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MIC AND BIOFILM HETEROGENEITY

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ABSTRACT

Recent investigations have shown that many biofilms have a heterogeneous structure with microorganisms aggregated in clusters, forming physiological microniches within the biofilm where local chemistries are distinctly different from the surrounding. The persistence of these highly localized conditions depends on the activity of the respective physiological groups of microorganisms. This paper presents selected advances in studying consequences of biofilm heterogeneity that can be linked to MIC.

THE CONCEPT OF HETEROGENEOUS BIOFILMS

Many research groups expressed dissatisfaction with the conceptual model of homogeneous biofilm [1-5]. The introduction of Confocal Laser Microscopy (CLM) and analysis of the first confocal images of biofilms precipitated the notion that the conceptual model of homogeneous biofilms was inadequate in some instances. These images showed that microorganisms in bioflms were aggregated in microcolonies, not uniformely distributed as previously thought[6] (Figure 1).

It has been documented that biofilm heterogeneity influences the rates of nutrient transport and consumption near and within biofilms[7-14] and, therefore, influences biofilm activity. To accommodate these observations, the model of homogeneous biofilms has been appended by a model of heterogeneous biofilms, displaying microorganisms aggregated in microcolonies instead of uniformely distributed throughout the matrix. At present both conceptual models, homogeneous and heterogeneous biofilms, are functioning side by side; the conceptual model of heterogeneous biofilms is preferred by life scientists, while the model of homogeneous biofilms is favored by the engineering community, mostly because it simplifies mathematical modeling of biofilm activity. There are signs that the gap between these two models is closing, however. Growing popularity of the model of heterogeneous biofilms among life scientists stimulates the engineering community to construct mathematical models of such biofilms. The task is difficult for many reasons, among them because there is little experimental data describing microbial activity distribution in heterogeneous biofilms and because heterogeneous biofilms need to be modeled in 3-D, which complicates the matter. New approaches to mathematical modeling of biofilms, cellular automata and neural networks, may bring a new generation of 3-D biofilm models, which include complex structure of the biofilm.

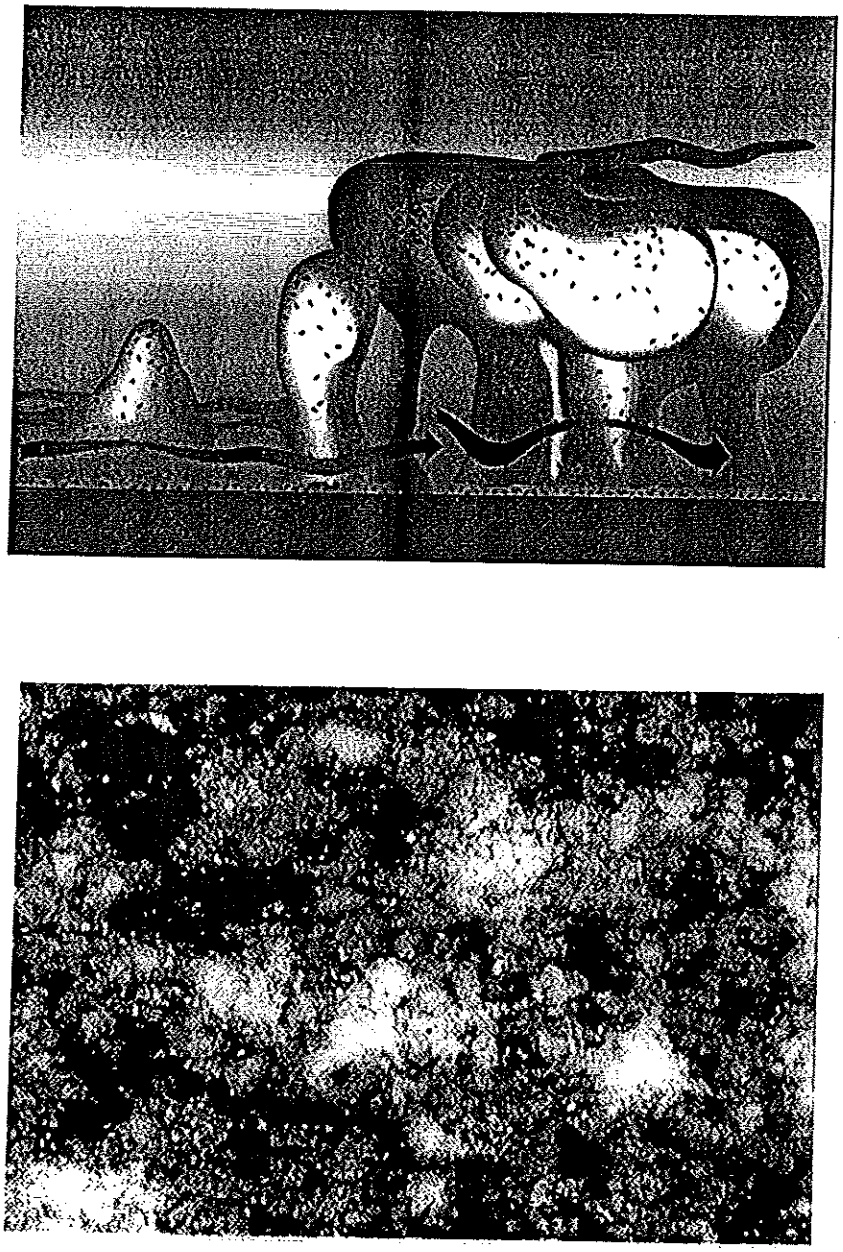

Figure 1. Heterogeneous biofilms. Top: diagrammatic representation of the structure of a hypothetical bacterial biofilm drawn from CSLM examination of a large number of mixed-species biofilms. A network of interstitial voids filled with water surrounds discrete microcolonies of microorganisms. The arrows indicate convective flow within the water channels. Bottom: light microscope image of a heterogeneous biofilm.

As expected, distribution of nutrients in heterogeneous biofilms is non-uniform; the images of nutrient distribution that were built for uniform biofilms became much more complicated. Figure 2 shows an example of an effect that structural heterogeneity of biofilms may have on oxygen transport through the biofilm matrix[2]. Oxygen is depleted within the microcolony while it freely penetrates the biofilm through the voids. If such a biofilm is deposited on a metal surface it may generate differential aeration cells.

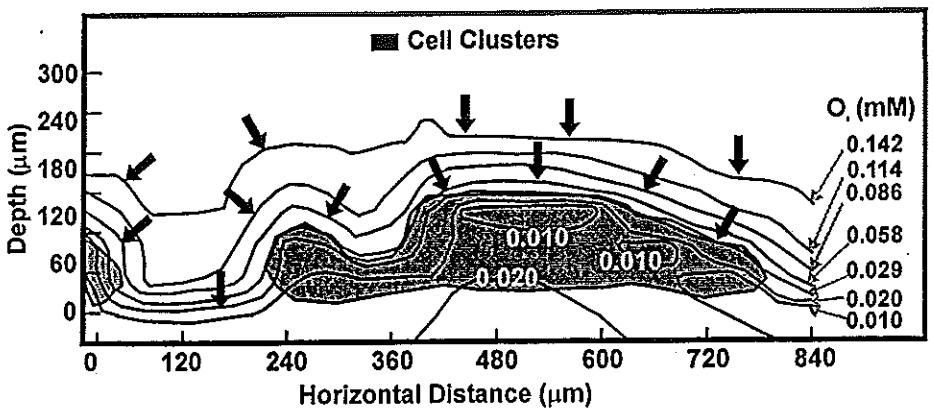

Figure 2. Oxygen concentration around a microcolony. Continuous lines are isolines of equal oxygen concentration, while arrows indicate the direction of oxygen fluxes, always perpendicular to the active surface. Notice that the microcolony is anoxic in the middle while oxygen is still detectable at the bottom, which demonstrates that oxygen near the bottom was transported there via channels and voids, not just by diffusion through the microcolony

We now understand that there are many levels of biofilm heterogeneity. The first level is the morphological or structural, non-uniform distribution of the biomass on a surface, microcolonies separated by interstitial voids. However, biofilm heterogeneity can be related to other factors beside the morphology. Figure 3 shows distribution of different physiological groups within a cell cluster of a biofilm, autotrophic ammonia oxidizing microorganism (lighter spots) form clusters within clusters of heterotrphic, carbon-oxidizing aggregates of microorganisms. This is an example of a physiological microniche within a cell cluster and such heterogeneity is termed "physiological".

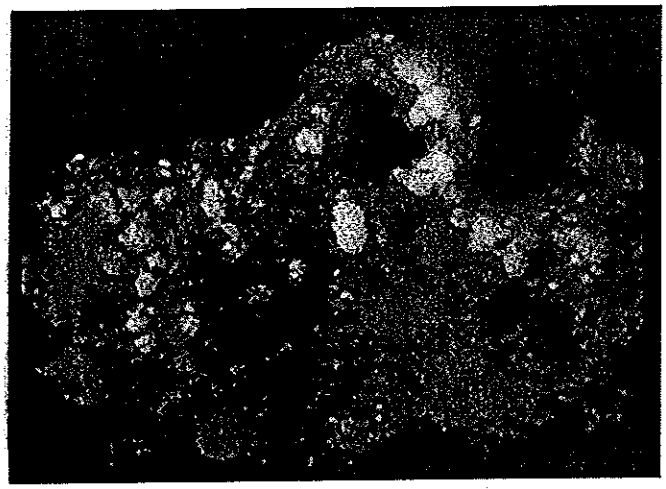

Figure 3. An example of physiological heterogeneity in biofilms: a heterotrophic biofilm with non-uniformely distributed autotrophic ammonia oxidizers (lighter spots). Ammonia oxidizers have been visualized by in situ fluorescent hybridization (FISH) probes.

MECHANISMS OF MIC

The major challenge in studying the MIC is distinguishing the circumstances where microorganisms modify the electrochemical reactions and accelerate corrosion from those where corrosion just proceeds in the presence of microorganisms. There are no universal rules here and in some cases the microorganisms are just spectators and do not modify corrosion processes. The only way to distinguish a case when microorganisms accelerate the corrosion rate from a case where microbial colonization is not affecting the corrosion rate is to understand the mechanisms by which microorganisms may affect electrochemical behavior of metals. Since there are many such mechanisms, the search for universal "fingerprints of MIC" is futile; there are many fingerprints, for there are many MIC mechanisms. As an initial approximation one may divide the effect of microorganisms on corrosion processes into two categories: nonspecific and specific effects. An example of a nonspecific effect of microbial colonization is formation of concentration cells: microorganisms colonize metal surfaces, physically obstruct mass transport of reactants near the surface, forming the cathodic and anodic sites on the surface. An example of a specific mechanism is microbial deposition of manganic oxides, which serve as cathodic reactants on the metal surfaces. Dividing microbial effects into specific and nonspecific categories may fail in some instances but it is conceptually convenient to determine the level of microbial involvement in MIC. It is interesting to notice that most of the mechanisms, specific and nonspecific are possible because biofilms are heterogeneous. If a biofilm, say an aerobic biofilm, were uniform, it should inhibit corrosion by depleting the cathodic reactant, oxygen. Some of the MIC mechanisms studied by our group are discussed here. One can expect that as time progresses we will find more such mechanisms.

Mechanisms #1: Differential aeration cells

This is an example of a nonspecific mechanisms, microorgansism in biofilms deposit extracellular biopolymers and the presence of these substances affect corrosion rate. Figure 4 shows an effect of depositing sodium alginate, an extracellular polymer that is a main constituent of biofilm matrix, on the surface of mild steel. Electrochemical effects were measured in real time using three closely spaced microelectrodes - dissolved oxygen, pH, and ion currents were mapped simultaneously and non-invasively above a mild steel coupon partially coated with biopolymer gels. Results indicate that depositing the biopolymer on the metal surface resulted in fixing the anodic sites under the deposits[15] (Figure 4). The initial hypothesis that alginates form metal concentration cell was rejected; we observed that corrosion occurred at approximately the same rate under two different biopolymer and the second polymer did not have the ability to bind metal ions. We concluded that corrosion was influenced not by the chemical properties of the biopolymer, but was controlled by the difference in mass transport rates of oxygen to the coated versus non-coated regions of the coupon; i.e., a differential aeration cell[15].

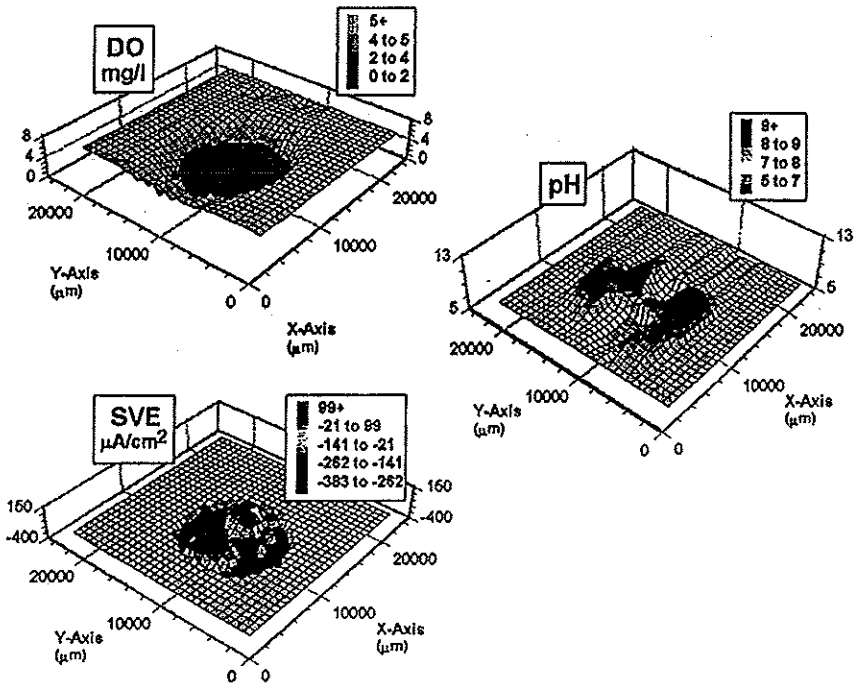

Figure 4. Two spots of calcium alginate deposited on the surface of mild steel fix the anodic sites.

Results in Figure 4 demonstrate that a mere deposition of a thin layer of calcium alginate, an extracellular biopolymer excreted by biofilm microorganisms, on mild steel is enough to fix the anodic sites and initiate corrosion. The mechanism of this process was further traced to differential aeration cells; the layer of the biopolymer locally impeded oxygen transport to the surface.

Mechanism #2: SRB corrosion of mild steel

This is probably the most celebrated case of MIC. In the absence of oxygen, sulfate may become the terminal electron acceptor and, as a result, sulfate ions are reduced to bisulfide and sulfide ions. The exact mechanism of SRB corrosion of mild steel is still debatable. It has been quite confusing in the past that the most notorious cases of SRB corrosion were reported in the presence of oxygen. Since the SRBs are anaerobic microorganism this fact was difficult to explain. According to the mechanism our group is promoting [16-17] this effect is based on oxidation of iron sulfides (resulting from the reaction between iron ions with sulfide and bisulfide ions) by oxygen to elemental sulfur; elemental sulfur is known as a strong corrosion agent. Biofilm heterogeneity plays an important role in this process. The mechanism applies because the central parts of microcolonies (Figure 5) are anaerobic while the outside edges remain aerobic. Figure 5 shows major steps in this mechanism for which a detailed description can be found in our papers[16].

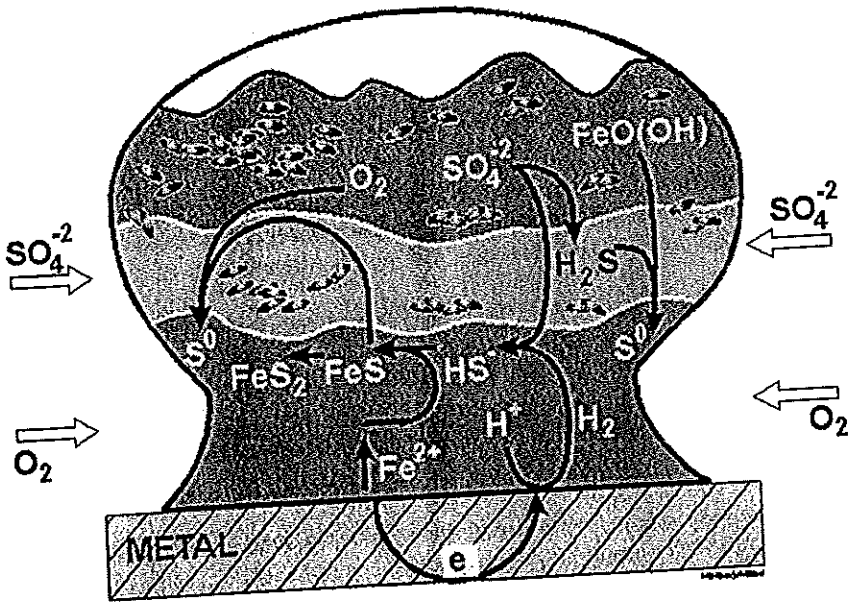

Figure 5. Effect of oxygen on SRB corrosion of mild steel

Mechanism # 3: Effect of Manganese Oxidizing Bacteria

This mechanism relies on the activity of Manganese Oxidizing Bacteria, MOB, in biofilms deposited on metal surfaces[18-20]. Biomineralization of manganese can be carried out by a variety of organisms including bacteria, yeast, and fungi,[21] but is particularly associated with genera of the so called iron and manganese group, *Siderocapsa, Gallionella, Leptothrix-Sphaerotilus, Crenothrix, and Clonothrix*. These bacteria accelerate oxidation of dissolved iron and manganese to form highly enriched mineral-biopolymer encrustations. Deposits form on submerged materials including metal, stone, glass, and plastic and can occur in natural waters with manganese levels as low as a 10-20 ppb[18-19]. Biomineralized manganic oxides are efficient cathodes and increase cathodic current density on stainless steel by several decades at potentials between roughly -200 and +400 mV_{SCE}. The extent to which the elevated current density can be maintained is controlled by the electrical capacity of the mineral which reflects both total accumulation and conductivity of the mineral-biopolymer assemblage (only material in electrical contact with the metal will be cathodically active). Oxide accumulation is controlled by the biomineralization rate (we have discussed the role of biomineralization in MIC elsewhere[22]) and by the corrosion current, in that high corrosion currents will discharge the oxide as rapidly as it is formed. It appears that this mechanism may result in

redox cycling of manganese on metal surfaces producing a renewable cathodic reactant[23], which agrees well with the notion that whenever biofilms accumulate on cathodic members of galvanic couples, a significantly increase in the reduction current can be expected [24]. This mechanism, again, depends on the heterogeneous distribution of the biomineralized manganese oxides in the biofilm. If the manganese oxides were deposited uniformely on the metal surface, the discussed mechanism would not be active.

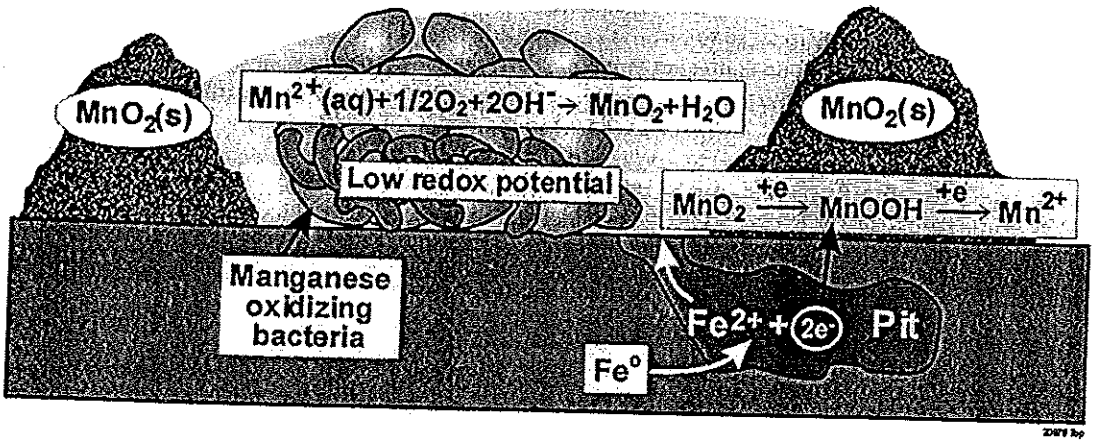

Figure 6. Mechanism of MOB corrosion of stainless steels. Biomineralized manganese oxides are deposited on the surface of a stainless steel which drives the open circuit potential of the stainless steel in the noble direction until it reaches the equilibrium potential of manganese oxides. The elevated OCP may, in the presence of chlorides, reach the pitting potential of the steel.

SUMMARY

We have discussed briefly three possible mechanisms of MIC: differential aeration cells, SRB corrosion of mild steel, and MOB corrosion of stainless steels. The first mechanism, differential aeration cells, we call nonspecific because it does not depend on the kind of microorganisms colonizing the surface nor metabolic process that supports the biofilm; it is initiated by microbial deposition of extracellular biopolymers on the metal surface. In fact once the biopolymer is deposited the corrosion process is accelerated even if the metabolic reactions ceased or even if the biofilm microorganisms were killed. The other two mechanisms, SRB and MOB corrosion, rely on the presence of specific group of microorganisms and specific metabolic reactions - therefore we call them specific. A characteristic feature of the latter mechanisms is that, in both cases, the microorganisms produce minerals that eventually serve as cathodic reactants. We have referred to only two such mechanisms here but, hypothetically, biomineralization may be a universal tactic that biofilms use to modify electrochemistry of metals. However, this modified electrochemistry does not necessarily lead to accelerated corrosion, in some instances it may be benign, in others it may actually inhibit corrosion. The consequences of the microbially modified deposition or dissolution of minerals on metal surfaces deserves special attention. Finally, it is characteristic that all three described mechanisms depend on biofilm heterogeneity.

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