

## REVIEW

# Adhesion of bacteria and diatoms to surfaces in the sea: a review

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**ABSTRACT:** The initial event in biofilm formation on marine surfaces is the adsorption of an organic layer. This is followed usually by microorganisms and more developed forms, but there appears to be no obligatory order in this succession. The influence of the substratum chemistry on the rate and the extent of adhesion of microorganisms is still not fully agreed because many types of organisms in differing physiological states have been used, making consensus difficult. Sometimes the surface chemistry of the substratum was not established, which further clouds the picture. Genetic evidence now supports the idea that surface proximity promotes the induction of new metabolic capabilities in microorganisms, however the environmental signal responsible for this is not agreed. We propose that restricted diffusion at a surface plays a role in the process. Support for the hypothesis that the biofilm matrix polymers are not the adhesives involved in the initial attachment of cells to surfaces is growing. Diatom adhesion is a more complicated process than that for bacteria and requires glycoprotein and protein synthesis as well as metabolic energy, i.e. it is not a passive process. Bacteria can utilize surface-bound substrates and it is possible that diatoms attached to sediment grains can exploit their attached existence also by moving along concentration gradients of dissolved organic substances in adjacent pore waters. Practical interest in the adhesion of marine microorganisms derives from their role in the degradation of man-made structures. A film of organisms only a few microns in thickness causes the hydrodynamic drag on a ship to increase considerably. Investigations of the means by which marine microorganisms adhere to surfaces have been taking place for more than 50 yr, yet we still do not understand completely the mechanisms involved.

**KEY WORDS:** Diatoms · Bacteria · Adhesion · Motility · Marine

## INITIAL EVENTS IN THE FORMATION OF A BIOFILM

Although, according to Marshall (1993), not the first to be published on this subject, Claude Zobell's work in the 1940s (e.g. Zobell 1943) proved seminal and re-alerted us to the fact that attached microorganisms occupy a specific ecological niche, now often termed a biofilm. A biofilm is a collection of adhered cells and their products at a surface (Characklis & Cooksey 1983). It has been accepted generally that the first step in the formation of a biofilm on a clean surface is the adsorption of an organic layer onto the surface from the aqueous milieu. In this context, let us emphasize that 'clean' means that the chemical functional groups presented to the aqueous interface are those of the substratum, not those of residues of substances used in the

process of 'cleaning' the surface. In the past, lack of appreciation of this has probably caused difficulty in the replication of experiments from laboratory to laboratory and from time to time in the same laboratory. The adsorption of the organic layer, often referred to as a conditioning film, is fast (seconds) and inevitable. The ways in which the surface chemistry of the substratum are changed by the adsorbed film have been discussed (Characklis & Cooksey 1983). For some years, it was held that there was an obligatory order in the succession of organisms that followed the adsorption of the conditioning film (e.g. Marzalek et al. 1979). Bacteria were considered to be the initial colonizers. These were followed by diatoms, other algae, and invertebrate larvae in that order. We now know that diatoms can attach to clean surfaces in the laboratory (Cooksey 1981) in the absence of bacteria, but many invertebrate larvae

respond to chemical settling cues generated by other organisms (Morse et al. 1979, Weiner et al. 1985).

It seems likely now that results concerning the obligate succession of organisms could have been artifacts of the methods used. For instance, Marzalek et al. (1979) found diatoms were evident on coupons submerged in Biscayne Bay, Florida, USA, only after 10 d. On the other hand, Cooksey et al. (1984), using identical equipment in the same place, showed that diatoms arrived on stainless steel or glass surfaces after only a few hours. Differences in enumeration technique between the 2 studies almost certainly accounted for this temporal difference. Whereas Marzalek et al. counted diatoms after preparation for electron microscopy, the other workers used either chlorophyll fluorescence or direct light microscopy to enumerate diatom cells. In the earlier study, the diatom counting technique would be insensitive to small numbers. The means for counting bacteria, however, was by colony-forming units. Even given the chance of missing unculturable organisms, this is a more direct technique than that used for the diatoms. It could be said of these 2 studies that one finds that for which one is looking!

It is important in kinetic studies of the numbers of cells on submerged surfaces to distinguish between the processes of colonization alone and colonization and subsequent growth of the organisms. Are the cells counted on a surface several days after its immersion the product purely of continuous colonization, or the results of growth of the initial colonizers? In a field experiment, this point was examined with algae since they do not usually grow in darkness. Cooksey et al. (1984) showed that after an initial colonization period of 48 h, logarithmic increases in the number of cells on glass or stainless steel surfaces in the sea took place only in daylight hours. Thus, *in situ* growth represented an important contribution to the number of algal cells on a surface after the first 48 h of submersion.

#### INFLUENCE OF SUBSTRATUM SURFACE PHYSICAL CHEMISTRY ON THE ATTACHMENT OF BACTERIA

It is difficult to find generalities in the literature in this subject, largely because of the wide variety of organisms studied, their varied physiological state at the time of the experimentation and the conditions of the incubation. Some examples will make this clear.

Dexter et al. (1975), using a natural population of cells from a harbor, showed that the relationship between the number of cells adhered to a substratum and its surface energy was not linear. The curve showed a minimal value for adhered cells at a critical

surface energy of  $25 \text{ mN m}^{-1}$  — a moderately hydrophobic surface. It is likely that the harbor waters were somewhat eutrophic and that this natural population was not seriously nutrient-limited. This paper was one of the first to try to explain how the surface energy of a substratum influenced the adhesion of cells when the conditioning film was interspersed between the substratum and the cells. These workers' suggestion was that the film, in some way, acted as a transducer of the chemistry that lay immediately beneath it, i.e. the orientation of the molecules in the film was influenced by the underlying chemistry. Fletcher & Loeb (1979) used a species of marine *Pseudomonas* NCIMB 2021 in laboratory experiments. Their results, in contrast to those obtained by Dexter's group, showed that hydrophobic surfaces were more rapidly colonized than hydrophilic surfaces.

The results of Christensen et al. (1985), which were obtained using the same marine *Pseudomonas* as Fletcher & Loeb, helped to explain this apparent contradiction. The group's findings indicated that the extracellular polymers in the *Pseudomonas* changed with the stage of the growth cycle. Cells attached poorly to hydrophobic surfaces during exponential growth but on entering stationary phase, there was a 25-fold increase in the number of adhered cells. This coincided with the production of a hydrophobic carbohydrate polymer quite different in structure from the one produced earlier in the growth cycle.

Paul & Jeffrey (1985) concluded after a series of adhesion experiments with hydrophilic and hydrophobic surfaces that the attachment mechanisms utilized by the marine *Vibrio proteolytica* were unique to each type of surface. Their rationale depended on the inhibition by proteolytic enzymes of adhesion of cells to hydrophobic, but not hydrophilic polystyrene. Thus, it seemed that adhesins (adhesive proteins) were involved in cellular interactions with hydrophobic, but not hydrophilic surfaces. Bacterial 'footprints' were left on surfaces after cells had been removed with proteolytic enzymes. If the proteolytic enzymes contained hydrophobic domains in their structure, they could adsorb directly to the hydrophobic surface. The result of this would be to block adhesive sites or to change the properties of the surface. The change in the surface physiochemistry could occur if the proteins contained both hydrophilic and hydrophobic domains, in which case the hydrophilic portions of the adsorbed protein would face the aqueous side of the polystyrene/water interface. Either of these phenomena is likely to alter the attachment of the *Vibrio* cells. Protein-surface interactions are less likely with heat denatured enzymes, so these are perhaps inadequate controls. These are speculations, and of course, do not prove Paul & Jeffrey were wrong in their interpretation of

their results. In the light of more recent ideas, however, concerning the footprints of bacteria remaining on surfaces after cellular removal, one could conclude that proteins were *not* involved in initial cell adhesion since the footprints (initial adhesive) were resistant to proteolysis (Neu & Marshall 1991). The results show, however, how careful one must be in dealing with multicomponent systems where the surface chemistry of all the components is not known.

Differences in the adhesion of marine bacteria to surfaces of differing surface energies was also noticed by Shea et al. (1991). The workers measured the numbers of cells of *Delacy marina* or a spontaneous mutant remaining on hydrophilic or hydrophobic surfaces after a gentle washing procedure. The wild type organism attached to hydrophilic, but not hydrophobic surfaces. In contrast, the mutant, which did not form mucoid colonies on agar, attached to hydrophobic surfaces, but more poorly to hydrophilic surfaces. Shea et al. suggest that the reduced ability to form extracellular polymers in the non-mucoid mutant revealed a second type of adhesion mechanism present in the wild type organism. They make the interesting point that cells may possess multiple mechanisms of adhesion and utilize whichever mechanism is appropriate for the surface energy of the substratum contacted. If this is true, then a strategy for the design of antifouling coatings will fail if it depends for its efficacy on materials which are within a narrow range of critical surface energy. When motile bacterial species attach to a surface, their motility either is reduced or they become stationary. This is not so for the so-called 'gliding bacteria'. In investigating the reaction of these organisms to surfaces of known surface energy, Burchard et al. (1990) found that gliding was inhibited on surfaces of very low surface energy and 'skittish', i.e. appeared uncontrolled, on hydrophilic surfaces. It seems that gliding is a function of the degree to which the cells interact physicochemically with the surface and that this interaction is probably mediated by some extracellular polymer.

In other work, McEldowney & Fletcher (1986) concluded that in mixed culture experiments, the adhesion of one organism interfered with that of another and that the processes involved were dependent on the nutritional status of the cultures. Similarly, Pedersen et al. (1986) found that adhesion of 3 marine isolates showed different responses to hydrophobic and hydrophilic surfaces, the cell density of the adhering population and nutrient concentrations. These observations help address another very important point of experimental protocol. Many investigations of the adhesion of bacteria to surfaces utilize washed suspensions of cells. This is to preclude growth of the organism in an experiment where only adhesion is of inter-

est. This approach can be traced to the days when we believed that cells in the absence of nutrients were quiescent. They were often called 'resting cell suspensions'. We know now that starved cells are not inactive. Washed cells in buffered salt solution, a frequent milieu for adhesion experiments, undergo a starvation or stringent response (Matin et al. 1989). These responses are known to cause, among other metabolic activities, changes in bacterial surface characteristics in less than 30 min (Nyström et al. 1990). Thus, by using washed cell suspensions, we are in effect stimulating cells to change their adhesive properties. Cellular metabolism here is not quiescent, it is dynamic. We do not believe this is fully appreciated by the community of marine microbiologists.

### DO BACTERIA 'SENSE' A SURFACE?

If we accept that polymers external to the cell are involved in bacterial adhesion, then it is appropriate to ask if the surface-attached way of life influences their synthesis directly. The polymers can represent a considerable fraction of the resources of the cell and it is certain that their synthesis is under regulatory control. Vandevivere & Kirchman (1993) have pointed out that the 2-stage adhesion process (reversible, followed by irreversible adhesion; Marshall et al. 1971), could be interpreted in terms of the provision of an extracellular signal during the reversible phase, its interpretation leading to extracellular polymer synthesis and, thus, irreversible adhesion. These workers were able to provide support for their idea with bacteria isolated from deep subsurface aquifers, but the results are likely to have relevance to the marine environment. They found that the addition of clean sand to shake-flask cultures induced polymer synthesis. Furthermore, extracellular polysaccharides were up to 5-fold higher on a cell protein basis for cells in sand packed columns than in freely suspended cell cultures. The process was reversible, i.e. when attached cells were resuspended, polymer production decreased to its preattachment level. This was a most important control since it eliminated the possibility that the surface (sand) selected a sub-population of cells that were particularly active in polymer synthesis, (but see later remarks).

Supporting molecular evidence for the idea that surfaces do provide environmental signals that initiate synthetic responses in bacterial cells has been provided by several groups. For instance, Davies et al. (1993), using reporter gene technology, showed that growth of *Pseudomonas aeruginosa* on a teflon surface activated a promoter gene specifically associated with alginate synthesis (Alg C gene). Alg C promoter activity was higher in biofilm cells than planktonically grown cells

at all phases of growth except lag phase. Alginate is known to be the major extracellular polymer of this organism. Davies et al. review the various studies of conditions enhancing alginate synthesis by pseudomonads and it is interesting that in all situations one kind or another of environmental stress (e.g. high osmolarity, ethanol exposure, low water potential, low nutrients) increases polymer production.

The work mentioned above was performed with a cystic fibrosis isolate but, nevertheless, is relevant to the argument we are developing. Somewhat similar work by Dagostino et al. (1991) was performed with a marine pseudomonad, but was not focused on a particular metabolic pathway. Dagostino et al. used transposon mutagenesis to introduce a cassette of genes in *Pseudomonas* S9 that were activated only on surface attachment of the cell. The Lac Z ( $\beta$ -galactosidase) gene was used as a reporter, but it was not tied to a specific metabolic response, as it was in the work of Davies et al. Nevertheless, the results of Dagostino et al. showed that surfaces can elicit genetically controlled responses in an attached cell that are not elicited in planktonic culture, or when bacteria are grown on the surface of agar. One could speculate that such a response may in fact be global and many genes, not just those responsible for alginate (or other exopolymer) synthesis, may be turned on by the proximity of surfaces. This may be responsible for the differential physiological status of cells on surfaces and those in the water column. Since the experiments of Dagostino et al. were carried out in nutrient-rich medium, they speculated that their results were not an indirect effect of utilization of nutrient that had accumulated at a surface. Their methods allow selection of general surface-effected mutants. It now will be possible to use these to investigate the induction by surfaces of specific metabolic effects.

Some bacteria have a structural response to a surface rather than merely a change in cellular metabolic properties. Certain *Vibrio* alter the number and type of flagella when confronted with a substratum. They change from swimming, polarly-flagellate cells with a single flagellum, to laterally-flagellate swimmers with many flagella. This metamorphosis has been researched in the laboratory of Dr Michael Silverman. The question that has occupied this group concerns the means by which the environmental signal (a surface) is transduced so that the appropriate genes (LAF genes) are expressed and the lateral flagella proteins are synthesized. The problem was approached by inserting a constructed genetic message into *Vibrio parahaemolyticus* so that when the LAF-gene promoter was activated it allowed the transcription not of the LAF-gene directly, but a reporter gene. The reporter gene used in these experiments was not LAC Z, but the LUX com-

plex from *Vibrio fischeri*. The products of these genes are responsible for bioluminescence of this bacterium. Thus, when the *Vibrio* containing the LAF promoter and the LUX reporter received the stimulus that the cell was on a surface, the bacterial cell produced light. The light could be quantified by a variety of laboratory instruments including a liquid scintillation spectrometer (Belas et al. 1986, McCarter et al. 1992). When various environmental signals were examined, it was found that only an increase in viscosity of the milieu promoted LUX gene induction. As the viscosity increased, the cellular motility engendered by the polar flagellum was impaired, leading these workers to propose that microviscosity in the external milieu was sensed by the polar flagellum. In addition to the change in viscosity at the surface, the environment must be iron-limited, i.e. unless the medium is deficient in iron, the wild type organism will not synthesize lateral flagella. The process described above does not appear to be unique and has been discovered in certain *Serratia* (Alberti & Harshey 1990), but at the moment it cannot be considered to be universal in flagellate marine bacteria.

#### IS THERE A TRANSDUCER OF SURFACE PROXIMITY?

It seems that the accumulation of greater concentration of extracellular polymers in surface-associated cultures relative to suspended cultures is common in bacteria (Sutherland 1980). Abu et al. (1991), for instance, demonstrated with the marine organism *Shewanella colwelliana* that polymer production was enhanced when cells were grown on a dialysis membrane (8000 Da molecular weight cut-off) lying on the surface of marine nutrient agar. The use of the dialysis membrane in this case was for convenience in harvesting uncontaminated extracellular polymer. However, in the experiments described by Schneider et al. (1991) positioning of a dialysis membrane overlay on solid media provided potentially important information concerning control of polymer synthesis. Using a non-mucoid strain of *Pseudomonas aeruginosa*, Schneider et al. demonstrated that the synthesis of extracellular polymer on the surface of dialysis membrane was dependent on the molecular weight cut-off of the membrane. When the membrane allowed diffusion of compounds of 30 to 50 kDa, no polymer was accumulated. With membranes of lower molecular weight cut-off, polymer was found. This suggests that compounds external to the cell and in the molecular weight range 30 to 50 kDa are important in the synthesis of polymer. However, it does not suggest a specific role for these compounds. The accumulated evidence is now suffi-

cient for us to believe that surfaces do influence polymer synthesis. The genetic evidence (Davies et al. 1993, Dagostino et al. 1991) is particularly convincing in this regard. No one yet has found a particular extracellular signal that turns on extracellular polymer synthesis. In anthropomorphic terms we would ask 'How does the cell know it is on a surface?' The signal may be physicochemical (microviscosity, water activity), chemical (adsorbed nutrient, conditioning film) or physical (surface as a diffusion barrier). We suggest that it could also be a combination of physical and chemical signals in the following manner. Let us assume that bacteria produce low levels of extracellular polymers constitutively. When the cells are in planktonic growth, these polymers diffuse away from the cell. When a cell is on a surface, diffusion from the cell is reduced by the proximity of the surface and local extracellular polymer concentration will thus increase. If such a local increase in polymer concentration is sensed by cell surface-bound receptors, it is possible that the signal so formed (receptor occupancy) could initiate increased polymer synthesis. Although this hypothesis fits the available information concerning increased polymer synthesis by cells on surfaces, there is no direct evidence to support it at the moment. This hypothesis has also been advanced for diatom adhesive synthesis (Wigglesworth-Cooksey & Cooksey 1992).

The general topic of surface-sensing by bacteria has been discussed by McCarter et al. (1992). In their paper, which is part of a volume of the journal *Biofouling* [Volume 5(3), 1992] dedicated to the subject, they make 2 points which we would like to reiterate here. Firstly, if cells sense surfaces, there is no need for the signal to be singular. In fact, it makes more sense for multiple signals to be used, since the commitment to the attached way of life involves expenditure of considerable cellular resources. The more information, the better the decision!

A second point made by these authors is contrary to all we have written here (and much that they have published!). They suggest that in spite of our hypotheses concerning sensing mechanisms, perhaps cells do not need to sense surfaces in order to adapt to life on them. They argue that any clonal population expresses considerable heterogeneity of traits. They provide evidence that mechanisms which generate a diversity of forms, including adaptation to surfaces, operate in bacteria. Thus, subpopulations would be selected by surfaces and so, concentrated there. Similarly, generation of less adhesive forms at the surface would result in cellular detachment. Thus 'continual generation of diversity coupled to the selective pressures of various environments results in matching the appropriate phenotype to a specific environment'. In other words, the process is purely stochastic.

### ARE THE MATRIX EXOPOLYMERS THE MOLECULES INVOLVED INITIALLY IN THE ADHESIVE PROCESS?

There is growing concern that the efforts expended in analyzing the extracellular polymers of marine microorganisms will not result in increasing our understanding of the *initial*, as opposed to the *permanent*, adhesive process. For instance, Allison & Sutherland (1987) measured the total extracellular polymer production by 2 freshwater microorganisms — a wild type and the mutant derived from it. Polymer production and adhesive ability were not correlated. They suggested that the polymeric material they had measured was extracellular matrix and not the material involved in the initial adhesive event. More recently Neu & Marshall (1991) and Neu (1992) have proposed that the material from beneath an attached cell that is left behind on the surface after the cell has been removed, may be the initial adhesive. If they are correct, then it is on these 'footprints' that our analytical effort should be concentrated.

Some evidence has been accumulated already that initial adhesive and matrix polymers are different. Fletcher et al. (1991) and Marshall et al. (1989) have investigated both marine and freshwater bacterial polymers by microscopical optical techniques and shown that indeed there are differences between initial and matrix polymers. In their experiments, the adhesion of cells that had been allowed to settle for only minutes was considered to involve the initial adhesive. For matrix polymers, cells that had existed in a biofilm for 24 h were investigated. The techniques used were interference reflection microscopy (IRM) and light sectioning microscopy (LSM). Fletcher et al. explain how their optical systems work and it is beyond the scope of this review to include detailed explanations. In brief however, IRM measures how close a cell is to a surface whereas LSM measures film (in this case biofilm) thickness. Thus, IRM responds to initial adhesive changes, whereas LSM responds to changes in the matrix polymer. From their results, it is generally true for a series of organisms that the initial adhesive and matrix polymers are distinct. It also appears that the polymers from marine and freshwater organisms are also different. This was deduced from treatments with cations and dimethyl sulfoxide (DMSO). Polymers from freshwater organisms appeared to contract in their presence whereas those from the marine *Pseudomonas* NCIMB 2021 did not.

### ADHESIVE EVENTS IN DIATOMS

These phenomena have been reviewed recently (Wigglesworth-Cooksey & Cooksey 1992). The adhe-

sive process has been dissected using various drugs and metabolic inhibitors in conjunction with an assay that measures the retention of the diatom *Amphora coffeaeformis* on a glass surface after a hydrodynamic challenge (Cooksey 1981). Cells retained after colonized surfaces were rinsed and were quantified by their chlorophyll fluorescence. By the use of this assay, we were able to show that adhesion, like motility, is  $\text{Ca}^{2+}$ -dependent. The process requires metabolic energy, protein and glycoprotein synthesis, but it is not light-dependent. The fact that  $\text{Ca}^{2+}$ -channel blockers that act at the cell membrane prevent adhesion in this organism, indicates that intracellular  $\text{Ca}^{2+}$  is required. Taken together, these results imply that adhesion can be characterized as a secretory process. The  $\text{Ca}^{2+}$ -chelator, EGTA, causes a cohesive break in the adhesive used to attach cells to glass surfaces suggesting a further, and extracellular, role for  $\text{Ca}^{2+}$ . So-called 'footprints' of the raphes of the diatom remain on the surface after EGTA treatment (Cooksey & Cooksey 1986).

There is no doubt that such an extensive set of biochemical and energetically-expensive events is under the metabolic control of the cell. At the moment, we have no idea how this is achieved. In fact, the study of transmembrane signalling in diatoms is in its infancy. So far as we are aware, cellular response to signals other than light has been studied only in *Amphora coffeaeformis* and a related organism (Cooksey & Cooksey 1988). There are no studies concerning the genetic control of the process in diatoms, such as those mentioned above for bacteria.

We have used the chemotactic system of *Amphora* to investigate environmental transmembrane signal transfer and its consequences. Diatoms move by gliding, i.e. they cannot move unless adhered. Therefore, those signals that induce motility, especially directed motility (chemotaxis), must induce adhesive mechanisms first. Chemotaxis in *Amphora* is receptor-controlled and probably a  $\text{Ca}^{2+}$ -mediated event (Cooksey & Cooksey 1988). There are at least 3 types of receptors for simple sugars and we can merely speculate how these may be involved in the sensing of a surface.

Earlier, we mentioned the so-called molecular marine conditioning film that is adsorbed to a clean surface seconds after its immersion in the sea. Since some believe the film to be glycoproteinaceous (Baier 1980), it may be that diatom cell surface receptors similar to the ones described for sugars are able to bind to the terminal sugars of the carbohydrate side chains of the adsorbed layer and set in motion the cascade of events that leads to the secretion of an adhesive polymer by the cell. This hypothesis is not entirely different from that described earlier for bacteria. Here, the source of the signal, but not its type, is different. For

bacteria, we suggest that bacterially-synthesized extracellular polymers, which as is well known contain sugars and sugar-like monomers, are the transducers of surface proximity.

## MIXED MICROBIAL FILMS

In the sea, a biofilm on an illuminated surface will contain many types of cells including bacteria and diatoms, yet as a rule, we study attached cells in axenic cultures. This is legitimately criticized by ecologists who insist we must study the real world to understand how it works. However, given the confusion that exists concerning even the study of cultures of a single bacterial species, it is reasonable to assume that studying a dynamic mixed attached microbial population would be extremely difficult. We proposed a compromise situation wherein it was possible to study algal-bacterial interactions in a defined attached population (Murray et al. 1986, 1987). Cells of *Amphora* and the marine bacterium *Vibrio proteolytica* were attached in known ratio to the surfaces of polystyrene Petri dishes. The uptake of  $^3\text{H}$ -thymidine was shown by autoradiography to be incorporated only into bacterial DNA and only when the mixed biofilm was illuminated. The incubation medium contained no added carbon or organic nitrogen source. Thus, we concluded that bacterial growth as measured by  $^3\text{H}$ -thymidine incorporation, was dependent on diatom metabolism. Much work on biofilms has been initiated by first harvesting the mixed cell population from a surface before physiological measurements were made. Using the defined consortium described above, we were able to show that this resulted in an artifactual increase in metabolic activity in the bacterial fraction of the consortium. The most likely reason for this was that cellular damage occurred during the harvesting process which provided the bacteria with assimilable carbon that was not available in the intact film.

In the experiments described above, the diatom and bacterial populations were allowed to attach to the polystyrene surfaces sequentially. In nature, both populations, in theory, would have the opportunity to attach simultaneously. It is now possible with microscopic image analysis techniques and flow chambers to gauge the influence of the attachment of one type of cell on another. Such a study is only possible in a laboratory situation, but would provide information concerning the competition for resources (if the surface can be regarded as a resource) and cell-cell interactions. We have initiated a cooperative venture between our laboratory and that of Dr H. Busscher at the University of Groningen, The Netherlands, to examine these ideas.

## THE SURFACE AS A SOURCE OF NUTRIENTS

In much of this review, we have considered only the initial colonization of a surface in the marine environment. However, colonization is merely the first step in the formation of a biofilm. Film thickness increases by growth of already attached cells. Various workers have suggested that surfaces, especially in oligotrophic environments, are sources of nutrients. For example, Power & Marshall (1988) investigated, individually, the growth of a marine pseudomonad and a vibrio species on glass and dialysis membranes. Each substratum was coated with stearic acid. Both types of cells were able to grow, supporting the contention that indeed surface-bound nutrients can be metabolized. It is interesting to note that the *Pseudomonas* and *Vibrio* were found to have evolved differing strategies in their metabolism of the surface-bound carbon source. Whereas the pseudomonad remained at the surface, vibrio cells detached before dividing. Once more this provided an example of the pitfalls of making ecological generalizations based on studies of one organism. Various other papers from Marshall's laboratory (Kefford et al. 1982, Humphrey et al. 1983, Hermansson & Marshall 1985) give considerable support to the hypothesis that the surface is a nutritionally-enhanced environment. If such surfaces were exposed to flow, surface-adsorbed nutrients would be continually replaced, allowing a large surface-bound population to result, i.e. a biofilm. This process should also take place to some degree with bacteria attached to free-floating particles in the sea. It is possible that the phenomenon observed by Maki et al. (1990) is related to that described by Power & Marshall. Maki et al. observed that the number of bacteria associated initially with metal surfaces exposed in Antarctic waters fell after about 1 h of exposure. It is possible that this reduction in surface-associated cell numbers was caused by bacterial scavenging of surface-bound nutrients followed by release of the cells from the surface.

These same arguments cannot be made for diatoms, since so far as we are aware, no similar experiments have been carried out. Furthermore, the consensus is that heterotrophy plays little or no part in diatom nutrition in the marine environment. Recent studies, however, may cause this to be re-evaluated.

The sediments in near-shore areas and adjacent wetland are frequently covered with diatoms, even when because of wave action and the character of the sediment, the water is turbid and light penetration is reduced. These sediments almost always contain more dissolved organic carbon in their pore water than the overlying water column. The role of the indigenous attached diatom population in the mineralization of the organic carbon pool has been controversial for the last

20 yr. Although in the laboratory, many diatoms will grow on compounds found in the pool, they do so usually at concentrations higher than those found in analyses of bulk sediments. Recently, we have discovered positive chemotaxis to a small number of organic compounds in 2 diatoms of the genus *Amphora* — organisms frequently found in sediments (Cooksey & Cooksey 1988). Chemotaxis to some of these compounds (D-glucose, L-glutamate) and their ability to be metabolized heterotrophically or mixotrophically is connected in some of the cases. If the possession of chemotactic ability is common in motile, pennate diatoms, then it may be suggested that they are able to use this behavior to move towards higher concentrations of nutrients and thus, may be much more involved in the biogeochemistry of sediments than current knowledge suggests. Although the term is not normally used in microbiology, this is in effect 'foraging'.

Although chemotaxis in diatoms is a relatively new finding, phototaxis is well known (Halldal 1962). Movement along a chemical gradient is, however, no different in concept (or probably in biochemistry), than moving towards a higher light level. It is common for a diatom attached to a sediment particle to be subjected to reduced light as the sediment is disturbed. Thus, a cell capable of mixotrophy or heterotrophy in a gradient of a metabolizable compound *and* in reduced light, is potentially presented with a choice. We are unable to speculate whether either of these 2 environmental signals will predominate.

Consider, for instance, the following information which was collected using several organisms (Cooksey & Chansang 1976, Cooksey & Cooksey 1978, 1988, Miller 1980, Wigglesworth-Cooksey & Cooksey 1992). The operation of chemotaxis requires the synthesis of specific receptors proteins, the differential occupancy of these receptors by chemotactically-active compounds, the transduction of the receptor signal(s) to the motility apparatus and, at least in *Amphora*, the differential interpretation of that signal at the 2 raphes, otherwise directional response (turning) would not be possible. This highly abbreviated and simplified description requires that a considerable degree of genetic, and ultimately metabolic, control be operating in these organisms. In view of its sophistication, it is hardly likely to be a redundant system. That chemotaxis is likely to be of importance in toxic avoidance has already been shown. The soft coral *Leptogorgia virgulata* remains substantially fouling-free in nature. Extracts of the coral prepared by Dr Nancy Targett, University of Delaware, caused negative taxis in *Amphora* (Wigglesworth-Cooksey & Cooksey 1991), as well as loss of motility and cellular detachment from the surface as concentrations were increased. The chemotactic threshold for glucose for *A. coffeaeformis*

is 1  $\mu\text{M}$ . A similar, but smaller *Amphora* grows heterotrophically at 5  $\mu\text{M}$  glucose and has a  $K_{1/2}$  for heterotrophic growth on glucose of 25  $\mu\text{M}$ . Uptake of glucose, however, in this organism has a  $K_{1/2}$  of 92  $\mu\text{M}$  and is inducible by glucose, but not by its non-metabolizable analogue, 3-O-methyl glucose. Further, chemotaxis shows specific desensitization behavior. Thus, a cell at low concentrations of glucose is able to steer towards higher glucose concentrations, grows and thus reduces the local concentration. It may then continue to move along the gradient. At sufficiently high local concentrations, the gradient will no longer be perceptible and chemotaxis will diminish. The distinction between responses of the cell during chemotaxis and the induction of transmembrane transport is significant. It is more important to have a specific uptake mechanism than a highly specific chemotactic mechanism. Thus, although a cell can detect a 3-O-methyl glucose gradient, 3-O-methyl glucose does *not* induce transport or support growth. This arrangement of physiological functions preserves cellular resources and maximizes environmental resource utilization.

The energy expenditure of chemically-directed taxis is not likely to be appreciably greater than that needed for random motility, which is carried out by unstimulated cells. Thus, the potential increase in resources available to the attached chemotactic diatom cell are achieved at no obvious additional metabolic cost.

These arguments concerning the role that chemotaxis could play in attached diatom autecology are based on the assumption that pore-water analyses of bulk sediment samples do not reflect local concentrations of organic compounds that could be metabolized mixotrophically or heterotrophically by the cells. Ideally, sampling and analyses aimed at understanding the chemotactic environmental signals, which may affect diatoms, should be made at the spatial concentration scale detected by diatoms. This is related to their length which in the case of the most organisms is in the range 10 to 100  $\mu\text{m}$ . No technology for sediment analysis currently in use has spatial resolution at this level. Microelectrodes are either not available (amino acids) or are not sufficiently robust, sensitive or small enough (glucose). Patchiness in sediment cores at the cm scale has been studied by others (Henricks et al. 1984, Bardige & Martens 1990) and found to be rather small ( $\pm 20\%$  between replicate cores for glutamate and dissolved free amino acids). Our current inability to measure microscale ( $\mu\text{m}$ ) patchiness is not critical to our argument however. For us to conclude that chemotaxis is possible, it would be necessary only to show that the average concentration of compounds known to be chemotactically active are greater than those needed for a threshold response. It would be hard indeed to argue that microscale patchiness did *not*

exist in sediments. For there to be no gradients present, a sediment would need to be completely mixed (as this term is used in chemical reaction kinetics) and the inputs and sinks would need to be equivalent — hardly a likely phenomenon at any scale in natural environment. Microscale gradients of dissolved gases (e.g.  $\text{O}_2$ ,  $\text{H}_2\text{S}$ ) and other nutrients ( $\text{NH}_4^+$ ) at the relevant scale have been demonstrated using microelectrodes in sediments, mats and biofilms (Revsbech & Jørgensen 1986). We are currently investigating these ideas concerning the ecological relevance of the attached state in diatoms.

### PRACTICAL CONSIDERATIONS

One of the driving forces for much of our interest in the ecology of marine biofilms derives from their nuisance value to man-made structures. The most commonly quoted example is the deterioration of the immersed surface of a ship which leads to increased drag, loss of performance, increased fuel consumption and corrosion. We have known for thousands of years that macroinvertebrates that cause calcareous fouling, e.g. barnacles, bear much of the responsibility for these problems, but it is only recently that we have come to understand that biofilms consisting only of microorganisms cause severe problems.

Although it was suggested from the results of fouled spinning disc experiments that biofilms could cause a large drag penalty (Loeb et al. 1984), it took trials with a ship to convince the skeptics that a microbial slime only a few hundred microns in thickness could cause hydrodynamic problems. Bohlander (1991) described experiments in which a U.S. Naval vessel was used. The bottom paint of the ship was 22 mo old and was fouled with a microbial slime, but the hull was substantially free of calcareous fouling. The propeller was cleaned and polished to remove effects of fouling on this structure and tests of speed and power consumption were made. The hull was then cleaned with an underwater scrubbing device using soft polypropylene brushes that removed the biofilm, but left in place the calcareous material. The sea trials were repeated and the same measurements taken. The results showed that after cleaning, as much as 18% less power was needed to maintain a given speed, maximum speed increased 1 knot ( $1.85 \text{ km h}^{-1}$ ) and fuel costs were reduced by US\$ 400  $\text{h}^{-1}$  at 26 knots ( $48 \text{ km h}^{-1}$ ). From these figures, it is evident that the consequences of adhesion and growth of microorganisms on the hull of a ship are very significant. The ship in these trials was painted with an ablative coating containing both cuprous oxide and tributyl tin oxide. At the time of the trials, this was considered to be 'state of the art' as far



as hull protection was concerned. Now, in most parts of the world, tributyl tins can no longer be used. It is evident that the materials used to replace them must control microbial adhesion as well as the adhesion of higher forms of life.

## CONCLUSIONS

In this review, we have endeavored not to be encyclopedic, but to point out that the design of experiments to measure the adhesion of cells to surfaces is not without its pitfalls. Furthermore, we have tried to indicate the ecological relevance of the attached state. In spite of the fact that Zobell in 1943 alerted us to the importance of the attached microbial community in the sea, we still do not understand completely how cells sense surfaces (or even if they do) nor the biochemistry behind the mechanism of adhesion.

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