Local mass transport rates and local activity of heterogeneous biofilms
by Kjetil Rasmussen

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Environmental Engineering
Montana State University

Abstract:
Biofilms have been modeled as homogeneous layers of cells and extracellular polymers covered by a uniform liquid layer. Modelers have assumed that water within and in close vicinity to the biofilm is stagnant. However, scanning confocal laser microscopy has shown that biofilms are highly heterogeneous, and consist of cell clusters separated by voids and channels. The heterogeneous structure makes the mass transport near and within biofilms very complicated. To be able to describe mass transport in biofilms mathematically some simplifying assumptions based on experimental data are needed.

Microelectrodes have been used here to measure oxygen profiles and local mass transfer coefficient profiles in biofilm clusters and interstitial voids. Both dissolved oxygen- and local mass transfer coefficient profiles were measured at the same locations to make it possible to superimpose the two profiles. From the oxygen profiles the effective diffusive boundary layer thickness was determined. The local mass transfer coefficient profiles provided information about the nature of mass transport near and within the biofilm. All profiles were measured at three different flow velocities to determine the influence of fluid flow on mass transport.

Convective mass transport was found to be active within the mass boundary layer and in the upper region of the biofilm, independent of biofilm's thickness and flow velocity. The effective diffusive boundary layer thickness, however, varied strongly at different locations when the same flow velocities were applied. Oxygen-and local mass transfer coefficient profiles collected through a 70 μm thick cluster revealed that the thin cluster did not cause any significant changes in local mass transfer resistance. The same conclusion was drawn from profiles measured through a void filled with a 200 μm thick extracellular polymer layer when the flow velocity was higher than 1.53 cm sec^-1. In a 350 μm thick biofilm cluster the local mass transfer coefficient decreased gradually and approached zero near the substratum.

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APPROVAL

of a thesis submitted by

Kjetil Rasmussen

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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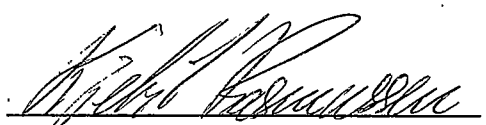
Signature 

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ABSTRACT

Biofilms have been modeled as homogeneous layers of cells and extracellular polymers covered by a uniform liquid layer. Modelers have assumed that water within and in close vicinity to the biofilm is stagnant. However, scanning confocal laser microscopy has shown that biofilms are highly heterogeneous, and consist of cell clusters separated by voids and channels. The heterogeneous structure makes the mass transport near and within biofilms very complicated. To be able to describe mass transport in biofilms mathematically some simplifying assumptions based on experimental data are needed.

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INTRODUCTION

Recent studies of biofilm architecture strongly influence our concepts of mass transport mechanism in biofilms. Images of living biofilms using confocal laser microscopy show that biofilms form cellular aggregates (microcolonies) separated by interstitial void spaces filled either with water or low density biopolymers as shown in figure 1 (Lawrence et al., 1991, Korber et al., 1994, Wolfaardt et al., 1994, Keevil and Walker, 1992). Structural heterogeneity of biofilms was demonstrated using scanning electron microscopy (Stewart et al., 1995), scanning confocal laser microscopy (SCLM) (Lawrence et al., 1991), and cryoembedding (Murga et al., 1994). It is well known that the nonuniform distribution of biomass in biofilms may influence the mass transport mechanism. The study of the nature of oxygen distribution within structurally heterogeneous biofilms clearly demonstrated the importance of biofilm architecture to oxygen transport (De Beer et al, 1994 a). Reports of such influence were published even before the importance of biofilm's architecture was fully realized. Siegrist and Gujer (1985) observed an increased average diffusion coefficient with increased biofilm thickness and hypothetically explained this as a result of irregularities of thick biofilms penetrating the boundary layer causing eddy diffusion. Similar explanation may be employed to the observations of Larsen and Harremoës (1994) and Horn and Hempel (1995) who

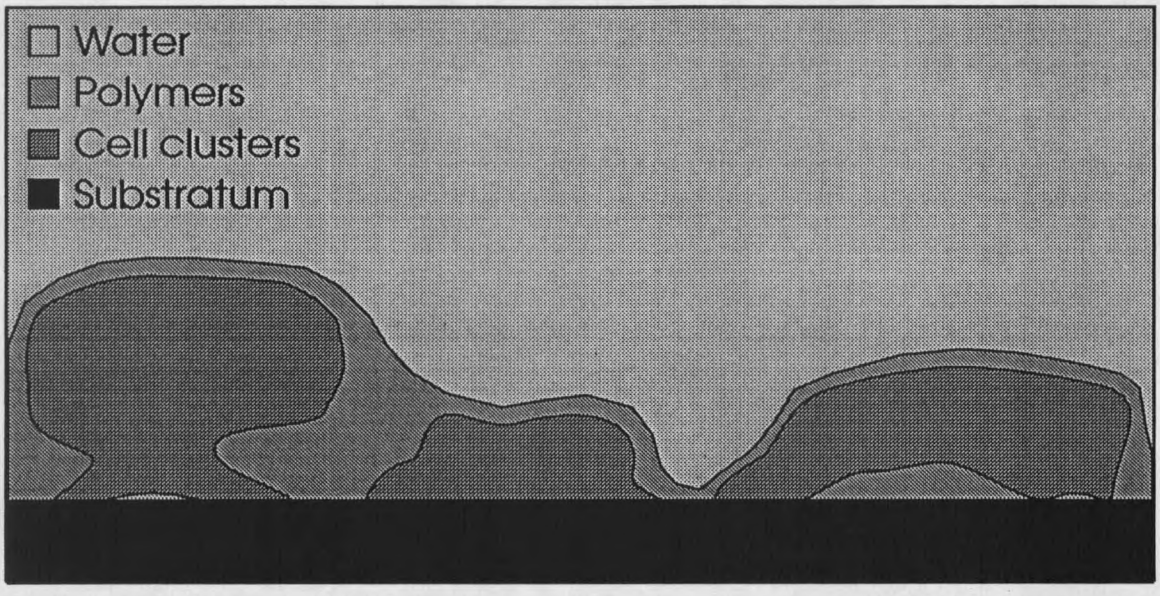

Figure 1. Biofilm structure; cell clusters separated by void spaces filled with extracellular polymers.

report that the oxygen diffusion coefficient in a biofilm is higher than in pure water. The concept of structurally heterogeneous biofilms constitutes an intellectual platform to accommodate those, otherwise difficult to interpret, observations. At the recent meeting of the International Association on Water Quality (IAWQ) Specialist Group on Biofilm Systems in Leeuwenhorst, the Netherlands, biofilm's heterogeneity was defined as "spatial differences in any parameter we think is important" (Bishop and Rittmann, 1995). Despite the awareness of biofilm heterogeneity there is no clear notion on what causes it and how the heterogeneity influences biofilm processes. Van Loosdrecht et al. (1995) discussed the influence of substrate loading rate, shear, and growth rate on the biofilm structure. Some qualitative opinions of how the process parameters influence biofilm structure have been established among biofilm researchers. A high shear rate tends to increase the biofilm's density and mechanical stability. Higher substrate loadings as well as presence of fast growing microorganisms result in thicker and less smooth biofilms. Some researchers initiated the quantification of parameters influencing structural heterogeneity. Zhang and Bishop (1994 a,b) determined the densities, porosities, specific surface area, and mean pore radius of biofilms. They determined the distribution of the tortuosity factor and the ratio of the effective diffusivity to the diffusivity in bulk solution of a biofilm. Fu et al. (1994) estimated the effective diffusivity in different layers of a biofilm using a dissolved oxygen microelectrode. Hermanowicz et al. (1995) used SCLM images to estimate the fractal dimension and biofilm morphology.

Biofilm reactivity is controlled by the rate of substrate consumption and by the rate of mass transport. Bird et al. (1960) describe two models for mass transfer; (1) the film theory; and (2) the boundary-layer theory. The film theory is a unnidirectional transport model, whereas the boundary-layer theory considers two-dimensional velocity profiles. The film theory says that a stagnant or laminar, fictitious film of fluid is present next to the boundary in which all resistance to transfer exists (Welty et al., 1976). Transport in this layer is only due to molecular diffusion. The mass transfer coefficient, k, is defined as the molecular diffusivity, D, divided by the film thickness, L_D:

$$k = D/L_D \qquad (1)$$

Although the disadvantages of the film model are well known, its conceptual simplicity and computational convenience are so appealing that it is frequently intermixed with the boundary layer concept. From its beginning biofilm modeling relied on the assumption that water near the biofilm, and within the biofilm, is stagnant (Atkinson and Davies, 1974, Rittmann and McCarty, 1978, Wanner and Gujer, 1984). It is a common practice to substitute the film thickness in the film model with the thickness of the mass boundary layer (MBL) determined with microelectrodes (Zhang and Bishop, 1994 c). This approach implicitly assumes that the mass transport in the mass boundary layer is due to molecular diffusion only. Figure 2 shows how a concentration profile through the bulk liquid, MBL, and biofilm should appear based on this concept. The assumptions that molecular diffusivity is the major factor influencing the mass transport rate inside the MBL and