Biofilms: their structure, activity, and effect on membrane filtration

Z. Lewandowski*, and H. Beyenal***

*Center for Biofilm Engineering, Montana State University, Bozeman, MT 59717, USA
(E-mail: *ZL@erc.montana.edu*; *beyenal@erc.montana.edu*)

**Department of Civil Engineering, Montana State University, Bozeman, MT 59717, USA

Abstract The goal of this presentation is to identify biofouling mechanisms that cause undesirable effects to the membrane separation processes of flux decline and pressure drop. The underlying assumption of this presentation is that biofouling is unavoidable and that the operator cannot eliminate it entirely. This premise justifies research efforts toward understanding the mechanisms by which biofouling affects the membrane processes, rather than expecting that technology can entirely eliminate membrane biofouling in the near future. An improved understanding of biofouling mechanisms may lead to better membrane design, better membrane modules, and better membrane cleaning procedures.

Keywords Biofilms; biofouling; filtration; membrane separation

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Nomenclature

C	Nutrient concentration in the biofilm (kg/m^3)
D_{fz}	Surface-averaged effective diffusivity of growth-limiting nutrient in z direction (m^2/s)
D_{sa}	The surface-averaged relative effective diffusivity of ferricyanide
D_w	Molecular diffusivity of growth-limiting nutrient or oxygen (m^2/s)
k	Local mass transfer coefficient (m/s)
k_{max}	The maximum local mass transfer coefficient (m/s)
K_s	Monod half-rate constant (kg/m^3)
X_{fl}	Surface-averaged biofilm density (kg/m^3)
$Y_{x/s}$	Yield coefficient (kg microorganism/kg nutrient)
Z	Distance from the bottom (m)
μ_{max}	Maximum specific growth rate (h^{-1})
ζ	Effective diffusivity gradient (dD_{sa}/dz; m/s)

Introduction

Membrane separation processes are increasingly popular in water reclamation and reuse, and in the future their popularity will increase further. One of the undesirable effects during membrane filtration is membrane fouling. As the filtration proceeds, membranes become covered with inorganic deposits, organics deposits, and microorganisms. These deposits are collectively called fouling deposits, and the process of their deposition is called membrane fouling. Membrane fouling can hinder even the best-designed operations, causing premature membrane flux decline and increasing the pressure drop. There are no easy remedies to prevent fouling. This paper discusses one component of the fouling processes, biofouling, which is caused by living microorganisms deposited on the filtration membranes; it comprises mostly bacteria but also algae and fungi.

This paper is written from the perspective of a biofilm researcher. Biofilm researchers are often faced with the necessity of preventing or reducing the effects of biofouling, and

membrane separation processes are a luminous example. Biofilm researchers have developed tools for studying biofilms, and these tools can easily be used to describe biofilms deposited on filtration membranes. There are also conceptual issues that biofilm researchers may help to address. For example, although the amount of biomass accumulated on the membranes is frequently monitored (Vrouwenvelder *et al.*, 2000; Vrouwenvelder and van der Kooij, 2001), it may not necessarily be the total amount of biomass accumulated on membranes as much as the structure of the biofilms that affects the filtration process; e.g. a highly porous biofilm may cause flux to decline to a lesser extent than a thin but continuous biofilm deposited on the membrane. Quantifying the structure and activity of biofilms deposited on membranes made of various materials may explain why some membrane materials resist biofouling more than others, e.g. why polyethylene membranes are less susceptible to biofouling than those made of cellulose acetate (Khedr, 2003). Also, membrane cleaning procedures can benefit from the insight provided by the research studying the effects of antimicrobials on biofilms. Some conclusions reached by operating membrane filtration modules, such as that chlorination enhances microbial growth and biofouling potential (Saeed *et al.*, 2000), can be explained by demonstrating how oxidizing biocides work on biofilms. In some instances, the efforts to construct and operate antifouling membranes (Coker and Sehn, 2000) can be interpreted using the knowledge generated by biofilm researchers in other areas, such as antifouling surfaces for implantable biomedical devices.

Although we cannot eliminate biofouling entirely at this time, we hope that the advances in biofouling research can help the designers and operators of membrane separation processes to understand the principles of biofouling, identify the components of biofouling that cause the undesirable effects, construct filtration modules that account for these effects, and rationally select membrane cleaning procedures.

Mechanism of biofouling and biofilm structure

In natural and engineered systems, suspended microbial cells are transported with the fluid flow to solid surfaces where they may adsorb. If environmental conditions are favorable, the adsorbed cells grow, replicate, and excrete extracellular polymer substances (EPS) which bind the cells together. The aggregates of cells, EPS, and other particulate matter accumulated at surfaces are termed biofilms (Characklis and Marshall, 1990). Biofilm morphology can be highly variable ranging from patchy, discontinuous colonies to thick, continuous films. To discuss biofouling and biofilm processes, such as biofilm activity and biofilm accumulation rate, it is convenient to use conceptual models of biofilm structure. Models of biofilm structure have evolved from displaying microorganisms uniformly and randomly distributed in the matrix of extracellular biopolymers (EPS) to so-called structurally heterogeneous biofilms, displaying microorganisms aggregated in microcolonies (Bishop and Rittmann, 1995; Costerton *et al.*, 1995; Walker and Keevil, 1994; Wolfaardt *et al.*, 1994). Conceptual models portraying biofilms as uniform layers dominated early mathematical models of biofilm activity and accumulation, and they still are in use, mostly because they are relatively simple, but also because the models of heterogeneous biofilms are not yet well developed. This may change soon, as much work has been devoted to constructing mathematical models of heterogeneous biofilms. Figure 1 shows the conceptual image of heterogeneous biofilms and the microscope image of a heterogeneous biofilm.

Most biofilm researchers agree that biofilms are composed of microcolonies, which are dense aggregates of microorganisms. A corollary to this is that a biofilm matrix is porous and the nutrients penetrate the pores and reach deep layers of the biofilm; this fact has many physiological consequences for microbial growth and biofilm accumulation.

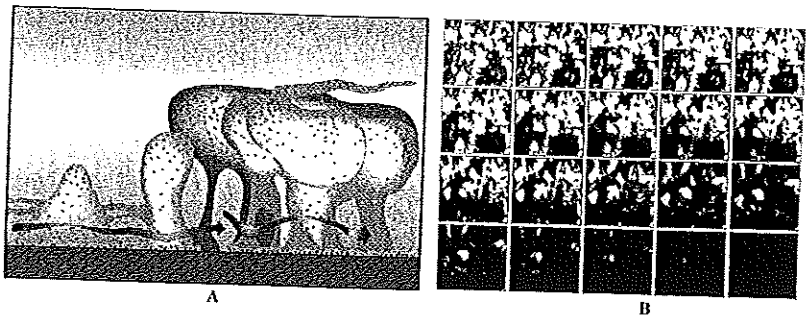

Figure 1 Heterogeneous biofilms. (A) Diagrammatic representation of the structure of a hypothetical bacterial biofilm drawn from confocal scanning laser microscopy (CSLM) examination of a large number of mixed-species biofilms. A network of interstitial voids filled with water surrounds discrete microcolonies of microorganisms. The arrows indicate convective flow within the water channels. (B) A series of confocal images taken at different heights, from the bottom to the top of the biofilm (the upper left corner to the lower right corner)

That biofilms are heterogeneous and porous indicates that some well-established concepts, directly applicable within the framework of uniform biofilms, are difficult to apply within the framework of heterogeneous biofilms. One such concept is shear stress: since flow velocity does not reach zero at the biofilm surface, and actually flows through the space occupied by the biofilm, the concept of shear stress is difficult to apply. Also, describing mass transport dynamics is more challenging in structurally heterogeneous biofilms than in uniform biofilms: one has to take into account the external mass transport (nutrient transport from the bulk water to the biofilm) and the internal mass transport (nutrient transport within the space occupied by the biofilm, within biofilm pores and within microcolonies).

At present the two conceptual models, homogeneous and heterogeneous biofilms, function 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. The growing popularity of the heterogeneous biofilms model among life scientists stimulates the engineering community to construct mathematical models of such biofilms. The task is difficult for many reasons, among them that there is little experimental data describing microbial activity distribution in heterogeneous biofilms and that heterogeneous biofilms need to be modeled in three dimensions, which complicates the matter. New approaches to the mathematical modeling of biofilms, cellular automata and neural networks may bring a new generation of three-dimensional biofilm models which include the complex structure of the biofilm.

The concept of biofilm heterogeneity was initially used in reference to the physical structure of the biofilm and the distribution of the biomass within the space occupied by the biofilm. We now understand that there are many levels of biofilm heterogeneity. The first level is the morphological, or structural, nonuniform distribution of the biomass on a surface, shown as microcolonies separated by interstitial voids in Figure 1. However, biofilm heterogeneity can be related to other factors besides morphology. Figure 2 shows the distribution of different physiological groups within a cell cluster of a biofilm; autotrophic, ammonia-oxidizing microorganisms (lighter spots) form clusters within clusters of heterotrophic, carbon-oxidizing aggregates of microorganisms. This is an example of a physiological microniche within a cell cluster, and such heterogeneity is termed 'physiological." Similarly, one may argue for the existence of genetic heterogeneity, chemical heterogeneity, and perhaps some other types of heterogeneity.

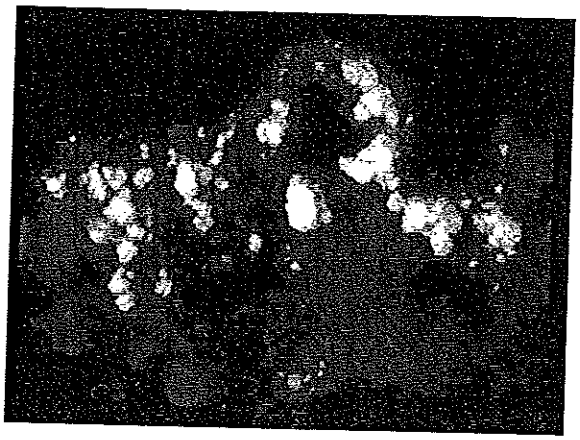

Figure 2 An example of physiological heterogeneity in biofilms: a structurally heterotrophic biofilm with nonuniformly distributed autotrophic ammonia oxidizers (lighter spots). Ammonia oxidizers have been visualized by in situ fluorescent hybridization (FISH) probes

As mentioned above, the term biofilm heterogeneity was used initially only in reference to biofilm structure. Biofilm structure is intensively studied by biofilm researchers because it seems to affect many biofilm processes. The structure of biofilms is affected by many factors. For example, biofilms grown at higher flow velocities have different morphology than those similar to the biofilm in Figure 1, with microcolonies including long filamentous streamers (Lewandowski and Stoodley, 1995). The reason that the microcolonies assume such a shape is the viscoelastic nature of extracellular biopolymers holding microcolonies together. When a single microcolony attached to the bottom of a reactor (as in Figure 1) is subjected to high shear stress and high flow velocity, the boundary layer separates downstream of the microcolony, causing the formation of a low-pressure zone. Because the microcolonies are held together by viscoelastic polymers, the material slowly flows, assuming elongated shapes known as streamers. In effect, the biofilm surface looks like the one in Figure 3A. Figure 3A shows biofilms composed of *Pseudomonas aeruginosa* (ATCC # 700829), *Pseudomonas fluorescens* (ATCC # 700830) and *Klebsiella pneumoniae* (ATCC # 700831) grown in a circular vinyl tubing (1.27-cm OD × 0.635-cm ID and 50 cm length). Figure 3B shows the variation of areal porosity with respect to the distance from the bottom. This figure shows the existence of

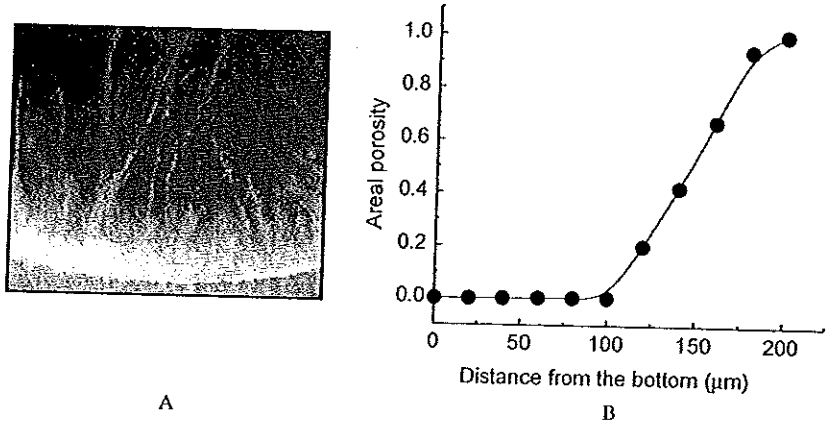

Figure 3 (A) Surface of a biofilm accumulated on the inner surface of a polyethylene tubing (Groenenboom, 2000). (B) Biofilm porosity distribution

a base biofilm (approximately 100 μm thick) and a surface film (approximately 100 μm thick). Figure 4 shows the variation of biofilm structure with respect to time. If the biofilm is allowed to accumulate, it eventually forms a thick layer of biomass and extracellular polymers. We do not know whether the base biofilm is formed by the microbes that grow near the substratum or by pieces of biomass that are sloughed off and physically trapped in biofilm pores.

In conclusion, biofilms are composed of microcolonies, dense aggregates of microorganisms embedded in gelatinous extracellular polymers. Biofilms may accumulate in discrete microcolonies and in continuous layers. As time progresses, biofilms tend to form continuous layers, at least near the surface. As a result, when biofilms are deposited on filtration membranes, they will decrease the flux and increase the pressure because water must pass not only across the membrane but also across the layer of gelatinous extracellular polymers.

Metabolic activity in biofilms

Metabolic activity determines the substrate conversion rate and the biofilm growth rate. A physiological corollary of the structural heterogeneity of biofilms is that substrate concentrations (e.g. dissolved oxygen) vary from location to location within biofilms (DeBeer *et al.*, 1994a; Lewandowski *et al.*, 1995). Bacterial cells within single-species biofilms actually grow in an almost infinite number of varying "microniches" in which they experience very different microenvironments (Costerton *et al.*, 1995). Figure 5A shows a CSLM image of a heterogeneous biofilm composed of *Pseudomonas aeruginosa* (ATCC # 700829), *Pseudomonas fluorescens* (ATCC # 700830) and *Klebsiella pneumoniae* (ATCC # 700831) along with measurement locations. Figure 5B shows oxygen profiles across a biofilm cluster and across an interstitial void, and Figure 5C shows a map of oxygen concentration across the measurement locations.

The oxygen concentration profile measured across the interstitial void (location B4) shows that at that location the space occupied by the biofilm is fully penetrated by oxygen, while oxygen is depleted under the microcolony just 150 micrometres away from the interstitial void (location B2). The apparent implication for the different profiles is the existence of a steep lateral gradient of oxygen between these two locations, a gradient of the same order of magnitude as the gradient perpendicular to the substratum. Consequently, the fundamental assumption for most biofilm models, a lateral gradient of substrate concentrations, is not representative of substrate concentration distributions in

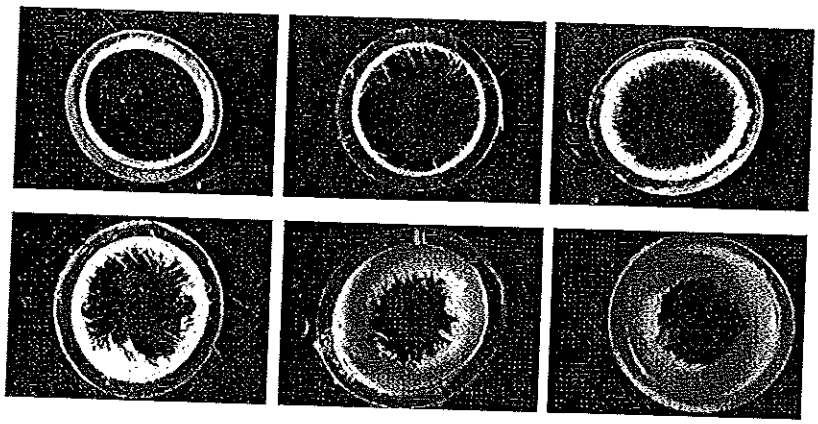

Figure 4 If the biofilm is allowed to accumulate, it may eventually form a thick layer of biomass and extracellular polymers (Groenenboom, 2000)

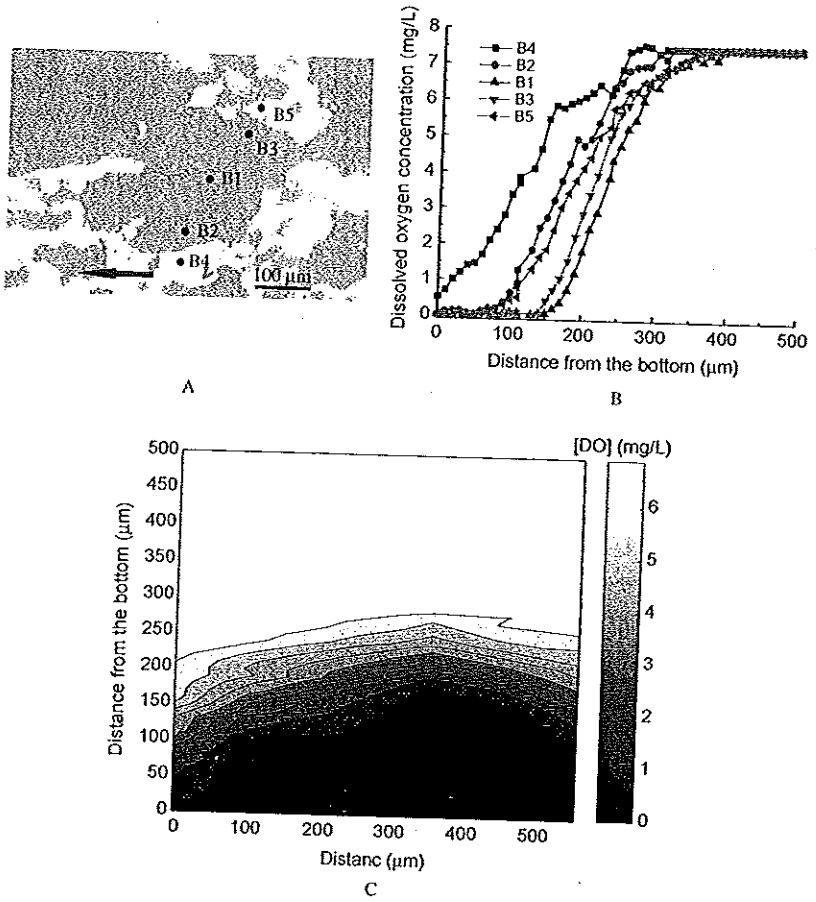

Figure 5 (A) Confocal image of the biofilm in which dissolved oxygen measurements were performed. Dark areas are cell clusters; light areas are water channels. We selected five sites for dissolved oxygen measurements. (B) Dissolved oxygen concentration profiles measured at the locations marked in Figure A. (C) Map of oxygen distribution across the measurement locations showing the influence of biofilm heterogeneity on oxygen concentration in the biofilm. A distance of zero on the X axis indicates the location of point B4, and all other distances are given as the distance from point B4 in the direction of the point B5. The average bulk flow velocity during the measurement was 2.1 cm/s

a heterogeneous biofilm. Therefore, it is necessary to accept that lateral mass transport in biofilms exists, and that its magnitude is comparable with the intensity of mass transport perpendicular to the substratum.

Hydrodynamics in biofilms

Hydrodynamics affect all metabolic processes in biofilms because substrate is delivered to microcolonies by convective mass transport and products of the metabolic reactions are removed by the same mechanism. Visualizing flow in a space occupied by biofilms is not trivial. Nuclear magnetic resonance (NMR) and serial photography of inert particles were used to demonstrate convective flow in water channels of biofilms (DeBeer *et al.*, 1994b; Lewandowski *et al.*, 1992; Stoodley *et al.*, 1994) in the same direction as the bulk flow but at a reduced rate. At low flow velocities the biofilm effectively smoothed the wall surface. Figure 6 shows two profiles of flow velocity measured by nuclear magnetic resonance imaging in a biofilm reactor (Lewandowski *et al.*, 1992).

The profile on the right was measured in a reactor without biofilm. The profile on the left was measured in the same reactor and at the same location but after the accumulation

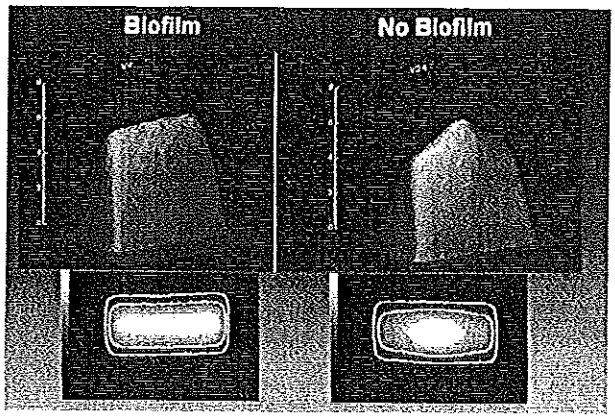

Figure 6 NMRI images of flow in a flat plate reactor in the presence (left) and absence (right) of a biofilm

of the biofilm. The image taken in the absence of biofilm indicates flow instability, as seen by the jet formed in the middle of the reactor. Since the two images were collected for the same set of hydrodynamic parameters, and the only difference between them is the presence of the biofilm, the conclusion that can be drawn from these data is that the presence of the biofilm decreased the hydrodynamic entry length in the reactor. This explains why a jet formed in the reactor without biofilm, while in the presence of the biofilm, the viscous flow was fully developed at the same distance from the entrance to the reactor. This can be further translated into the conclusion that the biofilm effectively smoothed the surface and that therefore the flow was more stable in the presence of the biofilm than in its absence. This conclusion and the preceding results clearly demonstrate that the popular notion that biofilms increase the surface roughness in conduits is in disagreement with experimental evidence.

The present understanding of flow dynamics in biofilms is summarized in Figure 6. Flow velocity does not approach zero near the biofilm surface, as required by the models of biofilm activity; instead, water movement is measurable throughout the entire biofilm down to the level of the substratum. This has clear and immediate consequences with respect to mass transport, which is affected by convection in the entire biofilm, not only above the biofilm.

Quantifying hydrodynamics near surfaces covered with biofilms is further complicated by the fact that biofilms are made of viscoelastic polymers. These polymers do not behave as rigid roughness elements but instead more like compliant surfaces. It is well known that biofilm buildup in conduits causes an increase in pressure drop, which is usually interpreted through hydrodynamics as the result of kinetic energy dissipation on surface roughness elements. Figure 7 demonstrates that this is not the whole truth: the viscoelastic properties of biofilm matrix may affect energy dissipation to an unknown extent.

Mass transport in biofilms

It is necessary to include mass transport in models of biofilm activity and accumulation. Mathematical models of biofilm activity (Atkinson and Davies 1974; Rittmann and McCarty 1980; Wanner and Gujer 1986) assume the existence of a thin diffusive boundary layer above the biofilm in which mass transport is dominated by molecular diffusion. Nuclear magnetic resonance imaging and particle velocimetry combined with confocal laser microscopy have demonstrated that this is not quite true: water actually flows through the space occupied by the biofilm. Also, because of biofilm heterogeneity, the intensity of mass transport varies from location to location. To quantify the distribution

Figure 7 The friction resistance and the buildup of pressure drop in conduits colonized with biofilm are related to biofilm structure and elasticity. Pressure drag exerted on microcolonies causes them to elongate, forming "streamers" (dark elongated shapes in the figure). The vigorous movement of the streamers is evidence that the kinetic energy of flowing water is being dissipated, resulting in an increase in pressure drop across the reactor

of mass transport intensity in biofilms (Yang and Lewandowski 1995) we developed a microelectrode to measure the local mass transport coefficient, providing insight into the dynamics of mass transport within the hydrodynamic boundary layer (Yang and Lewandowski 1995). Using both this microelectrode and other microelectrodes to quantify substrate concentration profiles in biofilms advances the understanding of the relationship between the rate of mass transfer and biofilm activity. Figure 8 shows an oxygen profile and a mass transport coefficient profile collected at the same location within a biofilm (Rasmussen and Lewandowski 1998).

It is well known that when the local nutrient concentrations measured across a biofilm are plotted versus distance from the surface, they form a nutrient concentration profile. It is expected that the shape of the nutrient concentration profile will follow the shape of

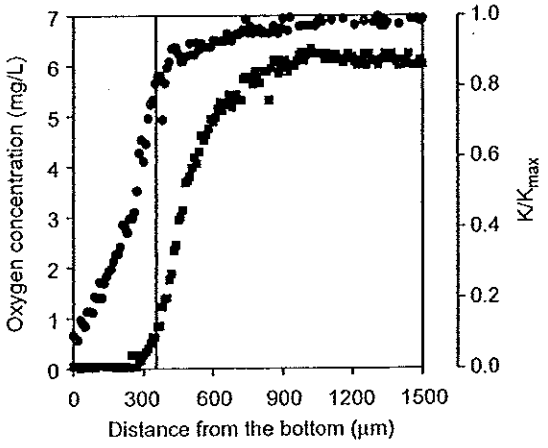

Figure 8 Superimposed oxygen concentration (■) and local mass transport coefficient (●) profiles. The local concentration of oxygen is about 6 mg/L in the bulk and about 0.6 mg/L near the surface, which accounts for a 90% decrease. However, the local mass transport coefficient decreases by only about 10% between these two locations (Rasmussen and Lewandowski, 1998)

the local mass transport coefficient profile when both are measured at the same location. It is also expected that, at locations where the local mass transport coefficient is high, the local nutrient concentration will be high as well, at least higher than at a location where the local mass transport coefficient is low. To verify these expectations we made the appropriate measurements. Figure 8 shows profiles of oxygen concentration and the local mass transport coefficient measured at the same location in a biofilm (Rasmussen and Lewandowski 1998).

The results demonstrate an unexpected deviation from expectations. It is clear from the data in Figure 8 that the changes in the mass transport coefficient do not correlate well with the changes in oxygen concentration. The oxygen concentration decreases rapidly as we approach the biofilm surface and reaches quite low levels at the biofilm surface, while the local mass transport coefficient remains quite high at that location. This observation is unexpected and difficult to explain; because there is no oxygen consumption in the bulk, the oxygen concentration profile should follow the shape of the mass transport coefficient profile much closer than it does in Figure 8.

To explain this apparent discrepancy we need to examine the procedure for measuring flow velocity (which is correlated to local mass transfer coefficient) in biofilms. All available flow velocity measurements in biofilms report only one component of the flow velocity vector, parallel to the bottom. Based on these results, we estimated that mass transport is controlled by convection near biofilms. However, the convective mass transport rate equals the nutrient concentration times the flow velocity component *normal to the reactive surface*. The component of the flow velocity *parallel to the surface* has nothing to do with the convective mass transport toward that surface. Consequently, the estimate of the mass transport mechanism based on flow velocity holds only in the direction in which the flow velocity was measured. Indeed, when the flow near a surface is laminar, the laminas of liquid slide parallel to the surface and there is little or no convection across these layers; the mass transport parallel to the surface is convective, while the mass transport perpendicular to the surface remains diffusive (Figure 9).

It is well known that effective diffusivity varies across a biofilm (Beyenal and Lewandowski 2002). We proposed the following equation to account for variable diffusivity and diffusivity gradients when modeling mass transport in biofilms (Beyenal and Lewandowski 2002):

$$D_{fz}\frac{d^2C}{dz^2} + \zeta\frac{dC}{dz} = \frac{\mu_{max}CX_{fl}}{Y_{X/S}(K_S + C)} \tag{1}$$

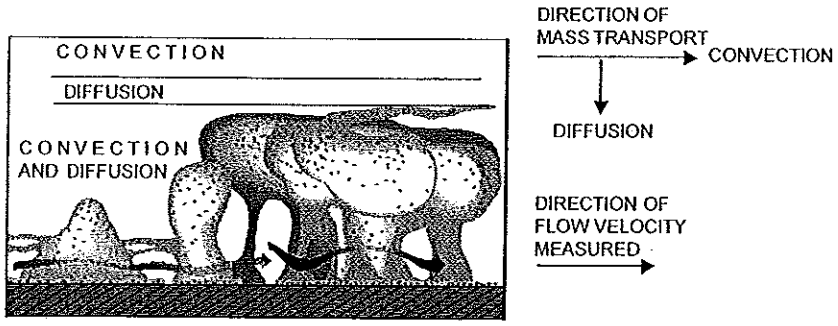

Figure 9 Alternating zones of convective and diffusive mass transport in heterogeneous biofilms. This hypothetical model of mass transport is consistent with the results in Figure 8. Mass transport in the space occupied by the biofilm is convective, but the amount of nutrient delivered to this space is limited by the diffusive mass transport just above the biofilm surface (Lewandowski and Beyenal, 2003)

Equation 1 reflects experimental results, which demonstrate that the average biofilm density increases as one approaches the surface. As shown in Figure 10, the surface-averaged effective diffusivity changes linearly with respect to distance from the bottom.

Resistance to antimicrobial agents

Wherever biofilms exhibit undesirable effects, there is a tendency to remove them using antimicrobial agents. However, one of the unique and puzzling features of biofilms is their unusually high resistance to antimicrobial agents (Brown *et al.*, 1995; Costerton *et al.*, 1987; Nichols *et al.*, 1989). Although the susceptibility of planktonic bacteria to biocides and antibiotics is well documented, the same microorganisms, when present as biofilms, require hundred-fold or even thousand-fold greater concentrations of the antimicrobial agent to kill them or to modify their metabolic states. For example, after an eight-hour incubation period with 50 μg/ml of tobramycin (an aminoglycoside antibiotic), no viable cells of *Pseudomonas aeruginosa* existed in their planktonic state, but 12-hr contact with 1000 μg/ml of tobramycin did not kill the biofilm cells of the same species (Nickel *et al.*, 1985). Lechevallier *et al.*, (1988) found that biofilm bacteria were 150 to more than 3000 times more resistant to free chlorine and 2 to 100 times more resistant to monochloramine than their planktonic counterparts (Lechevallier *et al.*, 1988).

Various hypotheses have been proposed to account for the resistance of sessile bacterial populations to antimicrobial agents. One hypothesis refers to the reaction-diffusion model (Costerton *et al.*, 1987; Hoyle *et al.*, 1992; Stewart 1994; Stewart and Raquepas 1995). It implies that antimicrobial agents are consumed, or deactivated, by intercellular polysaccharides or cellular constituents. It is also possible that the resistance to antibiotics is growth-rate-dependent (Roberts and Stewart 2004).

In the case of oxidizing antimicrobial agents, the resistance seems to be caused by the fact that strong oxidizers oxidize not only the bacterial cells but also the biofilm matrix and extracellular polymers. Therefore the penetration of antibiotic into the biofilm is retarded. As an example, temporal changes in hydrogen peroxide concentration profiles in biofilms (Liu *et al.*, 1998) are shown in Figure 11; it took about two hours to see considerable penetration of hydrogen peroxide into the biofilm.

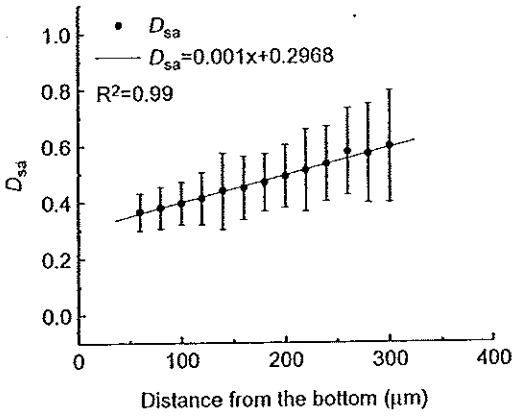

Figure 10 Surface-averaged diffusivity, D_{sa}, versus the distance from the bottom for biofilms grown at 7.5 cm/s flow velocity; $D_{sa}(= D_E/D_w)$ is relative effective diffusivity, where D_w is molecular diffusivity of the nutrient. Parameter ζ in Equation 1 can be calculated as the slope × D_w. The standard deviations increase toward the biofilm surface, which reflects the fact that biofilms are more heterogeneous near the surface than near the bottom. It is also worth noting that the effective diffusivity gradient (ζ) has the same dimensions as the mass transport coefficient (length/time); we termed ζ a secondary mass transport coefficient (Beyenal and Lewandowski, 2002)

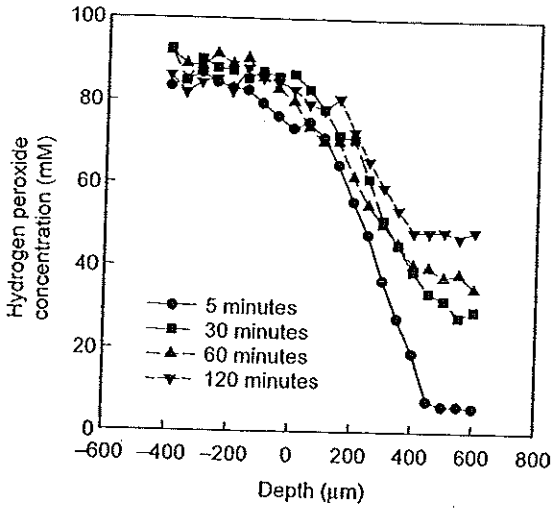

Figure 11 Hydrogen peroxide concentration profiles in biofilms (Liu *et al.*, 1998): with 0.3% HP treatment. Zero represents the interface of bulk fluid and biofilm, and negative numbers on the x-axis correspond to the bulk fluid. This result exemplifies the resistance of biofilms to antimicrobial agents. It took about two hours to see considerable penetration of hydrogen peroxide into the biofilm. Similar results have been published on the penetration of chlorine (DeBeer *et al.*, 1994a)

Conclusions

Biofilms accumulate at all surfaces immersed in natural waters. In some instances their presence is desirable, such as biofilms in bioremediated soils, and in some instances biofilms are not desirable, such as the biofilms deposited on filtration membranes. The existing technology can delay biofilm formation, but cannot prevent it. Therefore, the mechanisms of biofilm formation are studied to determine whether technological means can be used to lessen their undesirable effects. In membrane filtration, the present way of thinking is that by manipulating biofilm structure one can affect the extent of biofilm influence on flux decline and pressure drop increase. The hypotheses that have been put forward call for pre-colonizing filtration membranes with selected biofilms that are known to have highly heterogeneous structure and therefore affect the flux decline and pressure drop increase to a lesser extent. This approach, although promising, requires detailed knowledge of factors affecting biofilm heterogeneity. Intensive research effort is devoted to this problem. Although biofilm researchers cannot eliminate biofouling, they can help to mitigate the undesirable effects of biofilms on membrane filtration.

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