grow as saprotrophs associated with honeydew from aphids. A wide range of species from several fungal families, especially the Capnodiaceae and Chaetothyriaceae are involved. They form distinct, dense, dark hyphal networks on leaves often in the form of a thick felt and each fungus produces abundant conidia of often two or even three types as well as ascocarps.

9.11.1 Nutrient Sources

All the phylloplane inhabitants, the yeasts, the filamentous fungi and the bacteria, are chemo-organotrophs requiring organic nutrients

for growth. Some of their nutritional requirements may be met by organic substances absorbed or deposited onto leaves such as detritus trapped in their superficial wefts of hyphae, as also happens with fungi living on paint films or glass. Most of their nutrients, however, must be derived directly or perhaps indirectly from the host. A great variety of substances exude or leak out of leaves. These include free sugars, amino acids and inorganic ions which are all essential for fungal and bacterial growth. For example, water droplets placed on leaves exhibit an increase in conductivity indicating exudation from the leaf; increased growth of some fungi in these drops shows that certain of these exudates are of nutritional value. Two sources of added nutrients are from pollen and other spores. Nutrients also leak out of these. Pollen added to leaf surfaces stimulates the development of *Sporobolomyces* and *Cladosporium* and probably accounts for the sudden increase in their population shortly after flowering on leaves of plants such as rye. Conidia of *Botrytis cinerea* placed in droplets on leaves leak out amino acids and sugars in sufficient quantity for phylloplane bacteria to develop in such numbers as to inhibit germination of the conidia themselves. This all occurs on the intact surfaces of healthy leaves. On aphid infested leaves nutrients may be derived indirectly from the host. Host sucrose is converted to melezitose in honeydew and this is used by the phylloplane inhabitants.

The number of phylloplane inhabitants increases with the age of the leaf. This association between population density and age of the leaf is usually explained by increase in leaf exudates with ageing. It is also assumed that the restricted availability of the nutrients is one of the main causes of the relatively poor development of the phylloplane inhabitants on immature leaves. There is also evidence that some of these fungi can slowly degrade the surface waxes and cuticle and so gradually increase the permeability of the epidermis. Their numbers are also far greater on leaves infected by pathogenic fungi, such as rusts and mildews. Four to five times as many colonies of *Sporobolomyces* can be isolated from mint leaves infected with the rust fungus, *Puccinia menthae*, as from healthy mint leaves. Here the injurious effect of the pathogen, especially perhaps the changes in cell permeability, cause an outflow of additional nutrients.

The phylloplane inhabitants are also not uniformly distributed over the leaf surface. Most are more prevalent on the upper surface and are usually more predominant along the veins, frequently with their cells orientated to lie parallel with the vein axis. They also tend to align themselves along the anticlinal walls, as with veins there is a slight

depression there. They could be washed into these positions but there may also be more exudates released along the veins; also vein sheath cells may bring nutrients nearer the surface and thus facilitate exudation.

9.12 COMMON PRIMARY SAPROTROPHS

The common primary saprotrophs are unable to grow to their full extent in the phylloplane until the onset of senescence. Their pattern of development is restricted until senescence and several rarely or never grow on the green leaf. Their spores accumulate on the leaf prior to senescence and remain dormant until the death of the tissues. If they do germinate they do so only to a limited extent. On senescence they very quickly take advantage of the changing conditions. Sporing colonies of these fungi are ubiquitous on newly dead leaves of the majority of plants. The phylloplane inhabitants and the common primary saprotrophs by no means form distinct groups. *Aureobasidium* and *Cladosporium* have to be included in both groups because, although they grow and reproduce by conidia in the phylloplane, they develop to a much greater extent in the dead leaf. Other fungi, all conidial Ascomycotina, in this group include *Alternaria alternata*, *Botrytis cinerea*, *Epicoccum purpurascens* and *Stemphylium botryosum*. In the Tropics the list can be extended to include species of *Curvularia* and *Nigrospora*. Spores of a great variety of other saprotrophs may also be present on the leaves. They germinate only on the death of the leaves or sometime thereafter.

9.13 PATHOGENS

Two distinct categories can be recognized amongst the pathogens found on leaves. There are those from the Plectomycete Erysiphales, the powdery mildews, which are wholly restricted to the phylloplane except for haustoria in the epidermal cells of the host leaf. All their very extensive mycelium, conidia and ascocarps are borne on the leaf surface. The second category, covering virtually all other pathogens, infect leaves and grow almost entirely within them with only their reproductive structures having access to or being produced on the outside. These latter exhibit all gradations, from those which produce an appressorium from their spore and penetrate immediately, to those which have a prolonged and relatively extensive phase of epiphytic non-parasitic hyphal growth on the leaf surface before they penetrate.

The spores of many of these pathogens remain dormant for considerable periods only germinating as host resistance begins to fall prior to senescence or following a suitable change in the weather.

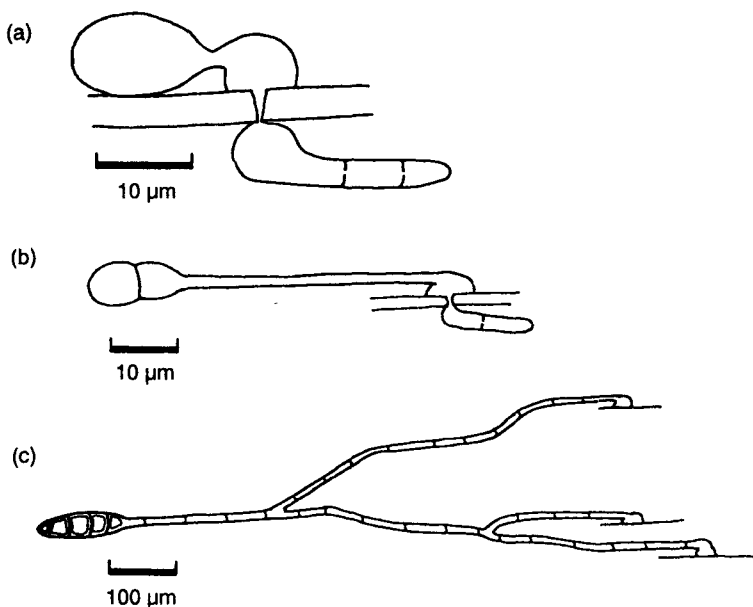


Figure 9.8 Three different types of growth shown by pathogenic leaf-inhabiting fungi on leaf surfaces. (a) *Botrytis fabae*. (b) *Mycosphaerella allii*. (c) *Cochliobolus sativus*.

Again no clear distinction can be drawn between pathogens of this latter type and some of the common primary saprotrophs. *Botrytis cinerea* is one of the latter but is also a necrotrophic parasite of some hosts under particular environmental conditions, such as prolonged very high humidities which favour it but not its host. Its conidia may then germinate on the leaf surface and after a phase of epiphytic growth penetrate and bring about a soft watery rot by means of its pectolytic enzymes.

9.14 EXOCHTHONOUS FUNGI

Spores of pathogens which are unable to infect the leaves on which they have landed may also be present. They may remain dormant or they may germinate before they recognize that they are on the wrong host. They may contribute, like pollen, to the nutrients available on the leaf surface. They could be included with the exochthonous or casual fungi as they are found on leaves but do not grow there. The latter are unable to gain any nutritional advantage from the habitat which is clearly a dead end for many but by no means all. Any soil fungi with air-borne spores may be trapped on leaves and later washed off by rain onto the soil beneath and so they are successfully dispersed.

Spores of many coprophilous fungi on herbivore dung are discharged onto grass leaves surrounding the dung and remain there until the grass is eaten by herbivores. Passage through the gut of a herbivore may be necessary to trigger-off their germination. Direct dung to dung dispersal is abortive. Thus impaction onto leaf surfaces is important if they are to complete their dispersal and life cycle.

9.15 FUNGI OF LEAF SURFACE

The leaf surface is a most inhospitable niche in both physical and chemical terms for fungi. Although transpiration may mitigate against extreme low levels of relative humidity, the fungi are repeatedly dried by the sun and wind and re-wetted by rain and dew. They are not insulated against temperature fluctuations and as such are subjected to marked and very rapid variations in temperature. Even in temperate climates in relatively still air, leaf surfaces may be 10-12°C above ambient in the sun at one moment and in the next 2° below ambient as a cloud passes over the sun. They are exposed to the harmful ultraviolet component in daylight. Nutrient sources must always be fluctuating and low and competition for them must be severe.

9.15.1 Microbial Interactions in the Phylloplane

Interest in the leaf surface as a habitat for fungi has centred mainly on the fact that it is here that any pathogen must spend a critical period of time until it can establish and infect. During this time it is not only subjected to such environmental stresses but it may also be subjected to antagonism from the phylloplane inhabitants as well as from the host itself. A great variety of microbial interactions occur in the phylloplane and in the applied field thoughts are turning to consider the possibility of achieving biological control of some leaf pathogens by building up sufficiently large populations of phylloplane inhabitants. This may be an exceedingly difficult objective to achieve but a consideration of some of the research which led to the development of such ideas gives further insight into the biology of phylloplane fungi.

9.15.1.1 Pollen as a nutrient source and competition for nutrients

The fact that phylloplane inhabitants such as *Cladosporium* and *Sporobolomyces* could benefit from nutrients leaked from pollen grains was convincingly demonstrated by Fokkema. Rye leaves from two separate plots, in one of which the plants had their inflorescences removed or covered, so that no pollen fell onto the leaves below, were taken from plants and washed, twice a week from early June to September, in 1968 and 1969. The washings were plated out onto

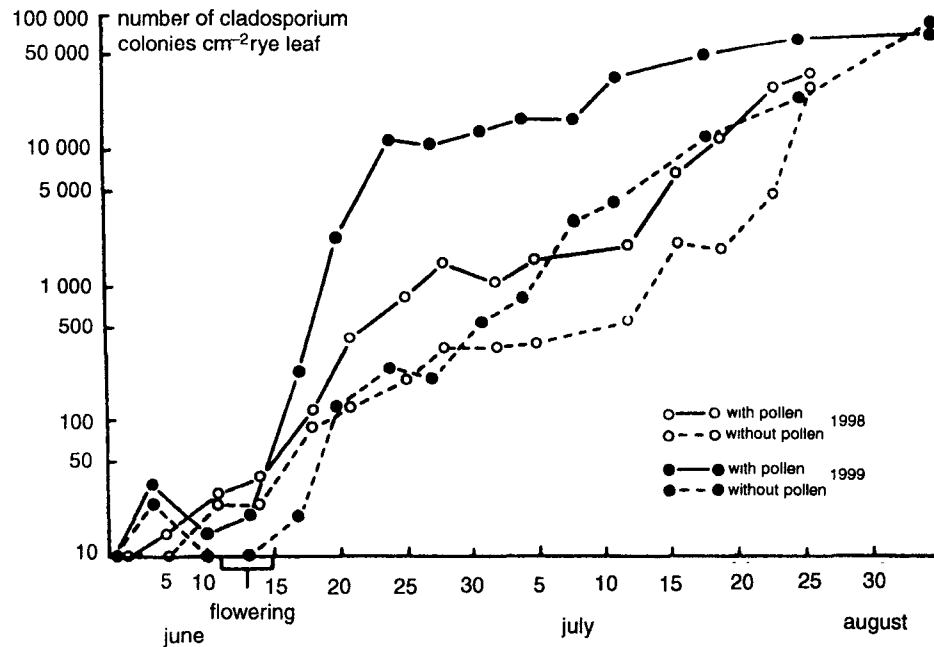


Figure 9.9 The successive changes in the number of *Cladosporium* spp. colonies per cm^2 rye leaf during the season. The data are the means of the numbers of colonies from washings of eight penultimate leaves.

nutrient agar and the colonies of *Cladosporium* spp. which developed were counted, the assumption being made that each colony arose from a single spore. In 1969, the number of colonies from leaves with pollen rose from 15 to 13 000 cm⁻² two weeks after flowering. On leaves without pollen the numbers were 10 and 550 respectively. On leaf senescence the colonies recorded from all leaves reached the

TABLE 9.1 Effect of pollen on successive stages of the infection process of rye leaves by *cochliobolus sativus*

Experiment	Pollen addition	Time after inoculation			
		2-3 days		7 days	
		Mean no. of germ tubes/ 100 spores	Mean mycelium length in mm ⁻²	Mean no. young lesions 10 cm ⁻²	Mean % necrotic area
1	—	23	0	17	2
	+	137	3600	113	58
2	67	250	22	2	
	+	102	3250	56	35

same levels. The leaves at this stage leaked more nutrients and the stimulating effect of the pollen wore off. The larger number of colonies of *Cladosporium* recorded in 1969 was shown to be due to more pollen on the leaves, a mean of 3450 cm⁻² as against 300 cm⁻². There was frequent rain after flowering in 1968 so that the pollen was washed off. Stimulation in the presence of pollen was not restricted to *Cladosporium*. *Aureobasidium pullulans* and *Sporobolomyces roseus* were also stimulated. For the latter, two weeks after flowering, 33 600 colonies cm⁻² were recorded from rye leaves with pollen and only 3800 colonies cm⁻² from rye leaves without pollen.

The Loculoascomycete *Cochliobolus sativus* is a leaf pathogen of rye which makes a variable amount of epiphytic growth before penetration into the leaf. Fokkema inoculated rye leaves with conidia of *Cochliobolus*, together with pollen and without pollen. The effect of the pollen on successive stages of the infection process and the resultant necrosis. Leaves inoculated with *Cochliobolus* and pollen had a significantly larger percentage of necrotic areas. He attributed this to the pollen leaking out nutrients which greatly stimulated the superficial growth of the mycelium. This increased the inoculum and so more young lesions per unit area were obtained and hence eventual necrosis.

In these experiments both the phylloplane inhabitants and the pathogen were relying, in part at least, on the same nutrient source and in nature they might well compete for such a source. Some degree of biological control could be achieved if the phylloplane inhabitants could markedly neutralize the stimulating effect of the pollen when inoculated with both it and the pathogen. This is exactly the effect that Fokkema later observed. The relative inhibitory effect on surface mycelial development of *Cochliobolus* on leaves was depressed by 72% and the extent of necrosis by 75% on rye leaves inoculated with *Cochliobolus*, pollen and *Aureobasidium*, as compared with leaves inoculated with *Cochliobolus* and pollen and *Cochliobolus* and *Aureobasidium* only. Effective competition for nutrients by *Aureobasidium* appears to be an adequate explanation for these reductions.

9.15.1.2 Antagonistic reactions

A number of such interactions have been reported but competition for nutrients is not the sole explanation for some of these. In some cases inhibitory substances produced by the phylloplane inhabitants may also be involved and normal leaf exudates rather than pollen may be

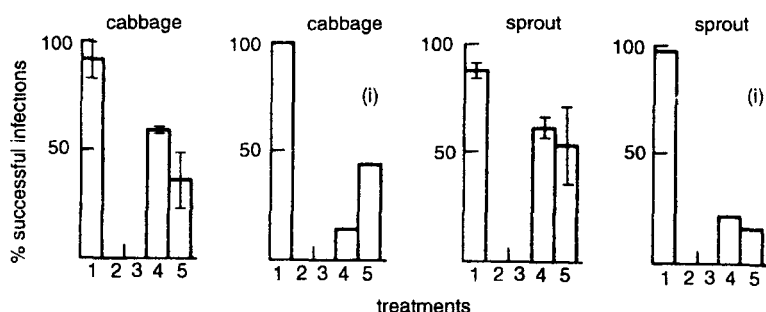


Figure 9.10 Percentage (mean of three values) of successful infections obtained on cabbage and Brussels sprout leaves. Treatments: 1 = *A/ternaria brassicico/a* alone; 2 = *Epicoccum purpurascens* alone; 3 = *Aureobasidium pu//ulans* alone; 4 = *A. brassicico/a* + *E. purpurascens*; 5 = *A. brassicico/a* + *Au. pu//ulans*. The vertical bars are the standard deviations from the mean. (h) = after pre-incubating the antagonist for 14 h.

the nutrient source. Pace and Campbell found that *Aureobasidium pullulans* and *Epicoccum purpurascens* were common in the phylloplane of *Brassica spp.* and that they were antagonistic to the wound parasite *Alternaria brassicicola* in culture. Their growing colonies inhibited mycelial growth of *A. brassicicola* and their germinating conidia inhibited the germination of its conidia. They inoculated leaves of cabbage and Brussels sprout after wounding with *Alternaria brassicicola*,

Aureobasidium pullulans and *Epicoccum purpurascens* separately and with *A. brassicicola* plus *A. pullulans* and *A. brassicicola* plus *E. purpurascens*. There was an 80-100% infection with *A. brassicicola* alone but none with the two saprotrophs alone. The percentage of successful infections by *A. brassicicola* was reduced when it was inoculated with either of the of the two saprotrophs. The reduction was greater when the saprotrophs were inoculated 14 h prior to *A. brassicicola*. Both *A. pullulans* and *E. purpurascens* were capable of active growth on the leaf surface so might compete with the pathogen for nutrients in the form of leaf exudates. This could explain why they were more effective when inoculated 14 h prior to the pathogen. But they may also produce inhibitory substances. Some evidence for this is that a 50% reduction in successful infections was obtained when conidia of *A. brassicicola* were suspended in a culture filtrate of *A. pullulans* and used instead of water for inoculation. The culture medium itself enhanced infection.

9.15.1.3 Towards biological control

These sorts of antagonism must obviously be having some effect in the field but the question to be answered in terms of achieving biological control is how can their effects be maximized? One approach is to manipulate the system to stimulate the phylloplane inhabitants. Biological control using the indigenous population, rather than introducing others, would be possible if a sufficiently large population of antagonistic phylloplane inhabitants could be built up. Bashi and Fokkema have shown that continuous high humidities and nutrients in excess of those exuded by leaves are necessary to maintain a phylloplane population of *Sporobolomyces* dense enough to have sufficient antagonistic potential to control *Cochliobolus*. *Sporobolomyces* is particularly sensitive to low relative humidities. Populations on leaves decreased markedly when maintained at 65% RH. Any added nutrients would have a stimulatory effect on the pathogen as well. To be effective in stimulating only the saprotroph nutrients would have to be added just prior to any significant build up of spores of the pathogen on the leaf surface. This would require accurate disease forecasting. But even so it would be almost impossible to maintain in the field the necessary continuous high humidities. However if such control was possible, it would be applicable only to those pathogens which rely on the absorption of exogenous nutrients to enable them to make superficial mycelial growth on the leaf before penetration. Pathogens which normally penetrate the leaf immediately after germination or which have a very restricted

superficial mycelial growth are probably less susceptible to competition for nutrients. The alternative approach is to allow the natural control measures to proceed and to avoid the indiscriminate use of fungicides which may affect the phylloplane inhabitants more than the pathogen. Pace and Campbell noted that the systemic fungicide Benomyl gave a good control of many diseases but not the leaf spot of brassicas caused by *Alternaria brassicicola*. The pathogen is resistant to it. The two antagonistic saprotrophs which they used, *A ureobasidium pullulans* and *Epicoccum purpurascens* are inhibited by Benomyl. Therefore use of this fungicide could make the disease worse. Fokkema found that *Cochliobolus* is also relatively resistant to Benomyl, and that inoculation of rye leaves with *Cochliobolus* just after flowering (i.e. with pollen), resulted in 60% less necrosis on water-sprayed leaves than on Benomyl-sprayed leaves. At the time water-sprayed leaves had a natural phylloplane population of 10 000 spores cm⁻² and Benomyl-sprayed leaves only 1200 spores cm⁻². This implies that the Benomyl had reduced the antagonistic capacity of the phylloplane inhabitants but it also provides direct field evidence for naturally occurring biological control.

9.15.1.4 Antagonism via lysis, antibiotic production or pH changes

Other forms of antagonism are exhibited in the phylloplane. Bacteria may lyse fungal spores. Chitinolytic enzymes are usually involved. Lenne and Parberry noted clusters of bacteria surrounding lysed conidia and germ tubes of the pathogen *Colletotrichum gloeosporioides* on leaf surfaces. Appressoria are necessary for penetration to occur. The bacteria failed to lyse these. They have melanized walls and there are numerous reports of melanized structures resisting the lytic action of bacteria. The production of appressoria was enhanced in the presence of bacteria but was reduced by added nutrients, such as 1 % glucose peptone solution. The stimulated production of appressoria in the presence of bacteria is a normal response of the fungus to a hostile environment. Desiccation and starvation also cause appressorial formation. This response serves as an important short term survival role during the infection phase. It should be noted that in this particular case added nutrients increased germ tube growth but fewer appressoria were formed. Since the latter are necessary for penetration, added nutrients may in this case enhance disease control.

Several phylloplane inhabitants, such as *Aureobasidium* and *Sporobolomyces* have been shown to produce antibiotics in culture although there is no direct evidence that they play a role *in vivo*. Although antibiotic production by bacteria on leaf surfaces does not

appear to be very widespread, some bacteria have been shown to produce antifungal peptides which, under experimental conditions at least, reduce incidence of disease caused by a number of species of *Colletotrichum*.

Some fungal leaf pathogens are very sensitive to pH changes. In *Septoria nodorum*, for instance, spore germination is inhibited below pH 6. Conidia of this fungus, placed around the edge of a growing colony of *Botrytis cinerea*, failed to germinate. The pH of the medium fell to below 6 in advance of the hyphal tips of *Botrytis*. Such a mechanism could operate in the phylloplane.

9.15.2 Fungistatic Substances Produced by Leaves

In addition to nutrients, leaves of many plants may exude fungistatic substances which cause inhibition of spore germination or restriction of germ tube growth. Phenols are the most widely known fungistatic substances produced by leaves. They are responsible for the inhibition of spore germination of the apple scab fungus, *Venturia inaequalis*, on some apple cultivars. Gallic acid has been identified as an antifungal component in droplets of dew obtained from sycamore leaves. Apart from these substances formed within the leaf cells and exuded onto the surface, some constituents of the cuticular waxes may also be fungistatic. An acidic ether-soluble fraction from the wax of apple leaves inhibits the growth of the apple mildew fungus, *Podosphaera leucotricha*. The properties of waxes on leaves will also affect the exudation of both nutrients and anti-fungal substances. Waxes with high proportions of more hydrophobic constituents will tend to limit the movement of exudates to the surface.

Thus at the leaf surface a series of complex interactions occur between pathogen/host/phylloplane inhabitants/environment. Numerous aspects and the outcome of many of these interactions are still to be discovered, but it is evident that the phylloplane inhabitants act in some sort of buffering capacity against some pathogenic fungi at least.

9.16 COMMON PRIMARY SAPROTROPHS

Eventually the leaf senesces either naturally or prematurely after supporting, in some cases, one or more pathogens. Of the multitude of fungal spores of a vast array of a species which are impacted onto leaf surfaces only relatively few succeed in colonizing the leaves as they senesce and grow as active saprotrophs within the leaf tissues after death. These common primary saprotrophs are virtually ubiquitous colonizers. On most leaves such as those of deciduous trees, shrubs,

herbs, grasses including cereals and even bracken, most are usually present and exceptions are difficult to find. Pine needles are a particularly selective substrate and of these fungi only *Aureobasidium pullulans* is ever at all common. In the tropics, *Alternaria alternata* is less common and is replaced by *Nigrospora* spp., especially *N. sphaerica*, and *Curvularia* spp., especially *C. lunata*, as is evident from examining senescent leaves of guinea grass (*Panicum maximum*) and banana (*Musa sapientum*). The differences are also reflected in the comparison of the dry air-spores of tropical and temperate climates. The association of these particular fungi has been noted on other substrates, such as cereal stubble and cotton fabrics exposed to the weather. The blackening of the ears of cereals in a damp season is caused mainly by *Alternaria*, *Cladosporium* and *Epicoccum*. Christensen and Kaufmann, in their studies on the deterioration of grain, designated these and others, such as *Chaetomium*, *Fusarium* and *Rhizopus* spp., as 'field fungi'. This is an appropriate term as they are almost always and constantly associated with exposed freshly decaying green parts of plants. On leaves they are usually associated with one or more other saprotrophs which are more restricted in the range of leaves which they colonize. These restricted primary saprotrophs may be confined to a particular host genus or a related group of plants. *Readeriella mirabilis* and *Piggotia stellata* appear to be restricted to *Eucalyptus*. Several species of *Leptosphaeria*, such as *L. microscopica*, are restricted to the Gramineae and *Fusicoccum bacillare* and *Sclerophoma pith iophila* are both very common on pine needles but the latter, at least, is also found on other coniferous leaves. In many of these substrate specificity might be synonymous with, and explained by, host specificity. Many of these, although very active saprotrophs, may have an additional advantage in that they can gain access as parasites. *S. pithiophila*, for instance, has been associated with the defoliation of the current year's needles of *Pinus sylvestris*.

As a group these common primary saprotrophs may be well-established in leaves long before leaf-fall. For example *Cladosporium herbarum* often colonizes and produces conidia on damaged necrotic parts of beech leaves in June, within two months of their unfolding. The duration of their persistence on leaves once they are in the litter is dependent upon many variables, one of which is the texture and another the composition of the leaves. In general, tree leaves, such as those of ash and sycamore, which decompose and disappear rapidly from the litter, support a more substantial growth of these common primary saprotrophs for a shorter time than do leaves of beech and

oak which persist much longer in the litter. On beech leaves, for example, *C. herbarum* persists in high frequency through the winter after leaf-fall until the following June and disappears after September.

Similar sequences can be found on other substrates. Primary saprotrophs are the first fungi to appear on flowering stems of cocksfoot, *Dactylis glomerata*. They are present on the basal leaves in early summer and progress up the stems as successive leaves senesce. They are well-established by July and August on the upper leaf sheaths and internodes of stems which flowered in late May and June and they persist there until the following summer. On nettles, *Urtica dioica*, primary saprotrophs colonize the upper leaves in August or September of the year of flowering, at the onset of basipetal senescence. They again persist throughout the winter until the following spring and summer.

9.17 ATTRIBUTES OF THE COMMON PRIMARY SAPROTROPHS

The intriguing aspect of this particular facet of fungal ecology is to ponder why so few of all the fungi are equipped to assume this role of primary saprotrophic colonizers of such exuberantly plentiful substrates.

9.17.1 Nutrients

In the well-known schema for fungal successions proposed by Garrett, the primary saprotrophs to invade are 'sugar fungi'. They are non-cellulolytic and rely upon readily available sugars, such as hexoses and pentoses, and other carbon sources simpler than cellulose, such as pectins and starch. These fungi also normally possess a high mycelial growth rate and a capacity for rapid spore germination. The classic example of such fungi is the Zygomycete Mucorales, common on herbivore dung. Primary saprotrophic sugar fungi are usually very ephemeral because of the transient nature of their substrate. The persistence of the common primary saprotrophs for months on leaves would suggest that they are not confined to such ephemeral substrates. The ability to utilize cellulose is often regarded as essential for saprotrophic fungi and the majority, except most Zygomycotina and Mastigomycotina, can do this. Of this particular group of leaf saprotrophs only *Aureobasidium pullulans* is non-cellulolytic. It probably relies on pectic substances for its carbon sources and this ability is often used to explain its role as a primary colonizer. None of the others is markedly cellulolytic when compared with some of the Basidiomycotina which

later colonize leaves in the litter layer. For instance, Hering inoculated oak leaves sterilized by γ irradiation with *A. pullulans*, *Cladosporium herbarum* and *Mycena galopus*, a Hymenomycete agaric from the leaf litter, and measured loss in mass after six months at 9-15°C. The two former brought about a loss of 2 and 4% respectively and the latter 15-20%. Not all the loss in mass was of cellulose but in the latter case the loss corresponded with the utilization of about one sixth of the total cellulose present. On filter paper cellulose, various isolates of *Alternaria alternata* brought about losses of 4-8% in 14 days and *Epicoccum purpurascens* about 4%. For comparison, under the same conditions the vigorously cellulolytic *Chaetomium globosum* brought about a 10% loss. It must be remembered that mass loss methods measure only the amount of substrate, in this case cellulose, respired and lost as carbon dioxide and water and not the amount incorporated into fungal material. The cellulolytic ability of these fungi thus varies and they all probably use simpler carbohydrates, such as sugars and starch, as long as they last and then go on to utilize cellulose even if to a limited extent and slowly. Thus they persist. This may be placing undue emphasis on their carbohydrate nutrition to the neglect of their nitrogen requirements. The nitrogen supply might be extremely critical in determining their distribution. In culture they can all use nitrate, ammonia or amino acids as their sole nitrogen source but nothing is precisely known as to what sources are available to them within the leaf. Indications of the overriding limitations of their nitrogen supply are seen when leaves are amended with an available source. Foliar applications of 5% urea solution, after harvest but before leaf fall, prevent ascocarp development in the apple scab fungus, *Venturia inaequalis*, on the overwintering leaves and is used as a control measure to limit the ascospore inoculum available to infect the newly emerging leaves in the following spring. Birchill and Cook in studying the mode of action of the urea demonstrated that both chemical and microbial changes occurred in the leaves after treatment. Marked alterations occurred in the composition and density of fungal and bacterial populations present on treated leaves. The urea in particular enormously increased the relative abundance, as assessed by the number of conidia produced, of both *Cladosporium spp.* and *Alternaria sp.* So marked was the development of the conidia and conidiophores of *Cladosporium* that they could be seen with the naked eye as olive green lawns. Many times more conidia were produced overall on the treated leaves suggesting that the added nitrogen enabled them to utilize more carbon sources and thus outcompete *Venturia* for substrate. Application of urea to fallen pine needles also dramatically

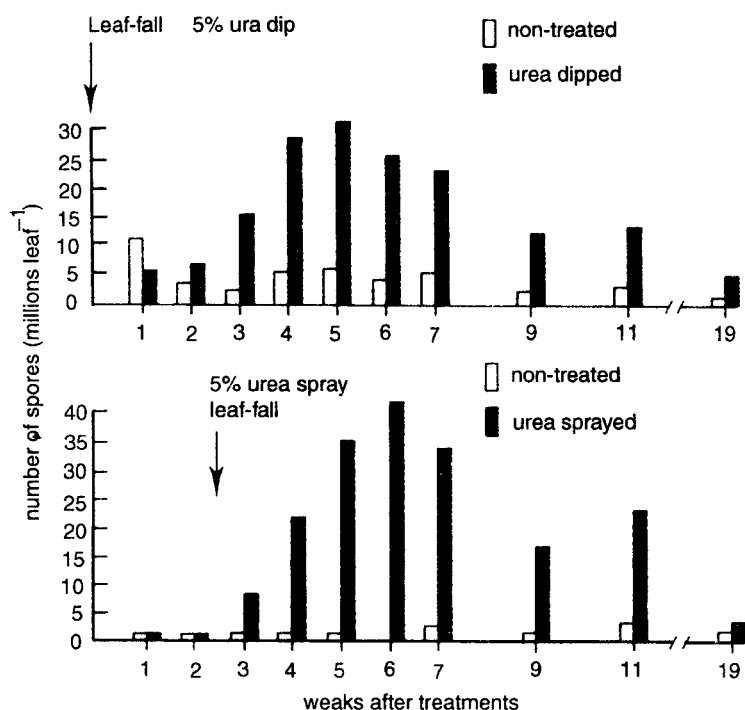


Figure 9.11 Effect of urea dip and urea spray applied 17 October 1967 on numbers of *Cladosporium* spores washed from overwintering leaves.

changes the fungal succession. *Cladosporium herbarum*, rather than being an occasional inhabitant, is again stimulated to develop to such an extent that its conidiophores may cover the needles as a dense felt and other common primary saprotrophs, such as *Epicoccum purpurascens*, which does not normally occur on pine needles, are stimulated to develop by the urea. The mode of action of the urea is not known in this case but is complex; its property of acting as an alkali may be one important aspect of its effect. For example, several agarics which have not been recorded from pine litter appear when plots are treated with urea or alkalis. An example is *Myxomphalia maura* which is characteristically found on the alkaline ash of bonfire sites on acid soils in coniferous woods. It is not found on woods on alkaline soils so is not a calcicole. *M. maura* is markedly encouraged by the addition of lime to pine litter. Pine needles treated with sodium carbonate are also colonized by *C. herbarum* and *E. purpurascens*. Urea and alkalis both produce similar effects on the litter. They both cause it to darken, become water-soaked and raise the pH from about 3.5-4.0 to 5.5-6.0.

They both bring about the release of ammonia from the litter and its use as a nitrogen source may be another important factor inducing these changes.

9.17.1 Growth Rates

These saprotrophs are not primary colonizers solely because they grow faster than any other would-be colonizers. Their only attribute with regard to growth rate is their great variability. *Aureobasidium pullulans* produces its slimy conidia very rapidly, but its yeast-like colonies are relatively slow growing. *C. herbarum* also sporulates rapidly but its growth rate is even slower. *Botrytis cinerea* grows very rapidly and *Alternaria alternata* and *E. purpurascens* not so rapidly but faster than *C. herbarum*.

9.17.2 Tolerance to Desiccation

Senescing leaves on the tree and recently fallen leaves are very prone to drying out and also subject to strong sunlight. Webster and Dix compared the growth rates, latent period for germination, germ tube growth rate at 100% RH and the lowest RH at which spore germination occurred in three primary colonizers with two later secondary colonizers, *Torula herbarum* and *Tetraploa aristata*. They found that there was little difference between the capacity of the mycelium of the various colonizers to grow at low humidities and the primary colonizers did not make better growth at low humidities. But it can be seen from Table 2.2 that under favourable humidities (100% RH), *A. alternata* and *E. purpurascens* not only grew faster than the secondary colonizers but also had a shorter latent period before germination and their germ tubes grew faster. These features, coupled with the fact that their conidia can germinate at lower relative humidities, would give them an advantage over the others in that their conidia would germinate under less ideal conditions of humidity and they would quickly exploit, by virtue of their more rapid growth rate, any changes to more humid conditions.

Because of the rapidly fluctuating conditions on the leaf surface, germinating conidia may rapidly dry out before penetrating into the leaf. Diem has investigated the survival at low humidities of germinating conidia of *C. herbarum*, *A. alternata* and some casual inhabitants of the phylloplane. He found that germinating pigmented conidia, such as those of *Cladosporium* and *Alternaria*, were more resistant than germinating colourless conidia of *Aspergillus* and *Penicillium*. The germ tubes of *Cladosporium* were remarkably resistant. Some 90% grew on at 100% RH after 8 h in a desiccator over anhydrous calcium chloride and 99%

TABLE 9.2 Mycelial growth rate, latent period for germination, growth rate of germ tubes at 100% RH and lowest RH at which spores germinated.

	Mycelial growth rate (mm day ⁻¹)	Latent period tube (h)	Germ growth rate (µm h ⁻¹)	Lowest RH at which germination occurs
Primary colonizers				
<i>Cladosporium herbarum</i>	2.96	6-1	4.2	89%
<i>Alternaria alternata</i>	6.41	3-6	29.1	89%
<i>Epicoccum purpurascens</i>	6.45	0-3	31.6	92%
Secondary colonizers				
<i>Torula herbarum</i>	1.41	12-18	0	Water
<i>Tetraploa aristata</i>	3.34	12-18	2.4	98%

did so after being kept at 40% RH for 8 h. This would indicate that if they germinated in the more humid conditions of the night and had not penetrated into the leaf by the morning they could survive the drier conditions of the day. Germ tubes of the conidia of *Alternaria* were equally resistant but some failed to grow on after periods at relative humidities below 65% but the conidia either germinated again from another cell or from a lateral branch from below the damaged part of the germ tube. In contrast, the germ tubes of the conidia of *Aspergillus* and *Penicillium* were no longer viable after periods at 85% RH. Species with coloured conidia are thus more likely to be successful in the phylloplane and as subsequent primary colonizers of the leaves. But it should be noted that the conidia of *Aureobasidium* and *Botrytis* are not pigmented. The biotrophic Erysiphales which produce their mycelium on leaf surfaces also have colourless hyphae and conidia. Thus pigmentation is a useful but not an essential attribute to possess.

Primary saprotrophs also show an equally remarkable tolerance to desiccation in their hyphal tips as distinct from germ tubes. Hyphal tips are very delicate structures but in these fungi they survive periods of extreme desiccation, some as long as three weeks, above a saturated solution of potassium nitrate. Thus they can rapidly exploit the return to favourable conditions of humidity with no apparent loss of previously synthesized biomass. Other fungi do not show this ability. This attribute may be critical in enabling such fungi to tolerate cycles of wetting and drying.

9.17.3 Survival Structures

Once established on the leaf surface, most of the common primary saprotrophs produce some form of pigmented survival structure: *Cladosporium herbarum* minute micro-sclerotia; *Botrytis cinerea* and *Epicoccum purpurascens* sclerotia; and *Aureobasidium pullulans* aggregates of chlamydospores. All have a pigmented mycelium. Such structures and pigmentation protect against desiccation, ultra-violet light and microbial lysis.

It is thus clear that the common primary saprotrophs possess a multiplicity of attributes by which they have become successfully adapted to this relatively inhospitable niche with each fungus possessing its own particular complex of attributes, not all necessarily the same.

As suggested, in temperate climates some of the common primary saprotrophs produce ascocarp initials in the late autumn in the year of leaf-fall as do a number of leaf pathogens, such as *Apiognomonia errabunda* on beech and *Venturia inaequalis* on apple. Ascospores are discharged from these over the period early April to early June. This is the time when the next crop of leaves is unfolding. The initially spore-free leaves become impaction sites for air-borne ascospores and under favourable conditions infection occurs. Such a life history is of particular significance in leaf pathogens with restricted periods of spore formation and release and where the host virtually frees itself of infection by shedding all its leaves prior to its dormant season. The requirement for an overwintering phase, a period of low temperature (5-8°C), before ascocarp initials mature is very common in these fungi. The maturation and release of the ascospores thus coincide with the breaking of bud dormancy of the host. Thus teleomorphic states of *Aureobasidium pullulans* (*Guignardia fags*) and *Cladosporium herbarum* (*Mycosphaerella tassiana*), which are common on fallen leaves, may be regarded as additional survival structures adding an ascospore inoculum to the conidial inoculum available in the spring.

9.17.4 Subsequent Colonizers and Leaf Decay

These initial colonizers gradually disappear, being replaced by other leaf-inhabiting saprotrophs which begin to reproduce in the late summer of the year after leaf-fall, reach a maximum in the autumn and persist over the winter, until the spring. These include a very wide variety of conidial fungi, such as *Polyscytalum fecundissimum* and *Chalara cylindrospora*, Ascomycotina such as *Microthyrium fagi*, and *Helotium caudatum*, and Basidiomycotina with minute basidiocarps, such as *Lachnella villosa* and *Pistillaria pusilla* on beech leaves. With

fragmentation in the final stages of decomposition, the fungal flora becomes dominated by typical soil-inhabiting fungi, mainly Zygomycete Mucorales, especially species of *Mucor* and *Mortierella*, and conidial fungi, such as species of *Penicillium* and *Trichoderma*, together with litter-decomposing Hymenomycete Agaricales, such as species of *Collybia* and *Mycena*.

The soil-inhabiting fungi grow up from the soil via the continuum of organic debris. The role which they play in the decomposition process has not been fully elucidated. At this stage the Mucorales are certainly not using any simple carbohydrates initially present in the leaves as these would have been utilized already. They could be living in association with the cellulolytic Agaricales as commensals by taking a share of the hydrolytic products of cellulose and thus acting as secondary saprotrophic sugar fungi rather than primary ones. Alternatively, they could be primary colonizers of the wealth of faecal pellets produced by the micro-fauna, especially mites, as they are on pellets of *Glomeris*. The H layer of the soil is particularly rich in chitin in the form of hyphal wall fragments and exoskeletons of insects or other chitinized remains of the micro-fauna. Species from several common genera of soil-inhabiting fungi, including *Mortierella*, *Penicillium* and *Trichoderma*, have the ability to break down this very resistant substrate and their activity may well represent one of the final stages of the mineralization of primary and secondary organic materials in the soil.

9.18 DECOMPOSITION OF PINE NEEDLES

The time period between leaf-fall and the final decomposition of a leaf varies enormously. In cool North temperate pine forests it may be 10 years or more, in ash and sycamore under 1 year and in tropical forests mere weeks. Pine needles are extremely durable and decay very slowly. Their decomposition most often results in the formation of a mor type of soil. The needles are shed mainly in August and September and there is an accumulation of considerable bulk of leaf litter, each successive leaf-fall burying the previous one so that a stratified litter layer is produced. The animals in the litter are sufficiently small not to disturb this stratification and such a litter layer well illustrates the diversity of organisms, fungi and animals, involved in the decomposition process. A very considerable amount of potential energy is available for micro-organisms in this litter. In *Pinussylvestris*, production of needles accounts for about one third of the total productivity and accounts for about 60-80% of the total litter. Although the decay process is a continuous, if fluctuating, one, it is convenient to recognize a number

of stages. The A horizon may thus be divided into L, F₁, F₂ and H layers. The L layer consists of freshly fallen, undecomposed needles, light brown to buff in colour and others somewhat darker in colour, which have fallen earlier. Needles remain in this layer for about six months. They all have a high tensile strength, a relatively low but fluctuating moisture content and form a loose, uncompacted layer on the litter surface. In this layer the needles are very susceptible to drying out and conditions are unfavourable for continuous fungal growth. In the upper parts of the F₁ layer, the needles are grey, becoming dark brown with depth but recognizable as needles. Their tissues become softened and they have a low tensile strength and a high moisture content. They remain in this layer for about two years. Below, in the F₂ layer, the character of the needles again changes. They are greyish, fragmented and compressed, but again still recognizable as needles. The mesophyll collapses and most bear dark amorphous faecal masses of the micro-fauna. Eventually the remains of the needles enter the H layer which consists of an amorphous mass of faeces and the remains of both the micro-fauna and fungi, the needles having undergone complete physical reduction. Below this layer is an intimate mixture of humus and mineral soil.

Two factors that may greatly influence the sequence of decomposer fungi on the needles are the time at which they fall and their previous history. Pine needles are far from being a homogeneous entity and at needle fall vary in age, physical structure, nutrient content and the presence or absence of fungal colonizers in or on the needles. The needles have a very thick, waxy cuticle and support a much sparser population of phylloplane inhabitants. *Sporobolomyces roseus*, although present on most attached needles, occurs in very low frequencies only. This contrasts markedly with its abundance on leaves of deciduous trees and herbaceous plants. It decreases rapidly on needle fall, whereas some other yeasts, such as *Bullera spp.*, increase in frequency and persist. A number of other mycelial fungi such as the conidial *Sclerophoma pithiophila* may grow and sporulate on the leaf surface.

Vigorous ones, such as *Lophodermella sulcigena*, an apothecial Ascomycete, and *Coleosporium senecionis*, a rust, cause premature needle cast, either directly or by predisposing first year needles to infection by secondary pathogens. For example, *L. sulcigena* infects young first year needles and predisposes them to infection by *Hendersonia acicola* or *Lophodermium pinastri* and finally *Naemocyclus niveus*, which cause the needles to fall in their first summer. Such weak pathogens

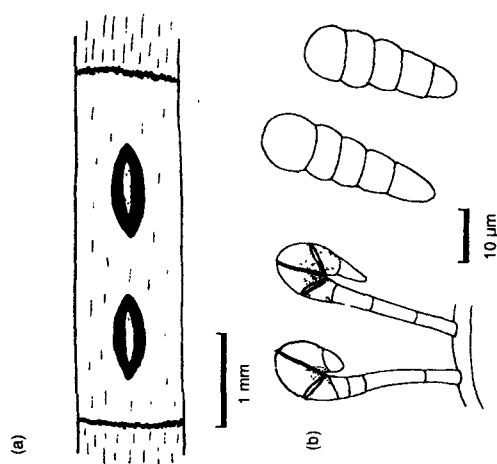
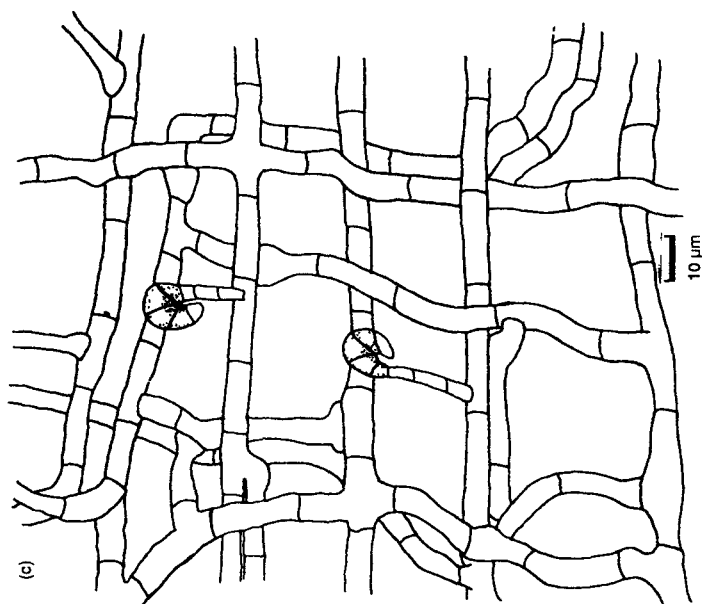
may colonize the needles directly but spread very little until senescence. They may also gain access via tissues damaged by insect pests. Living needles may also be colonized by *Fusicoccum bacillare* or *Sclerophoma pithiophila*. Needles infected by either of these two conidial fungi soon die and turn brown but remain attached to the tree. Such needles again fall in the summer. *S. pithiophila* is also a frequent colonizer of needles containing high nutrient levels, such as first year needles, shed while still green and of needles of felled pines. Clearly pine needles can fall at varying times of the year and may already be colonized by a variety of fungi which have already initiated the process of decomposition. *Lophodermella sulcigena* actively decomposes the mesophyll tissue and *Hendersonia acicola* may remove much of the cellulose, reducing the needle to a skeleton of epidermal waxes and lignified tissues. *Lophodermium pinastri* produces pigmented diaphragms across the needles delimiting the extent of its colonization. Such parts later escape extensive internal attack by saprotrophic needle-inhabiting fungi. This is often attributed to their inability to penetrate the melanized diaphragms but in culture at least *Lophodermium* produces powerful antifungal antibiotics and they may also play a part in restricting saprotrophic colonization. Such parts of the needles decay more slowly than uninfected parts and as a consequence accumulate in lower layers of the litter. The saprotrophic colonization of naturally fallen needles and needles shed after parasitic attack may thus be distinct.

Most needles, the bulk being second and third year ones, falling in August and September, are colonized by *L. pinastri* and somewhat fewer by *S. pithiophila*. Soon after needle-fall, a dark brown to black hyphal network develops on the surface of the needles. A number of fungi may be involved, including the conidial *Sympodiella acicola* and *Helicoma monospora* and, in drier situations, the ascocarpic *Kriegeriella mirabilis*. There is no apparent penetration of the needles by the surface hyphae although erosion of the needle surface does occur. The hyphal network shows marked linearity with the hyphae growing longitudinally along the cell boundaries. Internally the needles become colonized by *Desmazierella acicola*, which produces its conidial state from compacted, pigmented, hyphal cushions formed over the stomata. In spite of intensive grazing of the fungi by the micro-fauna, including mites, springtails and enchytraeid worms, all become more frequent as the needles become incorporated into the more moist regime of the F_1 layer. They persist for two years in the F_1 layer, that is for up to $2\frac{1}{2}$ years after needle-fall. *D. acicola* produces crops of conidiophores in both the first and second summers after needlefall. *L. pinastri* produces its ascocarps in

the L layer over the period January to May, providing the inoculum to infect further needles on the trees. After about 10 months in the L and F₁ layers, it too disappears. In the F₂ layer, which the needles enter in the third year after needle-fall, the micro-fauna assume more importance. The external feeders continue to graze upon the fungal hyphae and reproductive structures whilst the internal feeders rapidly comminute needles attacked by *L. pinastri* and *D. acicola*. Any needle fragments which escape extensive internal colonization become colonized by more general litter inhabitants such as species of *Penicillium* and *Trichoderma* and by pine litter-inhabiting agarics. Needles remain in this layer for about 7 years, by which time the fungi and fauna reduce them to an amorphous mass, typical of the humus layer. The role of agarics in the decomposition of pine needle litter has not been extensively investigated. It is usually assumed that they colonize the litter when it is in a relatively late stage of decay. This is not always so. The tiny agaric, *Marasmius androsaceus*, is very common in pine needle litter. It is often called the 'Horse hair fungus' because its stalk is shiny black, like horse hair and is of about the same diameter. *M. androsaceus* colonizes the needles very shortly after needlefall. Its delicate black, cotton-like rhizomorphs grow up from previously colonized needles below, binding them together in a loose tangle. Dense masses of basidiocarps may appear on the needles in the litter, any time from May to November. It is both strongly cellulolytic and ligninolytic and causes very extensive internal decomposition. The role of such Basidiomycotina should not be underestimated. Their mycelium is often prolific in both the L and F layers. Long lists of agarics have been recorded from pine woods. Richardson has estimated the total productivity of these in a woodland of *Pinus sylvestris* in Scotland, to be between 0.25-0.5 million basidiocarps $10^4 \text{ m}^{-2} \text{ y}^{-1}$. The majority are produced from August to September. However, because of our inability to distinguish species of Basidiomycotina from their mycelium and because we know insufficient about the biology of some of these, the problem is to assess the relative contributions of the litter decomposers and the mycorrhizal fungi. Since many agarics in the litter decompose both cellulose and lignin, it is probably delignification that reduces the needles to a greyish colour in the F₂ layer.

9.19 LITTER MICRO-FAUNA

As in other litter systems, the micro-fauna are important agents in the decomposition process. Mites and springtails cause considerable



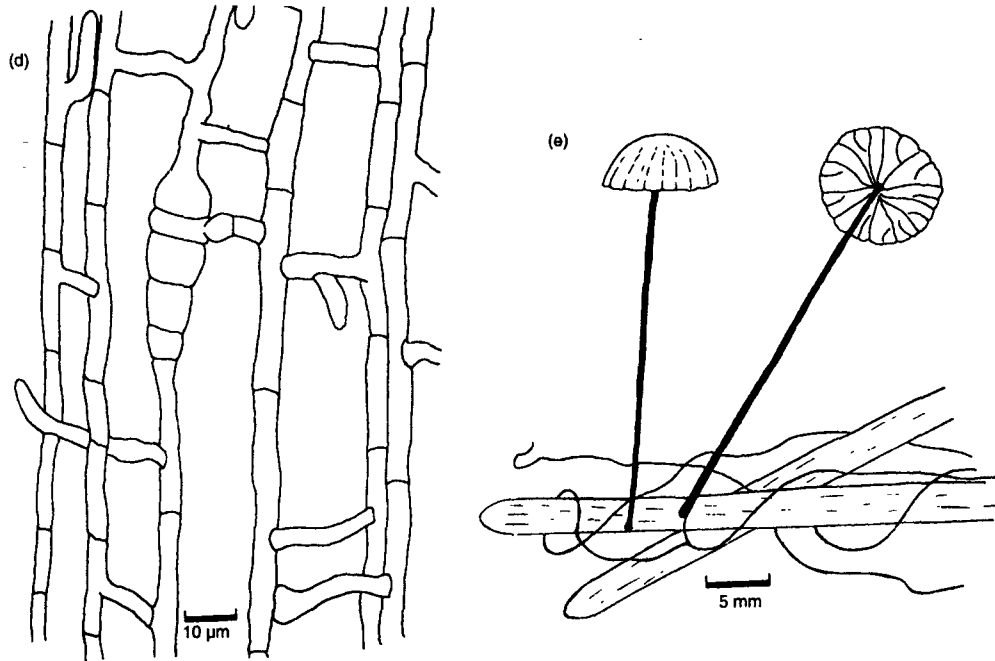


Figure 9.12 (Left) (a) Two ascocarps of *Lophodermium pinastri* and diaphragms across a pine needle. (b) Conidia of *Helicoma monospora* and ascospores of *Kriegeriella mirabilis*. (c) Conidia and hyphal network of *Helicoma monospora* on the surface of a pine needle. (Above) (d) Ascospore and hyphal network of *Kriegeriella mirabilis* on the surface of a pine needle. (e) Thread-like rhizomorphs and basidiocarps of *Marasmius androsaceus* on pine needle litter.

comminution of the needles and in so doing convert them to faecal pellets. It has been estimated that a pine needle with a surface area of 180 mm² would have a surface area of 1.80 m² after comminution to faecal pellets by micro-arthropods. Such comminution would present a much larger surface area to microbial enzymes and thus be expected to increase the decomposition rate. This may not always be so. Oribatid mite pellets persist longer than the source from which they are derived. This may be due to the nature of the substances cementing the particles of the pellets together and digestion by the animal of the more easily decomposable components of the litter. It is clear, however, that the water holding capacity of the pellets is higher and the rate of evaporation from the pellets decreased. This creates a higher and more stable moisture regime which again should favour microbial activity.

The enchytraeid worms, which are very abundant in podzols in northern coniferous forests, in addition to their grazing activities, play a vital role in the absence of earthworms in this mor type litter, in mixing the amorphous remains with the mineral soil. The major group of animals involved in the decomposition of pine needle litter are mites, many of which are strictly mycophagous, with some showing marked preferences for particular fungi. They are most abundant in the moister F layers where fungi are also more active. Protozoa and nematodes also occur in pine litter and feed on the contents of living fungal hyphae. There is no clear evidence to support or refute the hypothesis that mycophagy by the micro-fauna stimulate the growth of fungal mycelia.

Perhaps the most important effect of the micro-fauna is that they act as a reservoir of plant nutrients as they do in decaying wood, gradually making available the minerals which have become immobilized in the fungal hyphae. The large number of different species involved and their varying life spans mean that the nutrients contained in their tissues are only gradually mineralized.

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