Chapter 10

THE CONTROL OF FOULING BY NON-BIOCIDAL SYSTEMS

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10.1 INTRODUCTION

Fouling results in the loss of efficiency of ships, buoys, pilings, offshore platforms, sonar domes and a variety of piping systems (Haderlie, 1984). Fouling has been controlled traditionally by antifouling paints which prevent settlement of organisms through the release of biocides, chiefly metallic e.g. copper, or organometallic compounds e.g. triorganotins (Evans, 1981; Fischer et al., 1984). Recent studies on the environmental impact of antifouling paints (Alzieu et al., 1980; Waldock & Miller, 1983; Waldock & Thain, 1983) have led to renewed interest in the development of non-biocidal antifouling systems (see Milne & Callow, 1985; Gucinski et al ., 1984; Meyer et al., 1984). Non-biocidal antifoulings aim to utilize surface effects which result in non-adhesion of organisms or at worst, in weak adhesion so that fouling organisms are easily removed, for example, during passage of ships through the water or in the case of small yachts, by hosing.

The first event on exposure of a 'clean' surface to any natural aqueous environment (salt or fresh water) is the spontaneous adsorption of an organic layer principally of glycoproteins producing a modified substrate which, irrespective of the original surface characters becomes hydrophilic and negatively charged. This is then rapidly colonized by pioneer bacteria whose exudates in turn further modify the surface (Baier 1980, 1984; Baier et al., 1983). Larvae of macrofouling forms as well as members of the microfouling slime community (principally diatoms) attach to what has quickly become, a heterogenous substrate (Mitchell and Kirchman, 1984; Cooksey et al., 1984).

The adhesives produced by marine fouling algae range from various types of glycoprotein e.g. Enteromorpha (Callow & Evans, 1974), Ulva (Braten, 1975), Ectocarpus (Baker & Evans, 1973; Clitheroe, 1977), and Ceramium (Chamberlain & Evans, 1973; 1981) to the various types of substituted acidic polysaccharides produced by diatoms (e.g. Chamberlain, 1976, Blunn & Evans, 1981). The diatom mucilages are more closely related to those of marine bacteria (Fletcher, 1980) than to the adhesives of spores of macroalgae. The range of chemical

types of cell mucilages and adhesives is thus very diverse. The physical chemistry of bioadhesion has been extensively reported in the literature, particularly with respect to microbial systems (see Beachey, 1980; Berkeley et al., 1980; Allen, 1982). Bioadhesion is dependent on three major components, viz. the cell surface, the separating medium, and the substrate. Since it is not possible to alter either of the two former parameters, any attempts at non-adhesion of cells depend on modification of the substrate. Several approaches to surface modification have been investigated including surface charge (Marshall et al., 1971) and surface energy (Baier, 1980).

Three principle types of low energy surface have been investigated for use as non-biocidal anti-fouling systems. The development of fluoropolymers (Bultman et al., 1984; Griffiths, 1985) and the "tethering" of drag-reducing molecules such as polyox (polyoxyethylene) (Gucinski et al., 1984) are two systems being studied in the United States of America. The third system is that described in this paper viz. silicone elastomers (Milne, 1977; Milne & Callow, 1985). The silicone RTV polymers are based on a backbone of repeating (-Si-O-) units with the non-backbone valencies of the silicone attached to organic radicals (see Threadgold, 1985). The silicone polymers can be variously modified e.g. by inclusion of phenyl-methyl silicone fluid. Results of experiments on adhesion of the diatom Amphora to a range of silicone elastomers, in particular RTV elastomers and raft exposure trials of these substrates are described in this paper.

10.2 MATERIALS AND METHODS

10.2.1. Surfaces. Three groups of silicone elastomer were tested (A) Room temperature vulcanizing (RTV) silicone elastomers cured by the addition of tin catalysts referred to as RTV(i)-(iv). RTV(i) corresponds to the silicone elastomer described in Milne & Callow (1985). (B) platinum cured silicone elastomers referred to as PC(i)-(iii). (C) moisture cured acetyl tipped silicone elastomers referred to MC(i)-(iv). Modification of the elastomers was achieved by the addition of phenyl methyl silicone fluid (PMS) in the ratio of 100:5 (w/w) before curing. Elastomers were brush-applied to 10cm^2 formica panels. Glass microscope slides (75mm x 39mm) were coated using an applicator which produced a band of elastomer 18mm wide down the middle of the slide. Thus each experimental surface had an area of 13.5cm^2. Control surfaces were acid washed glass and P.T.F.E. (teflon). Raft trials were carried out at Newton Ferrers, South Devon, England during 1985.

10.2.2. Cell culture. Axenic stock cultures of Amphora coffeaeformis var. perpusilla were maintained under static conditions in Guillards F_2 medium

(Guillard & Ryther, 1962) in 2 dm^3 Ehrlenmeyer flasks. Instant Ocean artificial seawater was used to prepare the medium. Flasks were maintained at 20°C with continuous irradiance at a photon flux density of 15umol $m^{-2}s^{-1}$ and were subcultured weekly. For cell attachment experiments log phase cultures were shaken by hand and then filtered through nylon mesh, pore size 30um to remove clumps of cells.

10.2.3. Attachment experiments. Elastomer coated slides were randomized in white plastic trays to which a standard volume of log phase Amphora culture was added. The trays were incubated under the conditions described for cell culture for 8-16h. Slides were carefully washed using a standard procedure to remove unattached cells. The biomass of attached cells was then estimated by measurement of chlorophyll$_a$ or adenosine triphosphate (ATP) content. Previous experiments (Pitchers and Callow, in preparation) have established that a direct relationship exists between cell numbers, chlorophyll content and ATP content for log phase cultures of Amphora.

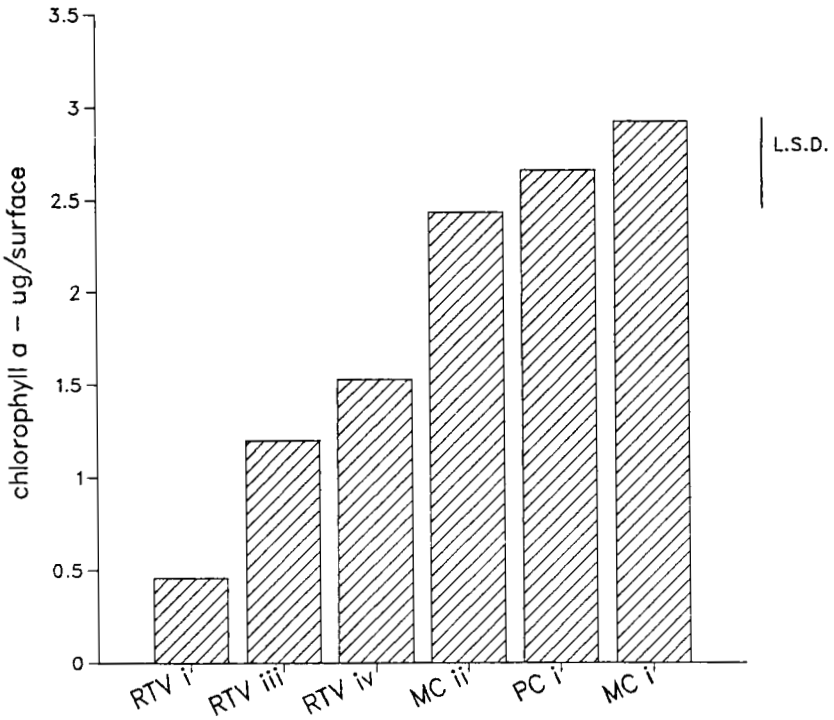
10.2.4. Chlorophyll$_a$. Cells were removed from the polymer surface on each slide (13.5cm^2) using a cotton wool bud which was immersed in 1cm^3 dimethyl sulphoxide (DMSO) to extract chlorophyll (Shoaf & Lium, 1976). Microscopical examination of surfaces after swabbing showed that >99% of cells were removed. After 1.5h in darkness at room temperature, tubes containing cotton wool buds were agitated and the absorbance of the DMSO extract determined at 630 and 664nm. Chlorophyll$_a$ was calculated following Holden (1976).

10.2.5. ATP: Cells were removed from the polymer surface as described above. The cotton bud was placed in 1cm^3 of extractant (0.2% v/v Triton X-100 containing 0.15% w/v disodium EDTA in distilled water) and gently agitated for 5 min. For luminometry 100mm^3 of extract were transferred to a reaction cuvette containing 700mm^3 0.1 M tris acetate buffer, pH 7.75 containing 0.075% w/v disodium EDTA and 200mm^3 luciferin - luciferase monitoring reagent (LKB). Readings were taken immediately after a ten second integration period using an LKB 1250 Luminometer. Readings are expressed as relative light units (R.L.U.) per standard area of surface.

10.3 RESULTS

The attachment of Amphora (measured as chlorophyll$_a$) to a range of silicone elastomers is shown in Fig.10.1. A one-way analysis of variance produced an F-ratio that was highly significant thus, the Least Significance Difference (L.S.D.) test (Parker 1979) was applied (Table 10.1).

Fig. 10.1. Histogram showing attachment of *Amphora* to a range of silicone elastomers.



RTV = Room Temperature Vulcanising ; MC = Moisture Cured ; PC = Platinum Cured

Biomass is measured as chlorophyll a

Table 10.1. Summary of the analysis of variance for data presented in Fig.10.1

Variance due to	Degrees of freedom	Sum of squares	Mean square	F-ratio
Polymer	5	23.13	4.63	37.29(***)
Error	24	2.98	0.12	
		(L.S.D. = 0.46)		

Means found to differ by more than the L.S.D. i.e. 0.46 can be considered significantly different. Thus, from the data expressed in Fig.10.1 three distinct groups appear to emerge in descending order of efficacy viz. RTV(i) : RTV(iii)/RTV(iv) : MC(i)/MC(ii)/PC(i).

An expanded range of silicone elastomers was tested for adhesion of Amphora using ATP measurement as an indicator of cell biomass. Two separate experiments were performed so a two-way analysis of variance was applied to the combined data (Table 10.2).

A highly significant F-ratio for the batch effect coupled with a non-significant difference for the interaction indicated that although a difference existed in absolute values between both experiments differences in ranked order between two experiments could be accounted for by random variation alone. Therefore, the pooled means of the two experiments were used and are shown (Fig.10.2). From the L.S.D. (i.e. 30.3) polymer RTV(i) is significantly different from all the other elastomers. Groups could be derived but there was considerable overlap between members of a group. However, it appeared that the tin - catalysed RTV elastomers exhibited a greater efficacy than either the MC or PC elastomers and application of a one-way analysis of variance showed this hypothesis to be correct (Table 10.3).

The effect of addition of PMS to RTV(i) was tested using glass and PTFE (teflon) as controls. Fig.10.3 shows the ranking of surfaces with respect to adhesion of Amphora cells. From a one-way analysis of variance (Table 10.4) there is no effect resulting from the addition of PMS although the RTV elastomers (+ or - PMS) are both highly significantly different from glass and teflon.

However, slides coated with RTV(i) with and without addition of PMS showed differences in anti-fouling performance when exposed in the marine environment for periods of 3 months or longer. Fig.10.4 shows typical slides after 16 weeks immersion. The PVC control is overgrown by macroalgal fouling, chiefly Ectocarpus siliculosus. RTV(i) bore a mixed diatom slime whilst RTV(i) + PMS is essentially free of all fouling organisms. Addition of PMS improves the antifouling performance of other silicone elastomers immersed for 16 weeks (Figs.10.5 and 10.6). Polymer RTV(ii) (Fig.10.5) had approximately 60% and 20% cover of slime and macroalgae respectively. The addition of PMS reduced the slime cover to 20% and no attachment of macroalgae occurred. Polymer MC(i) (Fig.10.6) had approximately 80% slime and 5% macroalgal cover whilst the addition of PMS resulted in 30% slime cover and no detectable attachment of macroalgae.

Table 10.2 Summary of two-way analysis of variance

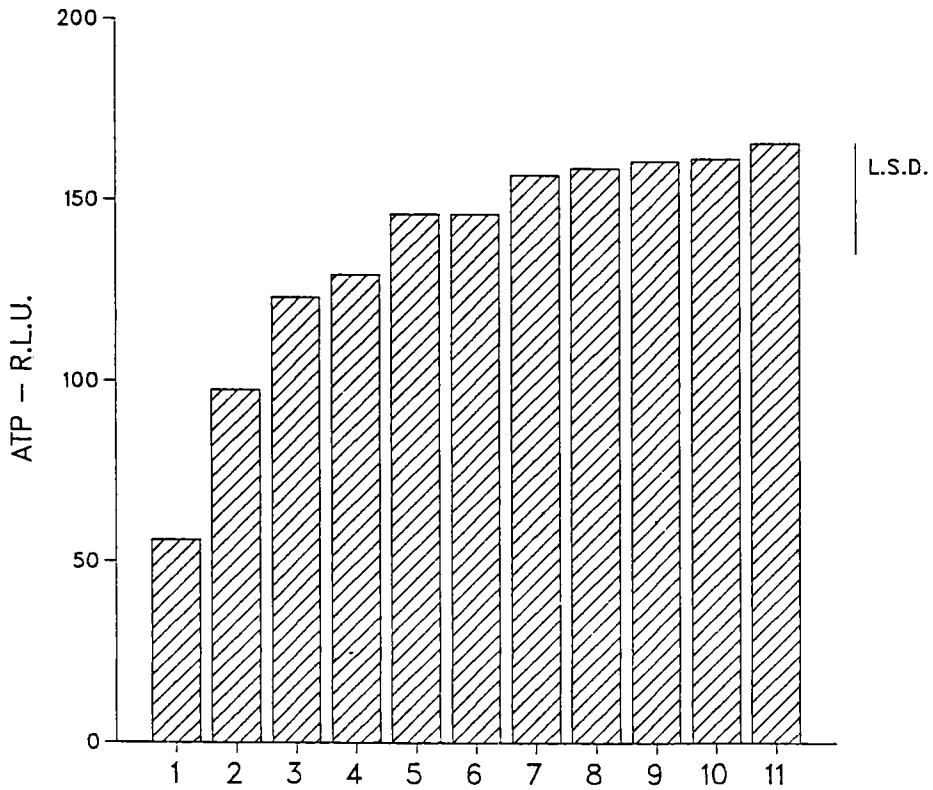
Variance due to	Degrees of freedom	Sum of squares	Mean square	F-ratio
Polymer	10	137704.0	13770.0	9.8 (***)
Batch	1	65901.0	65901.0	47.0 (***)
Interaction	10	22507.0	2251.0	1.6
Error	110	154382.0	1403.0	

(L.S.D. = 30.3)

Table 10.3 Summary of one-way analysis of variance to test the performance of tin catalysed RTV elastomers

Variance due to	Degrees of freedom	Sum of squares	Mean square	F-ratio
Catalyst	1	93418.3	93418.0	42.3 (***)
Error	130	287074.5	2208.3	

Fig 10.2. Histogram showing attachment of *Amphora* to a range of silicone elastomers

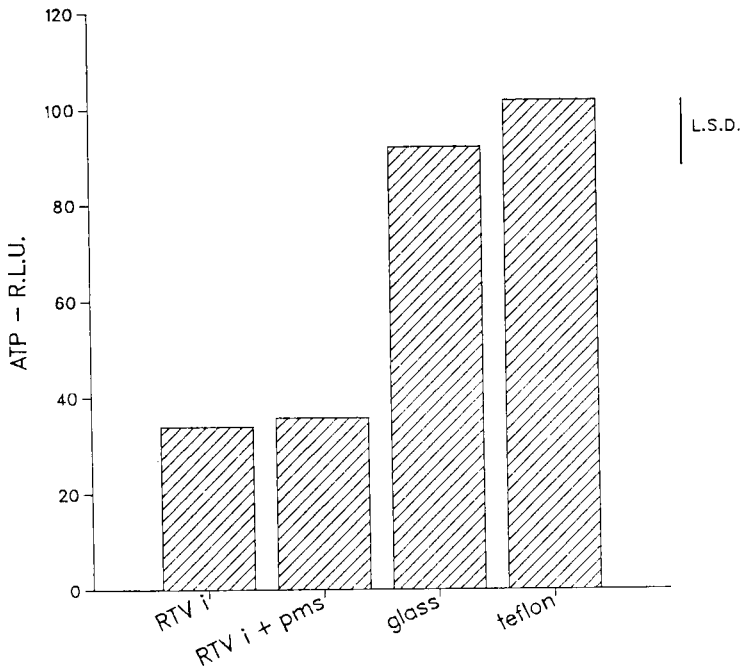


RTV = Room Temperature Vulcanising ; MC = Moisture Cured; PC = Platinum Cured

Biomass is measured as ATP and expressed as relative light units – R.L.U.

Legend	
1–RTV i	6–MC iv
2–RTV iv	7–MC i
3–RTV iii	8–MC ii
4–RTV ii	9–PC ii
5–PC iii	10–PC i
	11–MC iii

Fig 10.3. Histogram showing attachment of *Amphora* to various surfaces



RTV = Room Temperature Vulcanising ;
pms = phenylmethylsilicone fluid.

Biomass is measured as ATP and expressed as relative light units – R.L.U.

Table 10.4 Summary of one-way analysis of variance for data presented in Fig.10.3

Variance due to	Degrees of freedom	Sum of squares	Mean square	F-ratio
Surface	3	31987.2	10662.4	60.1 (***)
Error	29	5141.4	177.3	

(L.S.D. = 13.6)

10.4. DISCUSSION

Amphora was chosen for laboratory studies because of its importance in ship-fouling (Callow, 1986) and for its ease of culturing and rapid attachment to surfaces (Cooksey, 1981). In spite of being derived from a clonal culture, differences in the adhesive capacity of cells between cultures occurs. However, it has proved possible to pool data from separate experiments as shown in Fig.10.2 and Tables 10.2 and 10.3. The rapid attachment process and the continuing motility of cells after attaching led Cooksey (1981) to regard the type of adhesion displayed by Amphora as "temporary" as defined by Marshall and Bitton (1980).

The greatest reduction in adhesion of Amphora occurred on the tin-catalysed RTV silicone elastomers and RTV(i) was best overall. The addition of PMS to RTV(i) in the experiments reported here did not influence adhesion of Amphora although large visual differences in performance were apparent after 16 weeks immersion in the sea. Results previously reported (Milne and Callow, 1985) showed significantly fewer cells attached to RTV(i) whn PMS was added. It is not known whether these differences are due to cell variability or variability between batches of RTV polymer. Analytical studies on polymers are in progress. The techniques used here do not allow small differences in cell adhesion to be determined due to variability between replicates within any one treatment nor do they take any account of surface shear forces. The raft panels are exposed to surface shear stress caused by tidal flow (approximately 2 knots) and this may account for the differences in accumulated fouling between elastomers with and without the addition of PMS. Laboratory experiments where attached Amphora cells were subjected to shear in a radial flow chamber (Milne and Callow, 1985) suggested that the addition of PMS to RTV(i) resulted in enhanced cell detachment under conditions of shear. This line of investigation is being persued using a redesigned radial flow apparatus.

The silicone elastomers tested here are hydrophobic and possess low surface energy properties although to date these have not been quantitatively determined. Attachment and growth of macroalgal sporelings to silane-based coatings of low surface energy (Fletcher et al., 1984) resulted in altered morphology of the attaching rhizoids. In Enteromorpha, compact rhizoidal discs were strongly adherent on high energy surfaces in contrast to the loose filamentous rhizoids which only adhered poorly to the low energy surfaces. However, this response was not typical of all the algae studied (Fletcher et al., 1984). Macroalgae were not found attached to the best silicone elastomer viz. RTV(i) (Fig.10.4), although the PVC control was overgrown by Ectocarpus. It is also interesting to note that when exposed on rafts, those silicone

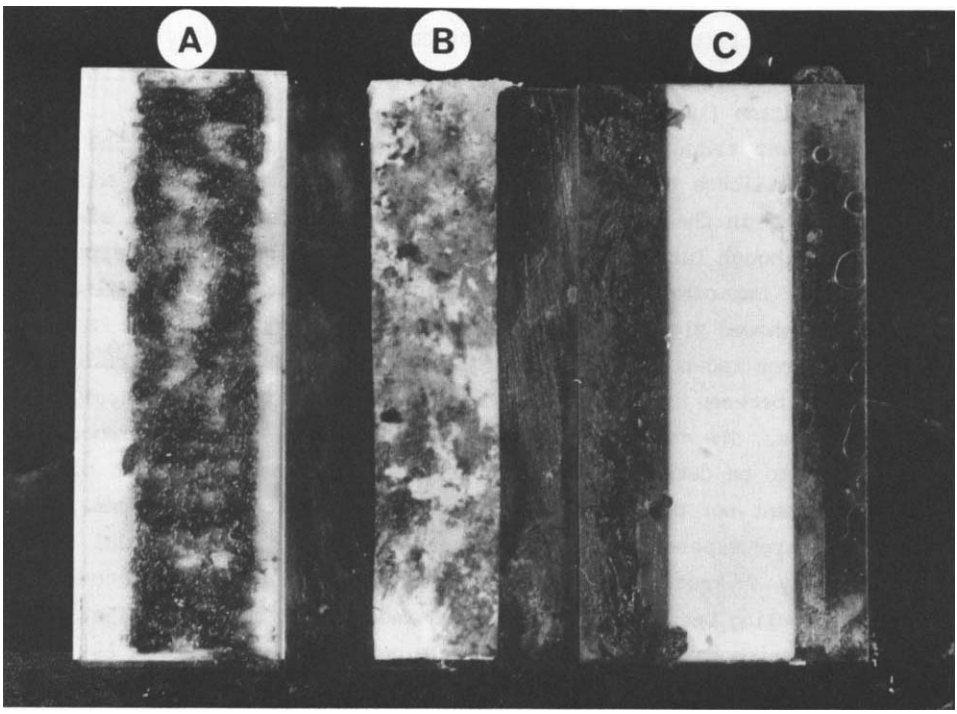
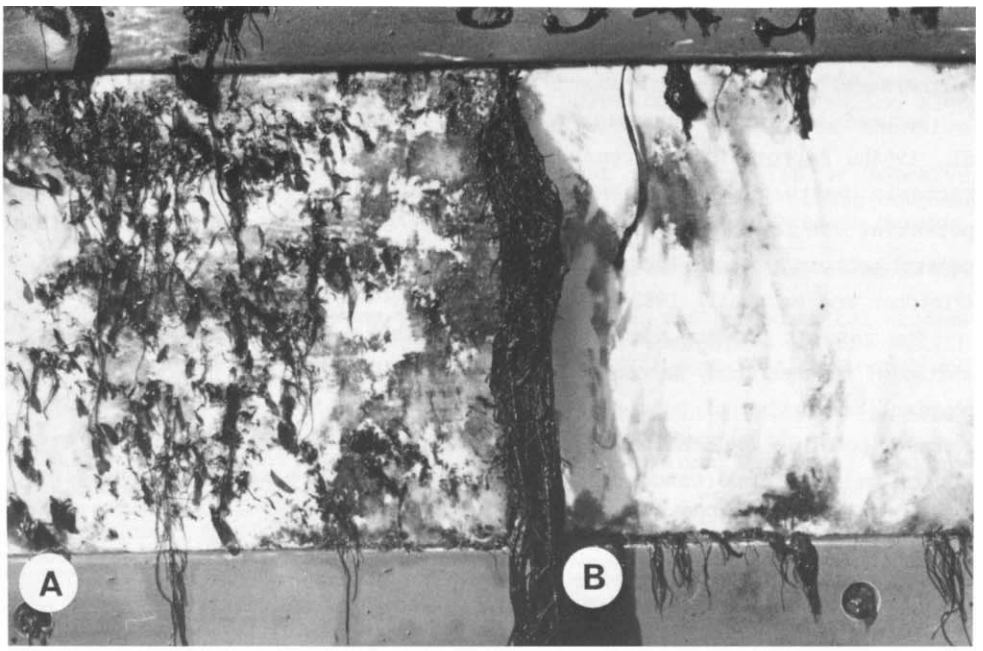
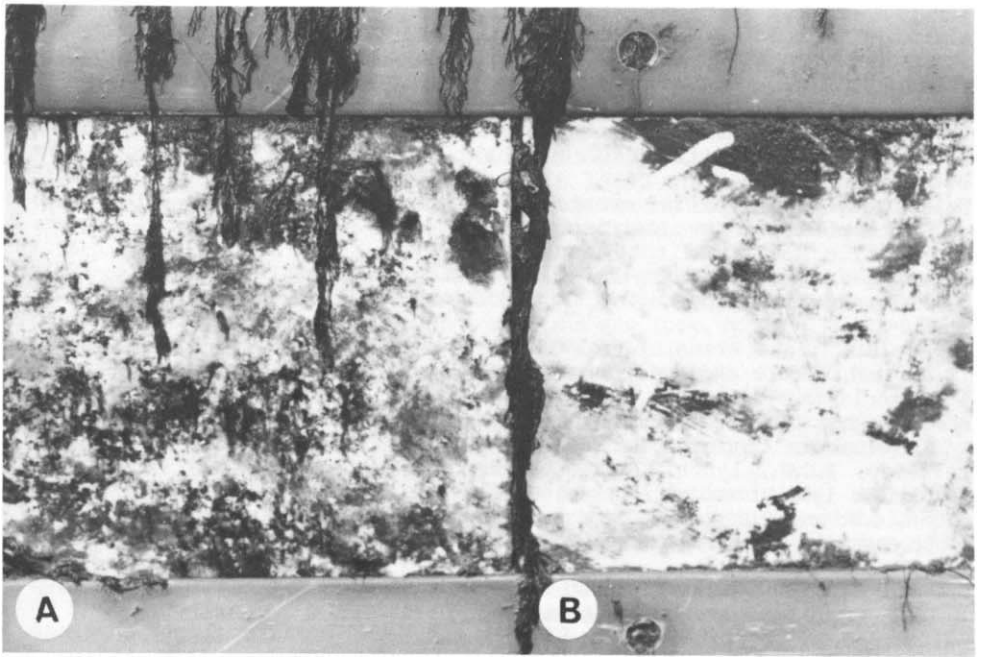


Fig. 10.4. Photograph of slides exposed on test raft for 16 weeks. Note that only the middle strip was exposed thus each side is free from fouling. A = control of PVC bearing a dense growth of Ectocarpus siliculosus and a few plants of Ulva, Enteromorpha and Ulothrix. B = RTV(i) bearing a thick diatom slime. C = RTV(i) + PMS bearing only traces of a diatom slime.

Figs. 10.5 and 10.6. Photographs of panels exposed on raft for 16 weeks. Fig.10.5 (top) A = RTV(ii) bearing a mixed macroalgal fouling growth of Enteromorpha intestinalis, Ulva lactuca, Ectocarpus siliculosus and Polysiphonia sp. and a mixed diatom slime. B = RTV(ii) + PMS bearing a diatom slime. Note - some of the slime has been removed by "brushing" caused by macroalgae attached to the wooden support holding the panels.
Fig. 10.6 (bottom) A = MC(i); B = MC(i) + PMS. Description as for Fig.10.5.


A
B


A
B

elastomers which performed less well than RTV(i) in the laboratory allowed growth and settlement of some macroalgae (see Figs.10.5 and 10.6). Furthermore, macroalgae were absent from the polymers containing PMS.

Reduced adhesion of mussels, barnacles, (Young and Crisp, 1980; Crisp et al., 1984)., Amphora (present communication; Characklis and Cooksey, 1983) and bacteria (Dexter, 1979) to a variety of low energy surfaces supports their potential use for fouling control. However, there are exceptions to this general pattern and some bacteria attach preferentially to low energy surfaces (Fletcher and Marshall, 1982).

The results to date with RTV silicone elastomers indicate them to be worthy of further study as non-biocidal anti-fouling systems. However, their physical properties of poor abrasion resistance and tear strength limit the range of possible applications to situations where such characteristics are not of primary importance e.g. aquaculture, offshore structures, piping systems and power station water intakes.

10.5 ACKNOWLEDGEMENTS

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