4 Macrofouling and Bioadhesion of Organisms on Polymers

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4.1 Macrofouling

A macrofouling community (consisting of 'soft' or 'hard fouling') develops and grows above the microfouling community. Soft macrofouling organisms include algae and invertebrates, including anemones, hydroids, soft corals, sponges, and tunicates. Hard macrofouling organisms include invertebrates including barnacles, mussels and tubeworms. The complete set of animals involved in macrofouling consists of amphipods, barnacles, bryozoans, corals echinoderms, hydroids, isopods, mussels, nemerteans, platyhelminthes, sea anemones, serpulid worms, sponges, and tunicates. Not all these organisms are found in all the oceans around the world. Some of them release a glue which helps them to attach on bare surfaces. Many of these organisms produce an adhesive which help them to attach to immersed surfaces. In some cases the attachment is irreversible and in others it is a reversible process [1].

Barnacles and mussels release small free swimming organisms, which settle and grow to maturity. The protein glue of the blue mussel (*Mytilus edulis*) contains polypeptides which are rich in dihydroxyphenylalanine, which crosslinks through an oxidative phenolic tanning type process. Barnacle larvae can attach even in areas where the water velocities are 2.4–2.7 m/s. The blue mussel prefers stable, low velocity environments. The barnacle's shell remains attached even after its death whereas the shell of a dead mussel breaks loose. Bryozoans, also known as moss animals or ectoprocts are tiny organisms which form a colony. The larvae that are produced attach *via* adhesive sacs to surfaces and undergo metamorphosis to the adult form. They grow as calcified or gelatinous encrustant on surfaces [2]. Hydroids are colonies of tiny stinging jellies, which are colonial in nature. The original polyp anchors itself to a solid substrate with an adhesive and forms a bud [3].

Tunicates, also called sea squirts, are a group of marine animals which spend most of their lives attached to rocks, surfaces or the undersides of boats. They are marine

filter feeders and once born, they immediately find a suitable place to live and become adults. They secrete an adhesive which helps them to attach head first to a suitable spot. Sea tulips, sea squirts, sea liver and sea pork are also from the same family [3].

Serpula are also known as calcareous tubeworms, serpulid tubeworms, fan worms, or plume worms. They are very common in the Pacific, Atlantic, and Indian oceans. They have a pair of calcium secreting glands. Like most tube building polychaetes, worms of the Serpula genus are benthic, sedentary and suspension feeders. They secrete and build a permanent calcareous tube attached to a hard submerged substratum [3].

Sea anemones are a group of water dwelling and predatory animals. A sea anemone is a sessile polyp. It is attached at the bottom to a bare surface by an adhesive foot, called a basal disc. It has a column shaped body ending in an oral disc. Anemones tend to stay in the same spot until the environment becomes unsuitable for it to stay. This may happen because of dry conditions, insufficient food or a predator attack. In such a situation anemones can release themselves from the substrate and use flexing motions to swim to a new location [3]. Most sea anemones attach temporarily to submerged objects, so their adhesive could be different to that of a barnacle.

The green alga, *Enteromorpha*, is the slippery grass-like plant that covers rocks in the intertidal zone and it is a major macrofouling alga. The cement produced by mature adult barnacles, consists of a complex of hydrophobic proteins which are unrelated to the blue mussel proteins and it is crosslinked *via* cysteine residues [3]. More details about the barnacle cement are given later in this chapter.

4.2 Effect of Macrofouling Organisms on Material

Macrofouling organisms form dense colonies. This means large quantities of nutrients and other material are removed from the water and deposited on or in the benthos. This deposition increases further fouling and silt. Macrofouling organisms can tolerate wide fluctuations in the environment and can adhere to submersed surfaces. They develop hard shells or exoskeletons, form dense colonies and produce planktonic larvae. Macrofoulers can attach to concrete, metals, wood, plastics and other synthetic polymers and also to other organisms [4].

Dense layers of macrofouling organisms can cause blockage or reduction in water flow in pipes, mechanical damage, corrosion, and failure of equipment. So macrofouling increases operational and maintenance costs. Macrofouling also changes the physical and chemical characteristics of submersed substrates. When an individual macrofoulant or colonies detach from surfaces their shells and exoskeletons cause

mechanical damage, blockages and corrosion to equipment, and submerged pipes and pumps as well as causing their failure [5, 6].

The quagga mussel (*Dreissena rostriformis bugensis*), and the zebra mussel (*Dreissena polymorpha*), cause damage by clogging screens and pipes and fouling hard substrates. It is reported that such damage cost the municipal water districts in Nevada, Arizona and California millions of dollars per year for additional maintenance and lead to several lake closures [7, 8].

Surfaces such as polyurethane (PU), polyester, silicon rubber (SR), syntactic foam (SF), glass glass fibre reinforced polymers (GFRP) and carbon fibre reinforced polymers (CFRP) become rough because of the action of wind, ocean currents and micro/macro fouling. Macrofouling can affect the tensile strength, elongation, hardness, roughness and contact angle of polymers, rubbers and composites after one year of immersion in the ocean. Tensile strength had decreased by 20–30%, hardness by 10–20% and surface energy by 30–50% (**Table 4.1**) [9].

4.3 Impact of Environmental Factors on Macrofouling

Dreissena mussels are dangerous macrofoulers because they can settle on and attach to hard surfaces even in the absence of a microbial biofilm. Generally such a biofilm is needed by many other fouling organisms. Macrofouling by the golden mussel (*Limnoperna fortunei*) decreases in regions of high flow and turbulence. Light has direct and indirect effect on macrofouling. *D. polymorpha* exhibits strong negative phototaxis, namely, a preference for shaded rather than sunlit surfaces. Light indirectly influences fouling by affecting the water temperature and the amount of phytoplankton growth in the water. The three-dimensional orientation of surface also affects macrofouling, although it is not clearly understood [10].

Chemical parameters such as pH, salinity, concentrations of calcium, magnesium, chlorophyll a, nitrogenous compounds, dissolved oxygen, hardness, organic and other macromolecules, colloidal matter and pollution can all affect macrofouling. The solubility and bioavailability of biocides (e.g., cuprous oxide) is influenced by pH and hardness which in turn affects macrofouling [11]. Rosin is used in the production of antifouling coatings and its solubility increases with increasing pH.

Table 4.1 Physical properties of polymers and composites (control and one year deployed samples)										
Name of the polymers	Surface energy (mN/m)		Root mean square roughness (nm)		Hardness		Mechanical properties			
							Control		After one year	
	Control	One year	Control	One year	Control	One year	Tensile strength (MPa)	Elongation (%)	Tensile strength (MPa)	Elongation (%)
Silicone rubber	21.43 ± 0.15	32.1 ± 2.50	168.49	188.97	17.0 ± 0.58	15.67 ± 0.47	3.32 ± 0.7	203.45 ± 2.40	2.33 ± 0.9	214.7 ± 3.33
PU	36.61 ± 0.20	40.5 ± 1.52	41.37	181.89	43.2 ± 1.58	41.00 ± 0.05	13.58 ± 0.12	276.05 ± 2.72	11.71 ± 0.27	318.55 ± 3.16
Polyester	34.46 ± 0.47	55.59 ± 1.33	28.95	102.75	43.6 ± 1.30	40.00 ± 2.12	54.29 ± 1.62	2.71 ± 0.53	46.94 ± 1.81	2.14 ± 0.47
Syntactic foam	38.32 ± 0.97	63.61 ± 2.31	62.92	317.05	41 ± 1.13	34.33 ± 0.47	28 ± 0.95	4.5 ± 0.39	26.64 ± 0.84	2.13 ± 0.27
GFRP	37.59 ± 1.84	54.75 ± 2.26	365.9	378.32	47.2 ± 1.53	44.00 ± 0.70	147 ± 3.46	5.5 ± 0.48	119 ± 2.39	6.3 ± 0.64
CFRP	47.63 ± 1.37	61.11 ± 2.48	324.8	338.12	37 ± 1.25	30.00 ± 0.02	–	–	–	–

There is a positive relationship between macrofouling and nutrient concentration. *D. polymorpha, D. rostriformis bugensis* and *L. fortunei* all require dissolved calcium to build a calcite shell. Dissolved oxygen reduces the antifouling properties of rosin-based, copper biocide antifouling coatings. The reasons for this behaviour include oxidation of dissolved copper(I) and the partial re-precipitation of copper(II) carbonate, copper(II)chloride or copper(II)hydroxide. Organic pollution affects nutrient and dissolved oxygen concentrations. Solubility of oxygen decreases with increase in dissolved salts. Organic and other macromolecule loadings influence the development of conditioning films. Particulate and colloidal matter adsorb biocides and can alter the reaction and diffusion rates that determine biocide release rates from coatings [12].

In the aquatic system, microalgae secrete extracellular polymeric substances, predominantly (from 40 to as high as 90%) containing a biopolymeric material known as exopolysaccharide (EPS). This extracellular polymeric substance also contains lipids, nucleic acids, and proteins. EPS plays an important role in the attachment and adhesion of cells to surfaces. Organisms that are attached to a surface increase their chances of survival when compared to ones that are in the unattached state. EPS forms a highly hydrated matrix and provides a layer of protection to the cells against toxic compounds or against predation or digestion by other organisms. EPS may also prevent cellular dehydration or damage caused because of ice crystal formation. EPS forms the architectural network of biofilms and aggregates, protecting the cells and assisting their intercellular communications and interactions with each other [13].

Season and monsoon conditions play an important role in the attachment of macrofoulants to surfaces. For example, studies carried out in the coastal water of the Bay of Bengal indicated that attachment of macrofoulants such as barnacles on low-density polyethylene (LDPE), high-density polyethylene (HDPE), polypropylene (PP) and polycarbonate (PC) were seen more during May–July 2009 and hydroids were seen more during the monsoon period (November 2009) than any other months. Apart from barnacles and hydroids, other foulants observed included polychaetes, bryozoans and tube worms. They were not seen in all the samples and their relative amount varied from month to month [9, 14, 15]. *Balanus amphitrite*, on PC, LDPE, HDPE and PP were dominant after the monsoon.

Season also plays an important role in total fouling load, total suspended solids, barnacle and polychaete attachment. Barnacle attachment on SR, PU, polyethylene terephthalate (PET), SF, GFRP and CFRP is highest in September and lowest in January–March (**Table 4.2**). Generally, July is the culmination of the south-west monsoon and November is the start of the north-east monsoon. The ocean temperatures may be lowest during the January–March season which may be one of the reasons for the low number of barnacles observed in the study. PU has the

highest and PET has the lowest barnacle attachment (**Table 4.2**), probably because PU is the most hydrophobic and PET is the most hydrophilic material. The amounts of polychaetes on these materials are highest in May or November. May is the start of south-west monsoon and November is the start of north-east monsoon [15]. Flow velocities of less than 1.2 m/s allow larval settlement. Once settled, these shell colonies can withstand velocities as high as 2.5 m/s. Flow velocities below 0.1 m/s are not sufficient to provide adequate food and oxygen for their growth [15].

Table 4.2 Effect of season on biofouling (micro and macro) on polymers/ composites placed for one year in the surface water of the Bay of Bengal (South India) (SR, PU and PET)

Name of polymers/ composites	Month	Total fouling load (mg/cm^2)	TSS (mg/cm^2)	Barnacle (Number/ plate)	Polychaetes (Number/ plate)
SR	September	38.73 ± 6.82	27.40 ± 6.51	21.7 ± 17.60	17.0 ± 5.00
	November	29.07 ± 6.78	4.24 ± 1.00	1.0 ± 1.00	5.0 ± 2.00
	January	42.40 ± 27.35	14.80 ± 0.40	0.3 ± 0.60	–
	March	34.93 ± 4.69	7.88 ± 1.50	–	0.3
	May	20.67 ± 9.93	16.20 ± 1.80	8.7 ± 4.00	26.0 ± 3.60
	July	36.38 ± 0.63	24.40 ± 2.40	0.3 ± 0.20	4.0 ± 2.30
PU	September	44.73 ± 14.40	22.18 ± 5.12	42.0 ± 8.5	7.0 ± 4.0
	November	37.07 ± 5.13	2.64 ± 0.96	6.0 ± 1.0	7.0 ± 4.0
	January	50.47 ± 7.06	15.40 ± 0.9	4.0 ± 3.0	2.7 ± 1.5
	March	35.22 ± 11.98	7.94 ± 1.8	2.0 ± 2.0	7.3 ± 4.9
	May	41.78 ± 21.44	21.70 ± 2.1	18.0 ± 10.0	35.3 ± 10.1
	July	100.58 ± 35.18	28.50 ± 4.1	14.3 ± 5.9	10.3 ± 2.1
PET	September	72.11 ± 24.02	37.73 ± 8.92	17.3 ± 11.3	13.7 ± 4.2
	November	39.53 ± 2.28	4.73 ± 0.7	5.0 ± 1.5	51.3 ± 9.2
	January	63.09 ± 13.02	15.93 ± 0.7	2.7 ± 1.5	2.0 ± 1.0
	March	86.73 ± 0.10	10.90 ± 0.0	–	2.3 ± 1.3
	May	53.67 ± 3.30	20.30 ± 0.9	9.5 ± 5.7	38.5 ± 12.0
	July	134.07 ± 46.90	29.40 ± 1.1	11.7 ± 5.0	5.7 ± 2.1

SF	September	112.04 ± 15.82	88.09 ± 7.4	38.3 ± 29.5	9.3 ± 0.6
	November	49.91 ± 3.48	6.47 ± 1.3	4.3 ± 1.0	24.3 ± 6.0
	January	144.53 ± 44.91	16.98 ± 0.9	7.0 ± 3.0	3.0 ± 2.6
	March	72.91 ± 20.22	11.90 ± 1.4	3.3 ± 3.1	4.7 ± 3.2
	May	125.00 ± 21.52	30.30 ± 3.1	13.0 ± 7.8	17.3 ± 13.0
	July	163.80 ± 28.26	28.40 ± 1.5	17.0 ± 3.0	8.7 ± 3.8
GFRP	September	49.42 ± 10.21	45.31 ± 10.28	43.3 ± 19.6	36.7 ± 3.2
	November	45.42 ± 5.51	4.80 ± 0.53	2.7 ± 0.6	44.0 ± 16.4
	January	78.51 ± 48.72	16.02 ± 0.8	7.0 ± 4.6	2.3 ± 1.2
	March	74.60 ± 23.35	15.23 ± 2.7	1.0 ± 1.0	10.3 ± 7.4
	May	64.38 ± 9.98	23.42 ± 1.8	13.7 ± 7.4	12.7 ± 7.3
	July	164.62 ± 36.25	25.20 ± 3.5	23.7 ± 9.1	3.7 ± 1.5
CFRP	September	79.51 ± 19.09	54.93 ± 14.0	44.3 ± 19.1	25.0 ± 8.7
	November	45.00 ± 0.01	5.53 ± 0.0	3.0 ± 0.0	20.0 ± 0.0
	January	116.04 ± 45.72	16.24 ± 0.9	5.0 ± 0.0	3.3 ± 0.6
	March	–	–	–	–
	May	–	–	–	–
	July	203.23 ± 38.70	27.5 ± 3.5	17.0 ± 5.7	12.5 ± 3.5
TSS: Total suspended solids					

4.4 Effect of Material Properties

Stiffness of the material plays a role in attachment. The maximum number of barnacles was seen on a stiff surface such as GFRP (23.7/plate) and the minimum number was seen on a flexible surface such as SR (0.3/plate) (**Table 4.3**).

Biofouling of material submerged in the marine environment is initially mostly governed by the surface properties of the virgin material and the bacteria rather than the biological processes. These polymers once placed in the marine environment are first covered by the conditioning film [16, 17]. This is followed by the attachment of microfoulers and macrofoulers. Microfoulers in turn, based on the season, affect the attachment of macrofoulers [18]. The settlement of bryozoans and barnacles on the surface of the polymers differ depending upon the study period [19]. The number of macrofoulers attached on various synthetic surfaces (polymers and composites) as a function of contact angle (an indication of the hydrophilic nature of the surface)

is shown in **Figure 4.1**. As the contact angle increases, the more hydrophobic the surface becomes, and the macrofouling decreases.

Table 4.3 Biofouling data (micro and macro fouling) after one year of immersion of various polymers and composites in surface waters of the Bay of Bengal, South India

Name of the polymer	Fouling load (mg/cm^2)	TSS (mg/cm^2)	Protein (mg/cm^2)	Carbohydrate (mg/cm^2)	Chlorophyll a (µg/cm^2)	Barnacle (Number/ plate)	Polychaetes (Number/ plate)
SR	36.38 ± 0.63	24.40 ± 2.40	1.02 ± 0.14	0.54 ± 0.04	0.54	0.3 ± 0.20	4.0 ± 2.30
PU	100.58 ± 35.18	28.50 ± 4.1	1.15 ± 0.25	0.48 ± 0.02	0.519	14.3 ± 5.9	10.3 ± 2.1
PET	134.07 ± 46.90	29.40 ± 1.1	1.25 ± 0.03	0.63 ± 0.04	0.6	11.7 ± 5.0	5.7 ± 2.1
SF	163.80 ± 28.26	28.40 ± 1.5	1.32 ± 0.07	0.67 ± 0.02	0.625	17.0 ± 3.0	8.7 ± 3.8
GFRP	164.62 ± 36.25	25.20 ± 3.5	0.97 ± 0.40	0.51 ± 0.10	0.867	23.7 ± 9.1	3.7 ± 1.5
CFRP	203.23 ± 38.70	27.5 ± 3.5	1.13 ± 0.04	0.63 ± 0.03	0.467	17.0 ± 5.7	12.5 ± 3.5

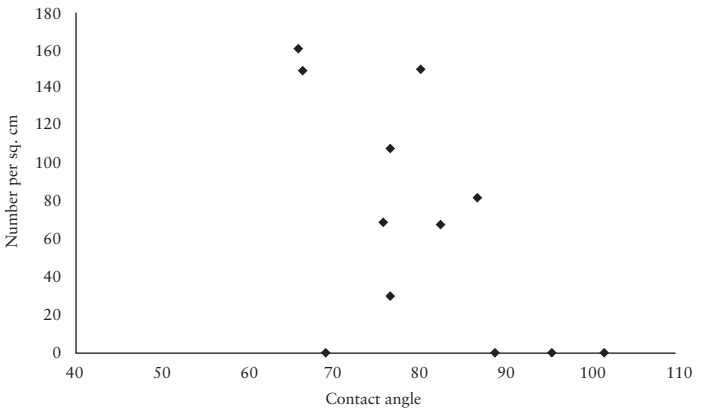

Figure 4.1 Number of macrofoulers at the end of three days placement, on polyvinyl chloride, PP, HDPE, PC, GFRP, Teflon®, SF, acrylic, silicone, PU, polydimethylsiloxane (PDMS) and polyester (correlation coefficient = 0.60)

The effect of biofouling on various polymers and composites such as: PU, SR, polyester (PET), GFRP, CFRP and SF placed for a period of one year in marine waters at a depth of one meter was studied by Sudhakar and co-workers. These materials are used widely in marine applications. SR with the lowest surface energy was the least fouled [11]. The maximum barnacle attachment was seen on a hard surface (GFRP) and the minimum was seen on a flexible surface (SR). Attachment of barnacles and polychaetes are positively correlated with surface energy. Fouling load is positively correlated with surface energy and hardness. The average temperature, pH, salinity and dissolved oxygen of the sea water at the location during the study period were 28 ± 2.04 °C, 8.18 ± 0.08, 30.67 ± 4.16 (ppt) and 4.57 ± 0.31 (mg/l), respectively [11]. Surface hardness also affects the fouling of the surface. Hard surfaces lead to a higher biofilm (**Table 4.4**), than soft surfaces (correlation coefficient = 0.68, $p<0.05$). Surface energy and surface hardness seem to positively affect the attachment of barnacles (correlation coefficient of 0.87 and 0.79, respectively, **Table 4.4**). Similar results were observed by Becker [20], on PC, polytetrafluorethylene (PTFE), and copolymers made from PTFE, and perfluoro compounds and by Crisp and co-workers [21] on slate. The literature mentions that hard hydrophilic surfaces enhance the attachment of barnacles [6]. Surface energy positively affects the attachment of polycheates.

Table 4.4 Correlation coefficients between surface properties of the substrates and biofilm at the end of 2 months of immersion of the polymers and composites mentioned in Table 2.1, in the Bay of Bengal, South India (*p* values <0.05 are reported here)

Biofilm parameters measured	Surface roughness	Surface hardness	Surface energy
Fouling load	–	0.68	0.93
Total viable count	–	–	0.58
Chlorophyll a	–	0.45	–
TSS	-0.73	0.57	0.48
Adenosine triphosphate	–	0.56	0.82
Barnacles	–	0.87	0.79
Polychaetes	–	–	0.72

Table 4.4 shows the correlation coefficients of the surface properties of the polymers/composites with hardness, surface roughness and surface energy on biofouling. A

positive correlation is observed between surface energy and fouling load (**Table 4.4**, r = 0.93), indicating that hydrophobic material gets fouled more than hydrophilic material. A positive correlation is also observed between surface energy and TSS. Adhesion of marine bacterial species on different surfaces have been investigated by several researchers and they concluded that bacterial adhesion is less on low energy surfaces and they are easier to clean because of weaker binding at the interface [22]. Kerr and Cowling [23] suggested that substrates with a surface energy between 5–25 mN/m will have minimum fouling. Baier and co-workers [24] and Baier [25] also showed that there was a relationship between the surface energy and relative bacterial adhesion. Similar findings were reported earlier by Sudhakar and co-workers [11]. Subsequent fouling of surfaces is governed by the initial conditioning film and not by the initial surface energy of the substrates.

A negative correlation (-0.73) was observed between surface roughness and TSS, indicating that smooth surfaces attract more suspended solids. A negative correlation was observed between surface energy and roughness in earlier field studies with LDPE, HDPE and PC [9, 14, 15] such a relationship with SR, PU, PET, SF, GFRP and CFRP is not noticed.

4.5 Barnacles and their Cement

Barnacles are sessile creatures (**Figure 4.2**). They spend their entire lifetime attached to a surface chosen during their cyprid larval stage. Generally, the attachment is an irreversible process. The major challenge it faces is the environmental dynamics that include intertidal current and winds and it has to overcome the possibility of being swept away by the ocean waves. The adhesion happens because of the attachment of its hard calcareous base plate to a fixed hard surface through a soft bio-organic adhesive layer. The interface is a few microns thick and the variation in stiffness changes quite drastically across the interface because of the presence of this adhesive layer. Two major characteristics of barnacle adhesion are: a) its structural attributes as a function of the different surfaces to which it attaches, and b) its biochemical characteristics. Barnacle adhesive is capable of adapting itself based on the substrate material to which it is attached to. These structures include surface roughness, texture, wettability, modulus, thermal conductivity, stiffness, and chemical composition. Adhesion studies have been reported on synthetic polymeric and metallic surfaces. Adhesion on biological surfaces including other marine creatures has not been well explored. It is known that marine creatures with textured surfaces suffer relatively less barnacle attachment. Though barnacles are commonly found attached to mussel shells (**Figure 4.2**), they can be easily peeled off which indicates a foul-release property of the mussel [26].

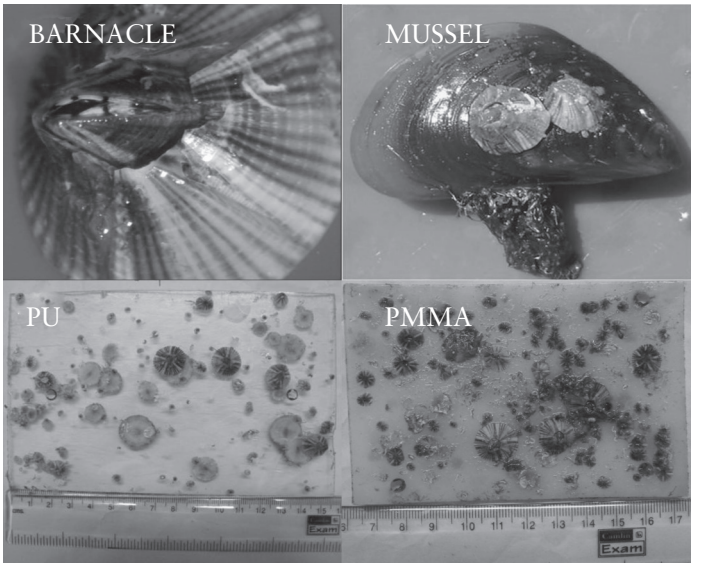

Figure 4.2 Barnacles attached to PU, polymethyl methacrylate (PMMA) and mussel surfaces placed in the ocean for one year.

Previous reports show that barnacles (*Chthamalus fragilis*) present on the leaf surfaces of the plant, *Spartina alternifora* replicate the epithelial cells and the other features present on the surface of the latter. The barnacle species, *Balanus eburneus*, grows on vinyl records mainly oriented along the direction of the grooves of the record, whereas on the smooth side of the record, the barnacles grow in random directions and not in a fixed pattern. So, it is postulated that the liquid cement secreted by the barnacles initially completely fills the surface fissures of both the base-plate as well as the substrate. Once solidified, it ensures a rigid interlocking with the surface. It is proposed from atomic force microscopy measurements that barnacle cement exists in layers and the outermost softest cement layer governs the shear strength of the adhesion. The microstructure of the bulk cement is a function of the supporting substrate material. Morphological crosslinking is more common in the cement detached from PMMA when compared to the relatively denser cement detached from a shell. The porous nature of the cement from the former is relatively absent in the latter. This suggests that the curing of the cement secreted on the periostracum (shell) is poorer in comparison to the cement secreted on PMMA or even on metals. On these materials fibrous or globular microstructures are well-developed. Absence of any crosslinked structures allow the peeling of the barnacle from the periostracum. The contact angle measurements suggest that the periostracum is more hydrophilic than the PMMA surface, but the attachment is stronger on the PMMA.

The microtopography of the periostracum of mussel shells has been shown to be capable of exhibiting foul-resistance and foul release properties. In addition the mussel may be releasing chemicals which may prevent biofouling. The elastic modulus of the periostracum of *Mytilus galloprovincialis* has been reported to be 100 MPa whereas that of PMMA is about 3.7 GPa which could also influence the interfacial strength [27].

During the growth of the barnacle its base-plate enlarges in a horizontal direction whereas the parietal plate grows in a downward direction. Because the cement cells of the barnacle are the modified epidermal cells, the secretion of cement is linked to its moulting cycle. The secretion takes place between consecutive moulting cycles at the periphery of the base-plate. The low viscous cement flows and spreads itself to fill any gap between the base-plate and the solid substrate. When the base-plate grows further, new active cement glands are formed at the periphery and the old ones stop secreting. So, they lay embedded in the hardened cement still carrying the non-solidified liquid adhesive inside them. So, the cured cement appears as discrete concentric rings throughout the base-plate. It has been reported that this underwater adhesive produced by a barnacle is a multi-protein complex and the underwater attachment is a multi-functional process [1].

The chemical composition of the cements retrieved from different barnacles is listed in **Table 4.5**. The lipid and protein content between each cement is significantly different. The table also shows the composition of the cement recovered from *Amphibalanus reticulatus* attached on PMMA and on mussel shell. Considerable differences in the composition are observed in the cements removed from different surfaces indicating that this organism produces cement of different composition depending upon the surface on which it attaches itself to. **Figure 4.3** shows the Fourier-transform infrared (FTIR) spectra of the cements removed from PMMA and a mussel shell [28].

Table 4.5 Chemical composition (%) of the analysis of three cements from *A. reticulatus* and their comparison with the literature reports from different barnacle species

Type of cement	Carbohydrate	Lipid	C	H	N	Protein	Barnacle
RC	19	0.33	50.63	4.71	4.71	89.4	*A. reticulatus*
P-C	4	0.10	33.5	3.76	9.29	58.1	*A. reticulatus*
MSP-C	0.25	0.10	27.46	0.96	1.3	28.2	*A. reticulatus*
Normal cement	2	8.2	41.05	5.96	11.15	69.7	*Lepadidae fascicularis*
Normal cement	–	0.69	–	–	–	84.4	*Balanus crenatus*
Normal cement	–	–	–	–	–	85.9	*Balanus nubilus*
Normal cement	–	2.74	–	–	–	70	*B. nubilus*

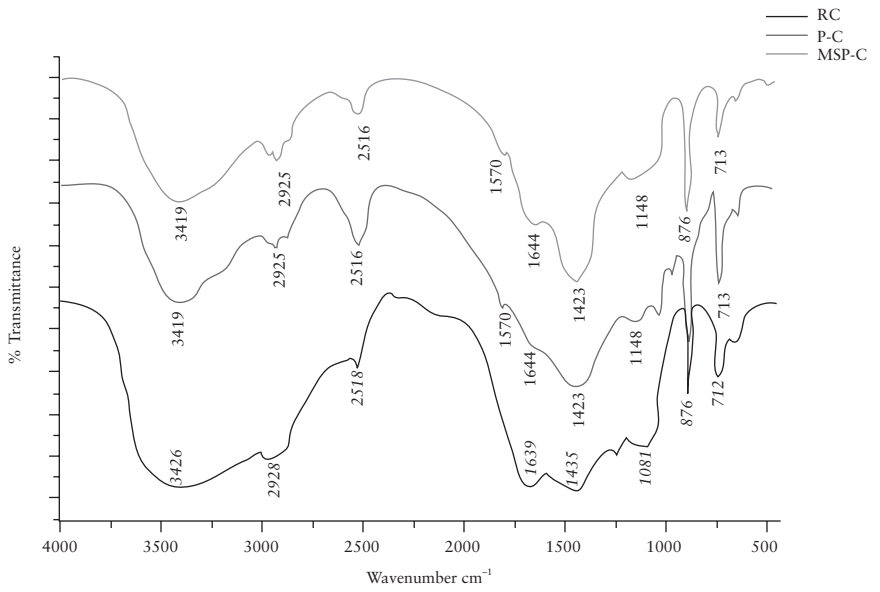

Figure 4.3 FTIR spectra of cements collected from barnacle, *A. reticulatus* attached on different substrates. RC: Rubber like cement from barnacle attached on mussel shell (thick and opaque), P-C: powder like cement from a barnacle attached on PMMA (thin and transparent) and MSP-C: powder like cement from a barnacle attached to a mussel shell

Sodium dodecyl sulfate – polyacrylamide gel electrophoresis (SDS-PAGE) (**Figure 4.4**) indicates the existence of polypeptides of varying molecular weights in the barnacle cement. Six proteins from the cement from the species, *Megabalanus rosa*, have been identified. The protein with the molecular weight of 19 kDa is a minor component of the cement and is believed to have functional roles in binding with the surface. Another minor protein with a molecular weight of 20 kDa was found to be calcite-specific in its adsorption behavior. Two major proteins, of 100 kDa and 52 kDa are believed to exhibit bulk functions. Homologous complementary deoxyribonucleic acid of some of these proteins have been discovered in several other genera of acorn barnacles (*Semibalanus balanoides*). The cement of *A. reticulatus* also has polypeptides of a similar molecular masses as those present in *M. rosa*. The predominant bands at 100, 80 and 20 kDa in *A. reticulatus* (**Table 4.6**) suggest that they may play a major role in the adhesion process. The layered nature of the cement and the interfacial cement layer replicating the substrate features suggest that each layer has a different functional role. Not just one polypeptide but combinations of several polypeptides appear to be involved in the formation of each layer [1].

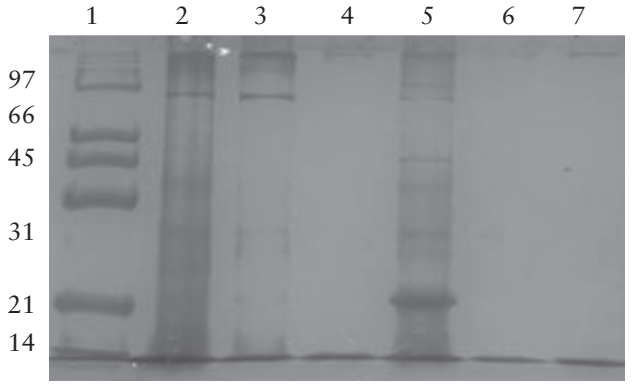

Figure 4.4 SDS PAGE analysis of the barnacle adhesive cement stained with silver staining. Lane 1: Molecular weight markers (Bio-Rad Laboratories), the numbers on the left-hand side indicate molecular masses (kDa). Lane 2: Barnacle cement protein (BCP) from GdnHCl-soluble fraction 2 (GSF2) showing 100 and 38 kDa. Lane 3: BCP from GSF2 showing 100 kDa protein band. Lane 4: BCP from a basal plaque with a base plate subjected to decalcification. Lane 5: Fresh barnacle cement from barnacles attached to mussels showing 100, 45, 38 and 19 kDa protein bands in which the 19 kDa protein is brighter and of higher concentration than the others. Lanes 6 and 7: Cement collected from the basal plate of barnacles attached to substrates. GdnHCl: guanidine hydrochloride

Table 4.6 Difference in the molecular weights (kD) of barnacle cement proteins observed on PMMA (P-C) and Mussel Shell (RC and MSP-C)

A. reticulates			*B. amphitrite*	*M. Rosa*	*E. Eburneus-*
RC	P-C	MSP-C	P-C	P-C	P-C
23	21	19	25	20	22
33	33	46	28	47	36
65	58	62	32	57	52
85	65	66	38	60	58
108	81	81	52	68	80
–	85	85	68	100	–
–	93	88	80	180	–
–	102	93	90	–	–
–	103	100	–	–	–
–	115	125	–	–	–

4.6 Controlling Biofouling and Macrofouling

Use of antifouling paint is the most common and best approach for protecting the parts of a ship that are under water. They contain water-soluble resins, pigments, metal salts and inert fillers. Copper oxide coatings are widely used. The basic insoluble-matrix, rosin-copper(I)oxide coatings (using vinyl, chlorinated rubber, and polyisobutyl resins) developed in the 1950s remain the same. These coatings are capable of a static barnacle resistance of over 90% for almost four years. Organotin coatings were introduced in early 1960s. There are two basic types of organotin coatings: coatings that incorporate organotin compounds and coatings based on film forming resins that contain a chemically bound organotin. Antifouling elastomers with a slow release toxic reservoir are reported in reference [7]. Current research towards identifying new marine-based products has led to the use of polyhydroxy sterols isolated from octocorals.

Chlorination is generally preferred to prevent the settlement of fouling organisms in the cooling water inlet lines of power stations. Macrofouling takes place when there is only intermittent chlorination or when chlorine levels are below the threshold value. Mussels and barnacles, once settled (during a period of no chlorination) are able to resist subsequent chlorination cycles. During the breaks in chlorination they feed and carry on their normal life [7].

Quaternary amine compounds and surfactants have been reported to act slowly on molluscs. These compounds do not trigger the chemoreceptors of these molluscs. The molluscs continue to ingest the antimicrobials throughout the exposure period, but they experience slow mortality. Pressure washing, water jetting, sonic devices, robotics, magnetic fields, reproductive control and cathodic protection are the other techniques that have been found to be effective to some extent and are used in some industries. Marine organisms are more sensitive to the ambient temperature than their terrestrial counterparts. Warm waters in the temperature range between 50–70 °C can kill nearly all organisms and it is found to be successful for localised fouling control [29].

Controlling biofouling by biological means is gaining attention because it is potentially cost effective, long lasting, less polluting, and a sustainable and green alternative. There are several examples of such an approach but they have still not been made available commercially for large areas. The common map turtle (*Graptemys geographica*) is known to feed on zebra mussels (*D. polymorpha*), one of the most prolific macrofoulers in American waters. The bull chub fish (*Nocomis raneyi*) can crush and ingest hard-shelled molluscan foulers. Blue crabs, hermit crabs and stone crabs prey on oysters (molluscs). Hydroids are eaten by some species of sea slugs, filefishes, puffer fish and moorish idol (*Zanclus cornutus*) [12].

Foul-release coatings are environmentally friendly and operate by two basic mechanisms: the hydrolysis of the polymer surface that 1) removes fouling with the eroded coating layer, and 2) minimises the initial attachment and the strength of attachment because of the properties of the coating surface. Coatings based on these foul-release mechanisms are effective and reduce the initial settlement and the strength of attachment. They are mechanically weak and are subject to failure because of detachment and abrasion. Heavy metal-based coatings are effective, and work by releasing biocides such as copper into the surrounding water, which may impact on the other flora and fauna. Coatings such as coal tar, epoxy resins or other anti-corrosion, anti-abrasion agents are not cost effective to use against mussels. Non-toxic coatings that rely on low-surface tension to create smooth/slippery surfaces, use of non-metal fouling repellants in traditional coatings, non-toxic foul-release coatings (ablative hydrophilic polymer films and low free surface energy films), and thermal spray coatings (in which slow dissolution of metal ions repels fouling organisms) are some current research areas. Extracts of *Pseudomonas* sp., incorporated into paints show good antifouling activity against bacteria, the *B. amphitrite* barnacle cyprid, and *Ulva lactuca* algal zoospores. A combination of neem oil/linseed oil treated Nylon fishing nets showed reduced macrofouling when compared to untreated nets after 20 weeks. Foul release agents such as PDMS, chitosan and polyvinyl pyrrolidone are incorporated into this concoction to give an antifouling mixture [8, 29–31].

4.7 Bioadhesive and Bioinert Surfaces

In order to surface engineer a substrate so that it turns into a low or non-sticking/non-adhesive one, the mechanism of bioadhesion needs to be understood. Strong hydrophilic, low energy surfaces appear to be promising for industrial and marine applications. Such an approach is also ecologically friendly. The adhesion bonding involves chemical (such as acid/base Lewis) and electrostatic interactions, Lifshitz – van der Waals forces, mechanical interlocking, diffusion and so on [32]. A thermodynamic approach considers the adhesion of bacteria to a surface as an equilibrium process and when the substrate is more hydrophobic the system is far away from equilibrium. Pedri [33] considered only dispersion forces to calculate free energy but a model which is based on the concept of 'theta surface' is later introduced which defines a critical surface tension range of 20–30 mN/m. Materials that are designed for strong bioadhesion should not be in this range and those that require easy foul release should be in this range ('theta surface'). Macrofooulers including barnacles, mussels algae and so on, make use of the concept of bioadhesion to bond to variety of wet surfaces in saline and turbulent conditions.

The well-known 'Baier curve' relates surface energy (water contact angle) or surface tension to the relative amount of bioadhesion. It indicates that the bioadhesion is

minimal at 22–24 nM/m [34]. Zhao and co-workers have suggested that an optimal surface free energy on which bacterial adhesion force is minimal should be between 20–30 nM/m [35].

Fracture mechanics theory states that elastic modulus is a key factor in bioadhesion and the ability for an organism to release from a surface. Adhesion correlates better with the square root of critical surface tension multiplied by elastic modulus than either of the terms independently [30].

The thickness of the biofilm on the surface seems to play an important part in the adhesion process. Below a 100 μm dry film thickness, barnacles can establish a strong adhesion with the surface. Surface roughness and topography also affects bioadhesion. The cement flows and spreads over rough surfaces. If the surrounding liquid is viscous the cement may not flow uniformly and fill all the crevices and when it solidifies it may cause stress concentration regions. Surface roughness may also cause changes in the surface wettability. Nanotopographies have been shown to exhibit foul repelling properties. Other properties that play a role in the bioadhesion process are material surface chemistry as in the case of silicon polymers which exhibit the lowest adhesion, slippage (peeling off) exhibited by silicon elastomers, friction and lubricity exhibited by oils. The type and amount of protein that initially form the conditioning layer alters bioadhesion. The lower the protein adsorption, the lower will be the bioadhesion [36]. So protein repellent surfaces are good antibiofouling surfaces.

4.8 Conclusions

Studies suggest that the surface properties (roughness, surface energy and hardness) determine the formation of the initial biofilm and further biofouling does not seem to depend on the original surface properties. Initial fouling on the surface is directly proportional (positive correlation) to the surface energy. Surface energy decreases at the end of one year. Biofouling seem to affect the physical properties of these material which include gravimetric and thermogravimetric weight loss, tensile strength, surface energy, and hardness. A seasonal variation of biofouling is observed. However, more investigations are needed to validate this finding. Biofouling is minimal on silicon rubber. Attachment of barnacles is minimal on it probably because of its flexible nature. However, stiff surfaces attract more attachment of barnacles and polycheates. For barnacles the nature of the material has a distinct role in controlling their adhesion mechanism. Biochemical differences exist between the various cements (powdery or rubbery) secreted by the barnacles that attach to different substrates, in terms of protein content, abundance of individual protein components and the chemistry of the polymerised cement. The doublet bands of >80 kDa proteins observed in these cements from different substrates suggest that they may be involved in the activation

of the structural and proteolytic precursors. These two components (>100 and >52 kDa proteins) are considered to constitute the bulk region of the cement. A small difference in amide I and II peak positions is observed between these two cements which suggests that some difference in the secondary structures of the protein occurs. Calcium detected in cement suggests that it may also play a major role in cement polymerisation as precursors or signalling molecules in the cement.

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