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The Microphysiology of Consortia Within Adherent Bacterial Population

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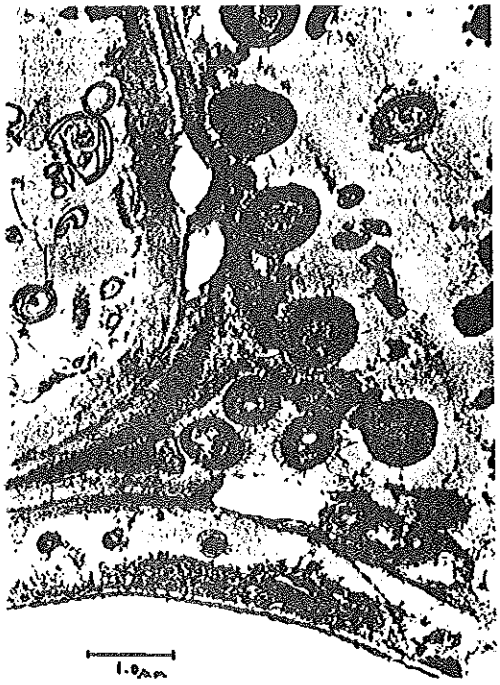
In coastal sediments, the decomposition of organic matter via sulfate reduction is thought to be mediated by a consortium of fermentative bacteria and SRBs. The fermentative bacteria hydrolyze the complex components of the biologically-labile fraction of detritus to low molecular weight compounds such as volatile fatty acids and hydrogen which, in turn, are utilized by the SRBs (Sansome and Martens, 1981b; Jorgensen, 1982). Efficient transfer of these volatile compounds from one population to the other is likely dependent on a spacial proximity and stability afforded by the polymeric material which anchors the cells to the sediment particles.

In the water column, where surface availability is reduced and diffusion rates and fluid shear forces discourage establishment of physical associations between microbial species, more complex mechanisms have evolved to facilitate interactions betwen cells. For example, a chemical attractant for free-living bacteria is excreted near the heterocyst-vegetative cell junction of some filamentous cyanobacteria (Paerl, 1982). The mucilaginous sheath surrounding the heterocyst provides a surface for attachment of the attracted bacteria. Acquisition of an epiphytic bacterial population leads to a local reduction in the oxygen tension around the heterocyst which then enhances nitrogen fixation by these specialized cells in an otherwise aerobic environment (Paerl, 1978; Paerl and Gallucci, 1985). Diverse, active, N_2-fixing cyanobacteria have been found associated with surfaces of marsh grass, seagrass, and seaweed (Goldner, 1980). Bacteria are always closely associated with cyanobacteria in these situations.

Cellulose degradation is an important process in the marine environment, particularly in coastal and estuarine habitats where marsh grass and seagrass are major contributors of carbon to the detrital food web. Although little is known about the community structure of microorganisms that participate in the attack of cellulose in these systems, some information on cellulose degradation has been obtained from studies in ruminant microbiology. Here, microbial attack of cellulose is mediated by bacterial populations that employ capsular polysaccharides to attach to the water-insoluble cellulose fibers (Patterson et al., 1975; Aiken, 1976). Electron microscopic studies have revealed very extensive glycocalyx-enclosed bacterial microcolonies on many fiber surfaces (Cheng et al., 1981). That the bacteria are often positioned in crevices that conformed to their own cell outline suggests that the original surface was eroded by the local action of extracellular cellulase enzymes excreted by the indivudal bacterial cells (Fig. 1). The appearance of 2 layers of morphologically-distinct bacterial populations, one attached exclusively to the surface and the other positioned on top of the surface-associated population, suggests that physical juxtaposition of different bacterial species is of pivotal importance to the complex digestive function mediated by cellulose-degrading bacteria (Fig. 2). Kudo et al. (1987) have recently demonstrated that coculture of cells of *Treponema bryantii* with cellulose-digesting adherent cells of *Bacteriodes succinogenes* accelerates the rate of cellulose digestion by +/– 9%. They suggest that these heterotrophic spirochaetes contribute to the activity of the primary cellulose digestors by withdrawal of the soluble products of this digestion from these functional consortia. In a similar manner, seagrass degradation is likely to involve interactions betwen several epiphytic bacterial species.

Consortia of sessile bacteria participate in the corrosion of metallic structures submerged in seawater. Respiratory activities of aerobic, slime-forming heterotrophs that colonize submerged surfaces reduce the local oxygen concentration at the liquid/solid boundary, thereby creating anaerobic conditions suitable for growth of SRBs



ig. 1. Transmission electron micrograph of barley raw partially digested by a natural population of umen bacteria. Primary cellulose-decomposing organisms, resembling *Bacteroides succinogenes* have olonized the cellulose substrata, by means of their idial glycocalyx polymers, and have begun to digest is structured material to form »pits«. Bar = 1.0 µm.

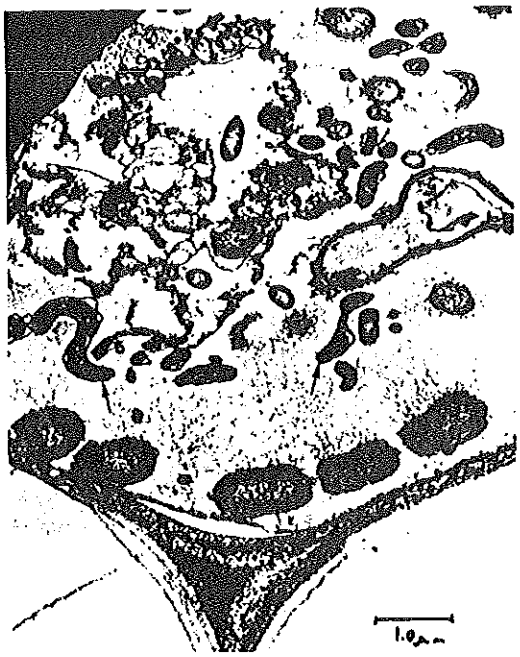


Fig. 2. Transmission electron micrograph of the preparation seen in Figure 1 showing colonization of the cellulose surface by similar primary cellulose decomposers and the juxtaposition of spiral cells (arrows) resembling *Treponema bryantii*. While these heterotrophic spirochaetes cannot digest cellulose their presence with cells of *Bacteroides succinogenes*, in coculture, increased the rate of cellulose digestion suggesting a functional cellulolytic consortium. Bar = 1.0 µm.

Tatnall, 1981a). The metabolic activities of RBs may then accelerate surface corrosion by emoving gaseous hydrogen at the cathode and/or lepositing ferrous sulfide and hydroxide at the node (Sharpley, 1973).

Slime-producing bacteria area also thought to reate differential aeration cells which can lead o pitting corrosion (Tatnall, 1981b). Other types of corrosion-causing concentration cells may also levelop on metallic surfaces under biofilms conaining populations or physiologically-distinct acteria. Limitations in non-destructive surface nalysis have compromised our ability to denonstrate reactions in or under biofilms.

That microorganisms differ in their ability to concentrate metal ions from solution suggests hat they may be capable of forming metal concentration cells. Bacteria employ various mechanisms to concentrate metals from solution. Some bacteria are able to accumulate metals intracellularly against a large concentration gradient (Strandbert et al., 1981), while others bind large quantites of metal ions extracellularly through interactions with cell wall polymers or exopolymers (Hoyle and Beveridge, 1983; Brown and Lester, 1979). Differences in metal-binding capacity are expressed in terms of a stability constant and binding site density. Copper-binding constants for different bacterial exopolysaccharides are presented in Table 1.

The FRI exopolysaccharide listed in Table 1 was isolated from a bacterium recovered from metal-laden sediments. This exopolymer is likely involved in cell adhesion since it is produced in cultures which exhibit cell aggregation (Platt et al., 1985). The polymer also retains a firm association with the cell surface.

Experiments utilizing Fourier transform infrared spectroscopy (FTIR) demonstrated that hydrated, cell-free exopolymer accumulated on submerged metallic copper surfaces. When a copper-coated, germanium internal reflection element (IRE), which samples only the aqueous boundary layer in contact with the copper, was submerged in an aqueous suspension of the exopolymer, an increase was observed with time

Table 1. Cu^{+2}-binding characteristics of bacterial exopolymers

Polymer	10g k[1]	MBA[2]
Klebsiella aerogenes exopolymer[3]	7.69	ND[4]
Xanthomonas campestris exopolymer (xanthan gum)[5]	8.26	123
FRI crude exopolymer[5]	8.86	37

[1] conditional stability constant.
[2] maximum binding activity (nmol/mg[dry wt]).
[3] from Rudd et al. (1984).
[4] no data.
[5] from Mittelman and Geesey (1985).

in the intensity of an ir absorption band of the exopolymer. The increase occurred only at the surface of the IRE. No increase was observed in the bulk solution based on determinations made with an uncoated zinc selenide IRE (Geesey et al., submitted for publication).

Furthermore, the thickness of the metallic copper film on the Ge IRE decreased in the presence of the FRI polysaccharide preparation. The decrease in film thickness was determined by the increase in intensity of the ir water absorption band at 1640 cm^{-1} as shown in Fig. 3. No detectable change in film thickness was observed when the copper-coated IRE was submerged in polysaccharide-free water. Other polysaccharide preparations also concentrated at the surface of the coppercoated IRE and promoted dissolution of the metallic film (Fig. 3). The different slopes indicate that each type of polysaccharide effected a different rate of copper loss from the surface. Recent work with J. Jolley at the Idaho National Engineering Laboratory has revealed, however, that not all exopolysaccharides of adherent bacteria cause deterioration of the copper film even though they bind to the surface. *Pseudomonas atlantic,* a bacterium commonly associated with marine biofilms produced an extracellular slime that did not attack the metallic copper surface in seawater.

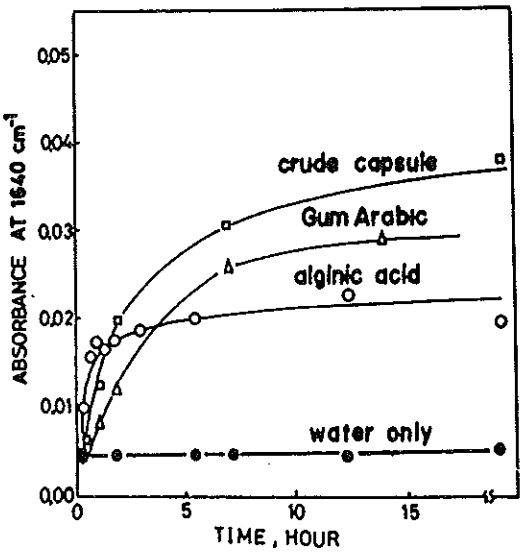


Fig. 3. Absorbance of 1640 cm^{-1} water band as a function of exposure time of copper-coated germanium IRE to 2% alginic acid (○), 10% Gum Arabic (△), 1% crude exopolymer from a sediment bacterium, FRI (□), and distilled water only (●).

We proposed that dissolution of the copper metal surface is due to binding of dissolved cupric ions by the FRI polysaccharide at the solid/liquid interface (Geesey et al., 1986). Two possible interacting mechanisms have been considered to date. High affinity binding of cupric ions by sites on the polysaccharide in the boundary layer adjacent to the metal surface may cause a localized reduction in the equilibrium concentration of the free dissolved metal ion. Ionization of metallic copper (i.e., surface dissolution) would then be favored in order to re-establish equilibrium between the 2 copper species. The different rates of metallic copper dissolution observed in the presence of the various polysaccharides may be related to differences in stability constants for the polymer-cupric ion interaction, while the extent of copper dissolution may be controlled by the number or density of binding sites on the polymer. A comparison of binding constants and copper dissolution rates for various bacterial surface polysaccharides is still needed, however, to assess the validity of this proposed corrosion mechanism.

Microbially-mediated acidification of the liquid boundary layer adjacent to the copper film may also have contributed to surface dissolution. The polysaccharides which induced this surface effect are acidic in nature with pKa values ranging from 3–5. Although the acidic groups of the FRI exopolysaccharide have not yet been identified, those of alginic acid and gum arabic are known to consist of carboxyl residues. Potentiometric titration of the FRI exopolysaccharide in the presence and absence of cupric ion revealed that binding of the metal ion resulted in the release of protons from the polymer (Mittelman and Geesey, 1985).

Corrosion damage caused by bacterial production of organic acids is not believed to be a very common problem (Sharpley, 1973). Acidic capsular polysaccharides have not been considered in this context, however. Nevertheless, acidic exopolymers are frequently associated with ses-

ile bacteria in aquatic environments. Sutherland 1980) found that 20 to 25% of the acidic exopolysaccharides isolated from freshwater and marine bacteria possess uronic acid subunits which contain reactive carboxyl groups. Thus, ocalized acidification of surfaces colonized by slime-producing bacteria is likely to be more prevalent than has been previously recognized.

In view of the evidence indicating the differential capacity of bacterial exopolysaccharides to 1) bind free cupric ions and 2) dissolve metallic copper surfaces, a corrosion reaction involving a metal concentration cell, formed as a result of the colonization, exopolymer production and microcolony development by a mixed bacterial biofilm has been proposed (Fig. 4). A metal concentration cell should develop over an area of surface where exopolymers exhibiting different cupric ion binding characteristics are excreted by bacteria in adjacent microcolonies. The area underlying bacteria (type A) that produce exopolymers containing high affinity cupric ion binding sites will be anodic to the area under bacteria (type B) which elaborate exopolymers containing binding sites of low affinity for the metal ion (cathode). Metallic copper at the anode dissolves in order to replace the free cupric ions taken out of solution by the polymer. The movement of electrons through the metal from the anode to the cathode and the relatively high concentration of cupric ion in the solution around the polymers with low cupric ion affinity will encourage reduced copper to be plated out at the cathode. This reaction should continue until all the cupric ion binding sites on the polysaccharide are saturated. Evidence of saturation is provided by the plateau achieved in intensity of the water absorption band after several hours exposure of the copper film to each polysaccharide in Fig. 3.

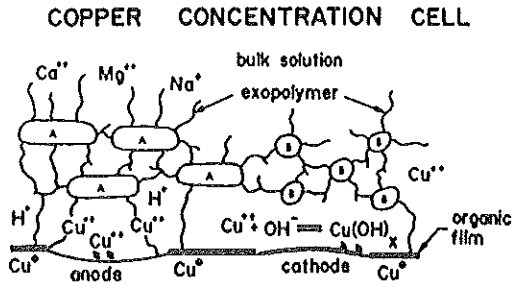


Fig. 4. Schematic representation of proposed copper concentration cell established on a copper surface coated with a biofilm of a mixed microbial flora. Exopolymers of a microcolony of Bacterium A possess high-affinity Cu(II)-binding sites. Free Cu(II) ion concentration in the water trapped in the biofilm in the area around cells of Bacterium A would be expected to be less than that in solution around cells of Bacterium B during biofilm growth. Carboxyl groups are depicted as the Cu(II)-binding sites on the exoplymer.

The net effect of a metal concentration cell is the formation of a pit in the metal surface under the colony of bacteria that elaborate exopolymer possessing the high affinity metal ion binding sites. Pits would not be expected to develop uniformly over the surface. Only under colonies of bacteria that possess exopolymers with metal-binding characteristics substantially different from those of adjacent bacterial colonies would a pit be expected to form. This is consistent with the fact that case histories of pitting corrosion often report that the pits are distributed irregularly over a surface.

Although these experiments directly demonstrate that cell-free preparations of exopolymer are capable of dissolving metallic copper surfaces, formation of metal concentration cell by a consortium of sessile, exopolymer-producing bacteria has yet to be demonstrated. This will require analytical techniques that are sensitive to very localized differences in metal dissolution rates under biofilms. Recent advances in FTIR microscopy, and Auger, and x-ray photoelectron spectroscopic surface analysis, however, may provide the spatial resolution required to distinguish differences in surface chemistry under bacterial microcolonies situated only a few micrometers apart.

In spite of the difficulties in evaluating potential interactions between physiologically-distinct populations of sessile microorganisms, microscopic studies in a variety of habitats, including the marine environment, suggest that consortial activities are required for many important microbially-mediated processes that are not favored or possible in the presence of single species populations. It is also clear that spatial considerations are important. Studies designed to evaluate activities of mixed populations of sessile bacteria should be performed in a way that preserves the physical positioning of the cells. Bacterial populations should, therefore, be encouraged to elaborate the holdfast exopolymers in laboratory cultures that they utilize in their natural habitat. Furthermore, laboratory cultures should be encouraged to develop in a heterogeneous rather than a homogeneous manner. Assay methods should be adapted to accomodate the non-random distribution of the populations in a culture or in a sample collected from the natural environment. Implimentation of these considerations will undoubtedly provide a better understanding of the activites of natural assemblages of microorganisms in the marine environment.

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