

## Role of Diatoms in Marine Biofouling

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Diatoms are one of the early colonisers on surfaces submerged in the photic zone of the environment. Their colonisation on man made structures, termed biofouling, has enormous implications in the marine industry. The processes involved in biofouling and the role of diatoms is presented. The article also provides an overview of the current and potential measures for the control of diatom fouling.

**Key words:** antifouling compounds, bacteria, chemosensory systems, diatom, EPS, fouling, microfilm, natural products, pennate

**Abbreviations:** CLSM, confocal laser scanning microscopy; EPS, extracellular polymeric substance; SEM, scanning electron microscopy; TBTO, bis (tri-n-butyltin) oxide

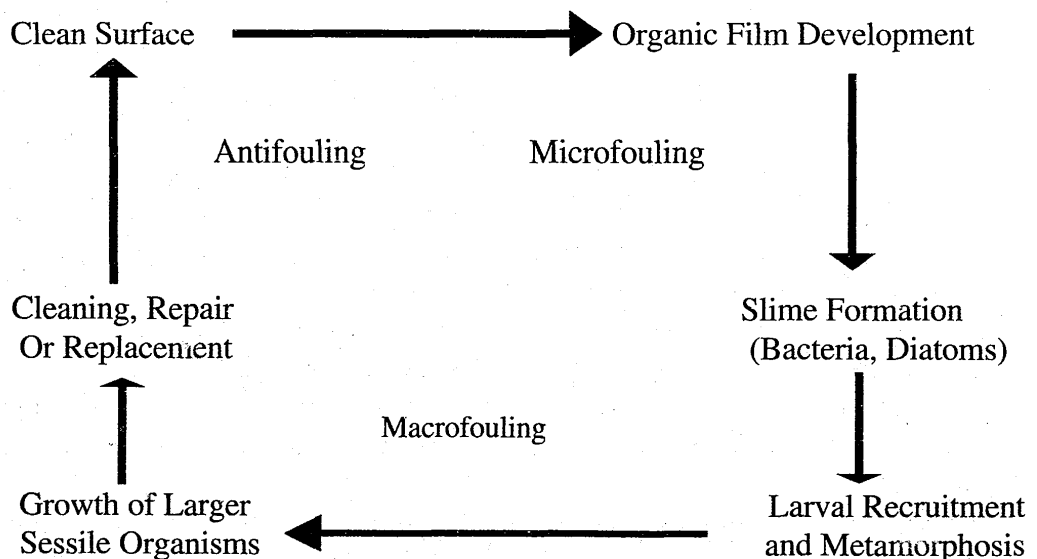
Materials used in the marine environment are prone to the phenomenon of biofouling. The term 'fouling' is commonly employed to distinguish the assemblage of plants and animals that grow on any artificial structure from those occurring on rocks and other natural objects. Marine biofouling is a nuisance for commercial and naval shipping and other marine activities all around the world. Biofouling increases weight and frictional resistance of the ship, thus affecting its hydrodynamics, speed and manoeuvrability (Rolland and DeSimone 2003). An increase in physical hull roughness of some 10 mm accounts for 1% increase in the fuel penalty (Lackenby 1962). The biofouling slimes that form on ship hulls are reported to raise fuel consumption by as much as 30% in some cases (Younqlood *et al.* 2003). This is significant as fuel costs account for upto 50% of transportation charges. The output of carbon dioxide, a green house gas, also increases proportionately (Anderson 2002). Thus, it is not far fetched to say that maintenance of clean hulls is not only beneficial to the shipping industry but is an essential component of global climate preservation.

In aquaculture, biofouling problems are of two types – on infrastructure (immersed mesh cages and trawls) and on stock organisms, particularly mussels, oysters and scallops.

With the emergence of multi-megawatt power plants, more and more nuclear and thermal power stations are turning to the sea to meet their cooling water needs. Heavy growth of macrofouling organisms like algae, hydroids and mussels can block their water intake pipes, thus bringing down the efficiency of these power stations and necessitating frequent cleaning which is an expensive affair. Also, thermal energy conversion plants are faced with the menace of biofouling. However, in this case, the concern over microfouling in heat exchangers is greater than the effects of macrofouling since microfouling brings down the efficiency of heat exchangers considerably (Anil 1986, Christie and Dalley 1987). Moreover, the biofouling of marine instrumentation also remains a serious problem in long-term studies related to climate change, pollution and other environmental issues. In addition to the above problems, biofouling on ship hulls is a powerful way of spreading species to new parts of the world oceans leading to bioinvasion, which is now recognised as a major threat to biodiversity (Anil *et al.* 2002).

It is now well known that the establishment of the fouling community can be differentiated into two different major phases: the initial phase consisting of the development of primary film or the organic conditioning film comprising of colloidal organic matter and molecules such as polysaccharides and proteins. This is immediately followed by an accumulation of microorganisms (bacteria, diatoms, fungi and other micro-algae) and the secretion of EPS at their cell surface during attachment, colonization and population growth. This process is known as microfouling and the resulting layer is termed as microfilm/biofilm. The microfilm subsequently induces settlement and growth of larger sessile marine plants like the green algae *Enteromorpha* and invertebrates such as barnacles, mussels, tubeworms, bryozoans, hydroids etc. thus leading to macrofouling (Anil 1986).

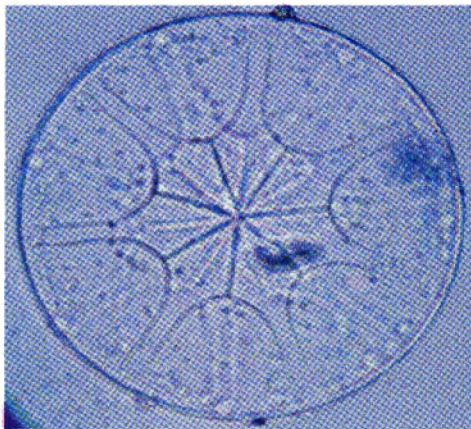
### MARINE FOULING CYCLE



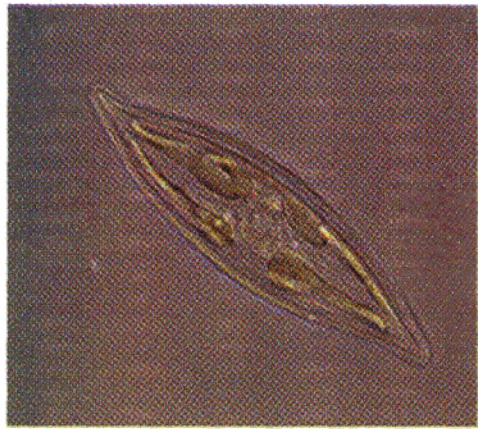
Although the microfilm comprises of different microorganisms, in the entire process of biofouling, diatoms are the major contributors to the microbial slime community (Evans 1988).

Diatoms are protists belonging to phylum Bacillariophyta and class Bacillariophyceae. Their cell walls are shaped like tiny glass pillboxes, with an amazing array of sizes, shapes and ornamentation. The rigid silicate frustule encases the vegetative protoplast. There are two basic body shapes of diatoms based on symmetry (Fig. 1), centric (round with radial symmetry, Order Centrales) and pennate (thin ellipse with bilateral symmetry, Order Pennales) and may be found in either the plankton or in the benthic environment. These two major taxonomic divisions also reflect a major ecological difference (Round 1971). Centric diatoms are mainly holoplanktonic or meroplanktonic, with only a few genera that are associated with substrates throughout their life cycles. Some pennate diatoms have a long slit (raphe) along the length of the frustule that helps in motility and attachment to substrata. Pennate diatoms can be divided into 3 types based on the presence and number of raphe (Fig. 2). Araphidineae (pennate diatoms without a raphe system) and Monoraphidineae (pennate diatoms with one raphe system on one valve) are attached to sand grains, rocks and biological substrates, whereas genera of the Biraphidineae (pennate diatoms with raphe systems on both valves) are almost completely attached to mud with only a few planktonic species.

Diatoms are the major primary producers (in both pelagic and benthic environments) and are thought to be responsible for upto 25% of the worlds net primary productivity (Jeffrey and Hallegraeff 1990) whereas total phytoplankton account for upto 40% of the global primary production (Falkowski 1994). Some diatom species are also reported to survive heterotrophically in the dark if supplied with a suitable source of carbon (Round *et al.* 1990).



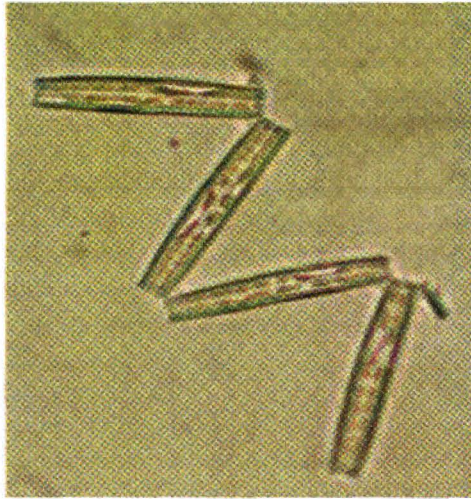
(a) Centric diatom – round with radial symmetry, e.g., *Asterolampra marylandica*.



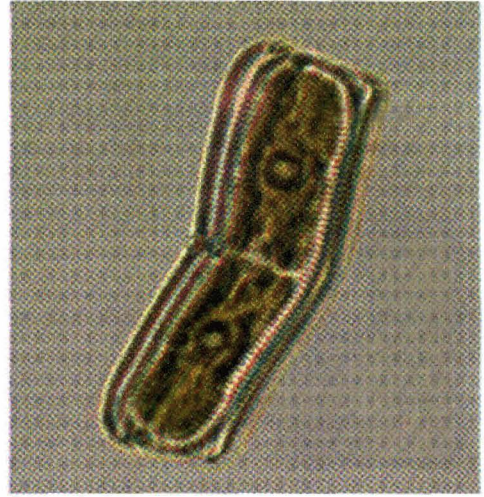
(b) Pennate diatom –elliptical with bilateral symmetry, e.g., *Pleurosigma naviculaceum*.

Fig. 1 — Two basic body shapes of diatoms based on symmetry





(a) Araphid, e.g., *Thalassionema nitzschioides*.



(b) Monoraphid, e.g., *Achnanthes subsessilis*.



(c) Biraphid, e.g., *Navicula transitans* var. *derasa* f. *delicatula*.

**Fig. 2 — Pennate diatoms classified based on the number of raphe present.**

Diatoms reproduce vegetatively by binary fission to form two new individuals within a single parent cell frustule. Each daughter cell receives one parent cell theca as epitheca; formation of a new hypotheca for each of the daughter cells terminates cell division. Diatoms may also, but not necessarily, pass through a seed-like phase known as the resting stage. Resting stages are of two different types, resting spores and resting cells. While, resting spores are thick-walled stages that are morphologically distinct from vegetative cells, resting cells are similar to vegetative cells, except for altered cytoplasmic characteristics (Anderson 1975, Hargraves and French 1975, Hoban *et al.* 1980, Sicko-Goad *et al.* 1986, Round *et al.* 1990). Resting spore formation is common in marine centric diatoms and rare in pennates. On the contrary, resting cell formation is observed more often in pennates

and freshwater diatoms (Sicko-Goad *et al.* 1986, Round *et al.* 1990). A number of external factors have been found to induce formation of resting stages and influence their development. These include the availability of various nutrients (N, P, Fe, Si), temperature, light and pH. In almost every species, nitrogen deficiency appears to be an effective inducer and, in some cases, the sole inducer. Only cells of a restricted size range that have the potential to become gametangia and in some centric diatoms, only particular stages in the size reduction cycle can form spores. Once formed, the resting stages behave as fine silt particles, often accumulating in high numbers on the sediment bottom. These resting stages can withstand periods of unfavourable conditions on annual and decadal scales to survive mass extinction and have been regarded as benthic. They are traditionally considered as overwintering forms, which could provide stocks for subsequent and seasonal blooms as well as for species dispersal (Lund 1954, French and Hargraves 1980, Garrison 1981, Hollibaugh *et al.* 1981).

### FOULING DIATOMS

Pennate diatoms often dominate the fouling (Fig. 3) as well as epibiotic population, e.g., *Amphora*, *Navicula* and *Nitzschia* spp. (Characklis and Cooksey 1983, Cooksey *et al.* 1984, Mitbavkar and Anil 2002, 2004, 2005, Patil and Anil 2000, 2005a, c), since they are capable of adhesion and attachment to substrates; this characteristic is not observed in majority of the centric forms.

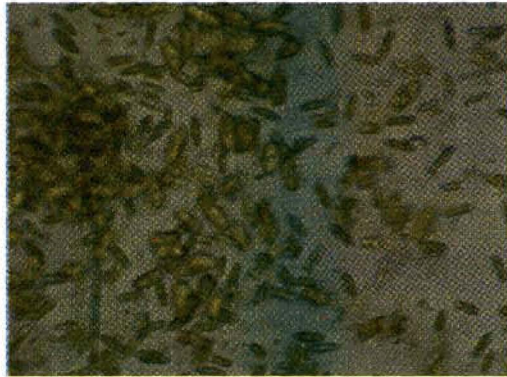


Fig. 3 — Photomicrograph of biofilm dominated by pennate diatoms *Amphora* and *Navicula* spp.

Adhesion of diatoms to surfaces is invariably associated with secretion of EPS from the raphe slits (Chamberlain 1976, Hoagland *et al.* 1993, Cooksey and Wigglesworth-Cooksey 1995). Diatom adhesion is governed by a 2-stage process: reversible ('initial contact' or the 'first kiss') followed by irreversible adhesion (long term or permanent adhesion; see review by Wetherbee *et al.* 1998) that is

facilitated by the production of EPS (Cooksey and Wigglesworth-Cooksey 1995). Experiments with *Amphora* showed that the mucilaginous material was a complex polysaccharide with uronic acid and sulphate substituents. Calcium ( $\text{Ca}^{+2}$ ) is necessary for secretion of the adhesive material since *Amphora* does not adhere or move in the absence of this ion (Cooksey and Cooksey 1980, Cooksey 1981).

Fouling diatoms are primarily native to the benthos (Mitbavkar 2003, Mitbavkar and Anil 2002, 2004, 2005) and are frequently resuspended into the surface waters through hydrodynamic processes such as waves, tides and currents (Breznak *et al.* 1985). Once resuspended, benthic diatoms need to survive in the water column until they settle to the bottom or find a suitable surface in the euphotic zone. Once attached, their growth and community structure is further dependent upon the physico-chemical and biological nature of both the ambient water and the surface. Studies reveal that diatoms have specific substrate preferences (Mitbavkar and Anil 2000, Patil 2003); higher diatom recruitment was observed on fibreglass, (a hydrophobic surface) than glass (hydrophilic) (Patil and Anil 2005a). Variation in communities developing on such surfaces may be due to subtle influences, for e.g., surface charge, surface tension or nutrient availability (Escher and Characklis 1980, Fletcher 1980, Fattom and Shilo 1984, Becker and Wahl 1991, Sekar *et al.* 2004, Holland *et al.* 2004). Biofilm diatom community structure also shows significant temporal variation that is attributed to physical, chemical and biological changes in the environment. Temporal variation in the tychopelagic diatom community structure (temporally dislodged benthic diatom community) of the surrounding water column also influences the biofilm community (Patil and Anil 2005a).

Marine biofilms contain high densities of diverse, fouling diatoms (Cooksey *et al.* 1984). In addition, their distribution in natural biofilms is heterogeneous, with micro-scale variations occurring within the biofilm. This presents problems in sampling and enumeration. Diatom species composition of biofilms can be evaluated either directly without disturbing the biofilm or indirectly by removing the biofilm and analyzing it microscopically. Direct observation of biofilms is achieved through various microscopic techniques such as CLSM, SEM, epifluorescence and light/phase contrast microscopy (Nagarkar and Williams 1997; Norton *et al.* 1998).

Indirect observation of biofilm after removal through brushing or scraping is the traditional method of enumerating biofilm diatom community from various types of substrata. Biofilm removal is the most critical step in this process. The brushing method is usually preferred because of brush flexibility (MacLulich 1986, Hill and Hawkins 1990, Sharma *et al.* 1990, Bhosle *et al.* 2005) whereas the scraping method can be used only for smooth surfaces because of the hard nature of the scraper. Patil and Anil (2005c) reported that a nylon brush was more efficient at recovering diatoms compared to a ceramic scraper. Although advanced microscopic techniques are available to quantify biofilms at higher resolution *in situ*, brushing or scraping methods are still practiced mainly because expensive equipment is not required. The advantage of brushing and scraping methods over direct methods is that quantification of larger areas of substratum is possible. The main disadvantages of



these methods are that cells may be lost or damaged thereby underestimating the results (Patil and Anil 2005c).

Generally diatom microfilms are considered to provide a foothold for macrofouling organisms to attach. Diatoms might offer differing cues to settling larvae of higher sedentary organisms, which are critical in determining the macrofouling community. Qian *et al.* (2003) hypothesized that changes in microbial community lead to changes in the recruitment pattern of marine invertebrates at the microscopic scale. Studies on acorn barnacle *Balanus amphitrite* Darwin, a dominant fouling organism, revealed that enhancement of metamorphosis depended on diatom species, density and composition of their EPS in biofilms (Patil and Anil 2005b).

Approaches towards control of biofouling should focus on prevention and control of the initial stages of fouling i.e., microfouling. Tackling biofouling at the first step itself would go a long way in preventing fouling problems in the marine environment.

## CONTROL OF DIATOM FOULING

A variety of fouling control measures have been tried which include mechanical scrubbing, heat, electromagnetic radiations and ultrasound. When cleaning (or scraping) becomes time consuming or ineffective, industries turn to perhaps the most widely accepted method of controlling and preventing biofouling by using antifouling coatings over submerged surfaces. Antifouling control agents interfere with the basic biochemical processes of fouling organisms. Most contain organic biocides, which are used singly or as an additional component to copper-based formulations. Such organic biocides enhance the efficacy of the formulation and are commonly referred to as 'booster' biocides. Some antifouling agents currently in use are cuprous oxide, bis (tri-n-butyltin) oxide, tri-n-butyltinfluoride, tri-n-butyltin acetate, irgarol 1051 and organometallic polymers. One of these, irgarol 1051, is a potent inhibitor of photosynthesis (Dahl and Blanck 1996). It prevents microfilm formation by diatoms thereby inhibiting the attachment of higher sedentary organisms. Khandeparker *et al.* (2005) have reported that biofilms treated with irgarol 1051 prevent metamorphosis in cyprids of the barnacle *Balanus albicostatus* Pilsbry and the serpulid polychaete *Pomatoleios kraussii* Baird.

Chemical antifoulants have several disadvantages. For instance, they pose a threat to non-target organisms (Readman *et al.* 1993). Organotins have a high leaching rate and one of them, bis (tri-n-butyltin) oxide or TBTO, has been proven to cause deformations in oysters and sex reversal (imposex) in marine gastropods. Imposex has also been recorded in *Cronia konkanensis* (Vishwakiran and Anil 1999) and *Gyrineum natator* (Vishwakiran *et al.* 2005), gastropods occurring along the west coast of India. In addition, organotins are potentially toxic to human beings.

The use of chemical toxicants may change the composition of the fouling community; it is likely that one may encounter new organisms not encountered

before the application of the biocide. In addition, new mechanisms of resistance to biocides are being revealed in marine organisms. These include slime formation, exclusion and secretion mechanisms and metabolic detoxification (Heinzel 1998). Due to current and expected future restrictive regulations on the use of TBT (Dalley 1987, Clare 1995) and probably other polluting compounds, there is a growing need to develop other methods to prevent/control biofouling.

Several other methods for antifouling control have been developed. These include electromagnetic radiations such as pulse laser irradiation (Nandakumar *et al.* 2003) and ultraviolet radiation, developed by Wheaton Inc. for oceanographic *in situ* fluorometer (Manov *et al.* 2004). Since radiation techniques are not species-specific and can harm non-target organisms, their application for the control of biofouling is limited to localized sterilization. Plasma pulse technology transmits energy directly into the water, which may cause harmful shockwaves or steam bubbles (Mackie *et al.* 2000). This process may create shockwaves that could affect cooling/heating systems. Pulsed electric fields, frequently used in pipes, create acoustic waves. Electric fields do not kill, but stun organisms, clearly lowering mortality of non-target organisms (Walch *et al.* 2000).

The concept of using naturally produced antifouling agents has been suggested as a promising approach towards fouling control. The search for antifouling compounds from marine organisms began in the 1970's and continued through the 1990's based on the observation that certain marine plants and animals naturally resist fouling. Typical examples of these are sessile organisms like seaweeds, soft corals, horseshoe crabs and marine sponges where barnacles and bryozoans do not attach to live organisms (Patil and Anil 2000, Thakur 2001, Thakur and Muller 2004). Fouling occurs only when these organisms die and presumably no longer produce the chemical defences. This initially suggested the presence of factors (metabolites) produced while the organisms were alive since there was no other physical mechanism for these organisms to rid themselves of attached foulers. Some of these metabolites are now being developed for use as natural antifouling agents (metabolites). They have several advantages over chemical antifouling agents. They are effective at low concentrations, are inhibitory (not toxic) to organisms and have a higher probability of degradation (de Nys and Steinberg 1999). Several kinds of natural antifouling agents that inhibit diatom growth have been isolated from marine organisms like bacteria (Holmstrom *et al.* 1996), marine algae (Abarzua *et al.* 1999, de Nys *et al.* 1996, Eng-Wilmot *et al.* 1979, Gross *et al.* 1991, Hellio *et al.* 2002, Ishida 2000, Murakami *et al.* 1991, Wu *et al.* 1998), sponges (Mokashe *et al.* 1994, Thakur 2001), coelenterates (Davis *et al.* 1989, Targett *et al.* 1983, Targett 1988), holothurians (Mokashe *et al.* 1994) and ascidians (Thakur 2001).

Though research efforts have produced numerous effective antifouling agents, no single chemical has yet entered the commercial stage. Most marine organisms that have been identified as producers of natural antifouling agents have symbiotic bacteria or algae associated with them. For e.g., sponges have epibiotic bacteria on their surface which contribute significantly to the chemical defence mechanisms of



the host (Thakur and Anil 2000, Thakur *et al.* 2003, Thakur and Muller 2004, Thakur *et al.* 2004). Where such associations exist, it is important to determine which of the two organisms actually produces the antifouling metabolite, or whether interaction between the two organisms is a prerequisite for metabolite production. These natural substances are complex in structure and the excessive cost of synthetic production make them economically non-viable as antifouling agents. In addition, more than one metabolite may be involved against the vast range of fouling organisms present in the environment. A promising approach would include determining the portion of the metabolite responsible for the observed activity and develop less complex analogs for testing. These can then be mixed and screened for additive or synergistic effects. Synthetic preparation overrides problems of variation in expression levels of metabolites with space, time and physiological status of the organism. Other requirements are ability to integrate well in slow-release coatings without decrease in stability, etc. (Targett and Stochaj 1994, Davis and Bremner 1999).

Many natural antifoulants have been isolated and ascribed a defensive role, but there is little evidence regarding the toxicity and biological degradation of these compounds. Phytoplankton are highly susceptible to herbicidal antifouling compounds due to the damaging effects on photosynthesis. Due to their short generation times and differential responses and susceptibilities to biocides, algae can be affected over time scales from hours to days, leading to changes in community structure. Studies on phytoplankton are particularly relevant when safe environmental regulations are to be established. These are typically based on unialgal tests, but responses of natural phytoplankton communities to toxic compounds are more environmentally relevant, yet such studies are scarce (Dahl and Blanck 1996, Nyström *et al.* 2002, Readman *et al.* 2004).

There are other aspects that need to be considered as well. Microfouling communities in nature are highly complex. Interactions between different trophic levels regulate community structure of fouling organisms. Bacterial biofilms, the first biological event in the series, are reported to provide cues for a range of succeeding fouling organisms. These include diatoms, polychaetes, seaweeds, etc. (Wiggelsworth-Cooksey *et al.* 2001). Some bacteria are reported to inhibit growth of several marine algae (Berland *et al.* 1972). Inhibition/Stimulation of *Enteromorpha*, a fouling green alga, by bacteria has also been reported (Thomas and Allsopp 1983). Bacteria, with inductive/non-inductive effects on larval settlement of a polychaete, *Hydroides elegans* have also been identified from marine natural biofilms (Lau *et al.* 2002). In contrast to the settlement of invertebrate larvae, very little is known about the means by which algal communities develop on natural surfaces with bacterial biofilms. Reports indicate that chemosensory systems are used to both attract and repel algae to surfaces (Berland *et al.* 1972, Thomas and Allsopp 1983, Holmstrom *et al.* 1996). Since bacteria produce many chemotactically active metabolites in the marine environment, they are likely to play an important role in controlling the specificities and attachment dynamics of fouling diatoms.

Among the signalling molecules produced by bacteria, lectins, a class of naturally occurring proteins or glycoproteins, are of considerable importance. They exist in almost all living organisms and can recognize and bind carbohydrates specifically and noncovalently (Khandeparker 2002, Khandeparker *et al.* 2002, Khandeparker *et al.* 2003). Since glycoprotein-binding sites for lectins are found in a variety of algae (Holmstrom and Kjelleberg 1999), they appear to be important in algal settlement. It is likely that diatom attachment to bacterial biofilms is mediated by lectins. Several levels of specificities may exist. Thus, bacteria may attract/repel different diatoms based on signaling specificities. Diatoms are also reported to provide signaling cues to polychaete larvae (Lam *et al.* 2003) and barnacle larvae (Patil and Anil 2005b). These intricate signaling processes between different fouling organisms – both micro- and macro-foulers compound the problem to a multi-dimensional scale. These aspects have to be taken into consideration when delineating measures for prevention of biofouling.

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