Kinetics of biofilm detachment
by Brent Michael Peyton

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Chemical Engineering
Montana State University

Abstract:
A biofilm is a matrix of cells and cellular products attached to a solid substratum. This biological matrix can increase operational costs and/or decrease product quality in a variety of industries, including oil and paper production, semiconductor manufacture, and drinking water distribution. Beneficial biofilms contribute to hazardous waste bioremediation and waste water treatment, and can offer significant improvements in bioprocessing technology. Understanding the processes which control the rate and extent of biofilm accumulation is critical to the control of both beneficial and detrimental biofilms.

One of the least understood processes affecting biofilm accumulation is detachment. Detachment is the removal of cells and cell products from an established biofilm and subsequent entrainment in the bulk liquid. The purpose of this research was to determine the effects of shear stress and substrate loading rate on the rate of biofilm detachment.

Mono-population Pseudomonas aeruginosa and undefined mixed population biofilms were grown on glucose in a RotoTorque biofilm reactor. Three levels of shear stress and substrate loading rate were used to determine their effects on the rate of detachment. Suspended cell concentrations were monitored to determine detachment rates, while other variables were measured to determine their influence on the detachment rate. Results indicate that detachment rate is directly related to biofilm growth rate, and that factors which limit growth rate will also limit detachment rate. No significant influence of shear stress on detachment rate was observed.

A new kinetic expression which incorporates substrate utilization rate, yield, and biofilm thickness was compared to published detachment expressions and gives a better correlation of data obtained both in this research and from previous research projects, for both mono- and mixed population biofilms.

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Brent Michael Peyton

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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Date June 9, 1992

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ABSTRACT

A biofilm is a matrix of cells and cellular products attached to a solid substratum. This biological matrix can increase operational costs and/or decrease product quality in a variety of industries, including oil and paper production, semiconductor manufacture, and drinking water distribution. Beneficial biofilms contribute to hazardous waste bioremediation and waste water treatment, and can offer significant improvements in bioprocessing technology. Understanding the processes which control the rate and extent of biofilm accumulation is critical to the control of both beneficial and detrimental biofilms.

One of the least understood processes affecting biofilm accumulation is detachment. Detachment is the removal of cells and cell products from an established biofilm and subsequent entrainment in the bulk liquid. The purpose of this research was to determine the effects of shear stress and substrate loading rate on the rate of biofilm detachment.

Mono-population *Pseudomonas aeruginosa* and undefined mixed population biofilms were grown on glucose in a RotoTorque biofilm reactor. Three levels of shear stress and substrate loading rate were used to determine their effects on the rate of detachment. Suspended cell concentrations were monitored to determine detachment rates, while other variables were measured to determine their influence on the detachment rate. Results indicate that detachment rate is directly related to biofilm growth rate, and that factors which limit growth rate will also limit detachment rate. No significant influence of shear stress on detachment rate was observed.

A new kinetic expression which incorporates substrate utilization rate, yield, and biofilm thickness was compared to published detachment expressions and gives a better correlation of data obtained both in this research and from previous research projects, for both mono- and mixed population biofilms.

INTRODUCTION

A biofilm is a matrix of cells and cellular products attached to a solid surface or substratum. At the substratum, cells grow, reproduce, and produce extracellular polymers and other byproducts. Biofilms are found in most natural and industrial aquatic systems and account for much of the overall microbial activity in these systems. Biofilms reduce heat transfer in heat exchange equipment and reduce flow capacity in pipelines leading to increased energy consumption and increased costs. Biofilms also contribute significantly to corrosion, oil field reservoir plugging and petroleum souring, drinking water deterioration, and computer chip contamination. However, biofilms present positive opportunities in bioremediation of hazardous and toxic substances in ground and surface water and waste water treatment. Biofilm and other immobilized cell reactors offer significant advantages in bioprocessing, such as increased process flow rates without washing the organisms from the reactor. Engineers and scientists are just beginning to realize the significance of biofilms on process industries, natural aquatic systems, and medical technology. Integrated knowledge of microbiology, chemistry, and engineering is necessary to fully understand the processes affecting biofilm accumulation and activity.

Relevance of Biofilms

Biofilms are found in both natural and manmade aquatic systems. In streams and rivers, a large proportion of the microbial activity occurs in attached films.

Wuhrmann (1971) estimated that 90 to 99.99 percent of the bacterial activity in shallow streams is associated with biofilms. Biofilm organisms are responsible for the transformation and degradation of natural and manmade organic compounds in the water.

In industrial systems, biofilm research is usually aimed at the reduction of biofouling (unwanted biofilms). Many deleterious effects of biofilm formation have been reported. Heat transfer efficiency in heat exchangers and condensers is reduced by biofilm accumulation (Characklis et al., 1980 and 1984), and oilfield water injection systems plug with material detached from biofilm. Hydrogen sulfide "souring" of oil reserves may be partially attributable to attached sulfate-reducing bacteria which produce hydrogen sulfide in the oil-bearing formation. With secondary oil recovery becoming standard practice, souring of oil fields has become a major problem whereby the quality of vast oil reserves may be seriously compromised through microbial production of hydrogen sulfide. In a study of a gas storage and production facility (Dziewulski et al., 1990), indigenous microorganisms were implicated in the souring of natural gas when supplied with nutrients by the flow of water in and out of the field during injection and production. Biological plugging of oil sand reservoirs has been examined (Geesey et al., 1987) to determine the potential of the water source to induce biofilm-related plugging of the formation. The highest cell densities and EPS concentrations were found in the region where the water entered the sampled core. This may be the result of higher nutrient concentration at the core inlet or because of filtration of biomass which detached upstream in the process. In the oilfield, plugging around an injection well results in decreased injection rates and/or higher injection pressure. Despite the plugging phenomenon, some researchers (Crawford, 1966;

Lappin-Scott et al., 1988) have applied biofilm technology in an attempt to enhance oil recovery. By plugging the larger pores with biofilm, oil remaining in the smaller pores is presumably exposed to more flow and more oil is recovered. In the laboratory (Torbati et al., 1986), selective plugging of larger pores in sandstone was accomplished using a mixed population with a sucrose-mineral salts medium. The largest pore sizes were reduced from 59 to 69 microns (10^{-6}m) before plugging to 20 to 38 microns (10^{-6}m) after microbial plugging.

Detachment

Biofilm detachment is the process of removal and entrainment in the bulk liquid of biomass from an existing biofilm and is most likely the process which limits the extent of biofilm accumulation on a substratum. Detachment is also one of the least understood processes in terms of the variables which affect the process rates. Computer simulations indicate that the detachment rate coefficient is the most sensitive variable affecting the predicted rate and extent of biomass accumulation, but no expressions have yet been proposed which accurately predict the detachment rate for a wide range of environmental and operating conditions.

The prediction of detachment rates has been a priority in understanding biofouling in drinking water distribution systems, the computer chip industry, paper production, and oilfield injection water pipelines. In each of these industries, detached biomass reduces final product quality and/or increases operating costs. Biofilms in drinking water distribution systems may harbor pathogenic organisms (LeChevallier et al., 1987). Detachment of these organisms and entrainment in the bulk liquid can result in failure to meet drinking water quality standards. The computer chip industry

requires ultrapure water. Biofilm detachment (White et al., 1990) can lead to contamination of delicate components where a single bacterial cell is enough to cause a failure in a computer chip. In the paper industry, detachment of macroscopic biofilm particles results in defects in the paper that can lower product value and reduce the strength of the unfinished paper. This reduction in strength leads to expensive downtime when the paper sheets tear on the paper machine. In oilfield injection water pipelines, increased biomass detachment speeds fouling of pre-injection filters or plugging of the formation itself. This results in more frequent maintenance or replacement of the filters, while formation plugging around an injection well results in decreased injection rates or higher injection pressure. For all these industries, decreasing biofilm detachment rates would reduce operational costs and/or improve product quality.

The experimental program described herein was conducted to determine the influence of two important variables, shear stress and substrate loading rate, on the rate of biofilm detachment and to evaluate mathematical expressions for the prediction of detachment rates.

Research Goal and Objectives

The long term goal of this research is to determine the kinetics of biofilm detachment.

The objectives established for reaching this goal are as follows:

1) Determine the effects of shear stress and substrate loading rate on biofilm accumulation, biofilm density, and, specifically, biofilm detachment.

2) Differentiate between sloughing and erosion as separate detachment processes

based on detached biofilm particle size distributions.

3) Observe the influence of step changes, such as pH, on the biofilm and the detachment phenomenon.

BACKGROUND

Biofilm Processes

The progression of biofilm accumulation typically follows a sigmoidal-shaped curve in terms of biofilm mass, cell numbers or thickness (Figure 1).

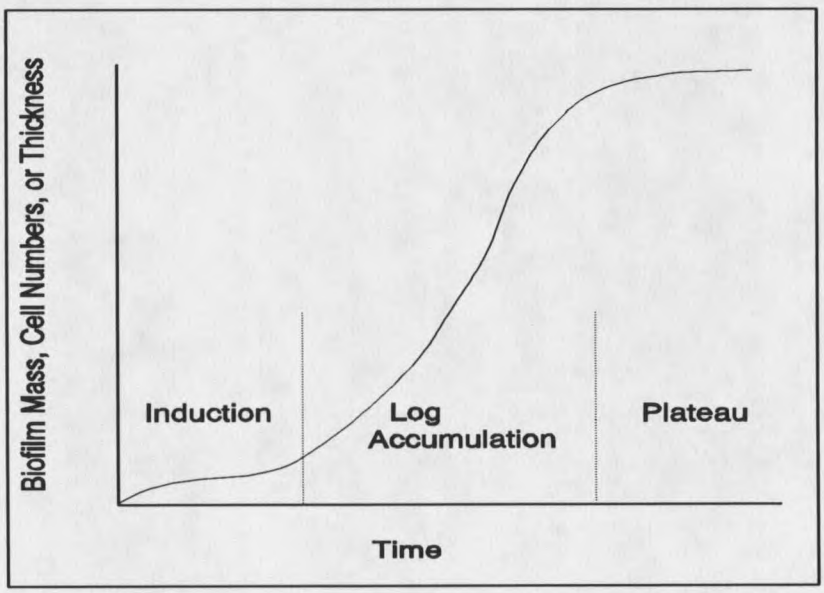

Figure 1. The characteristic progression of biofilm accumulation with time.

Biofilm accumulation is the net result of various processes which can be identified and quantified (Figure 2):

1) Adsorption - the initial colonization of a clean substratum by cells.

2) Desorption - the reentrainment into the bulk fluid of a cell adsorbed to the substratum.

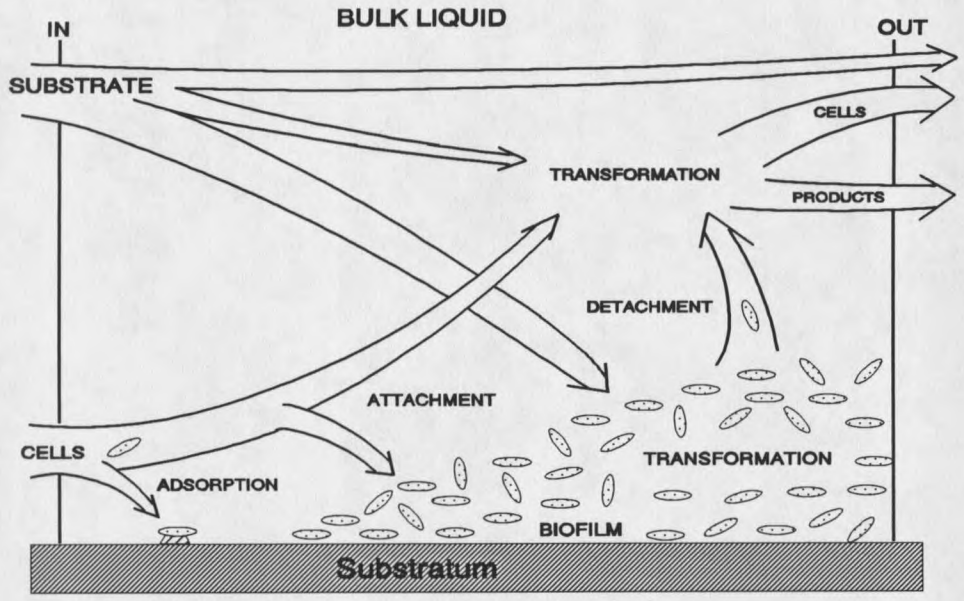

Figure 2. Schematic illustration of processes contributing to biofilm accumulation and activity, including substrate transport and transformation; cellular adsorption and attachment; and detachment of cells and products.

3) Attachment - the acquisition of cells from the bulk liquid by an existing biofilm.

4) Detachment - the entrainment into the bulk fluid of cells from an existing biofilm.

5) Growth - the increase in the number of biofilm cells as a result of replication.

6) Death - permanent loss of a cell's reproductive and metabolic activity.

7) Endogenous decay - biofilm cell metabolism of internal cellular materials.

8) Product formation - the production of polymers and metabolic byproducts in the biofilm.

To mathematically model the accumulation of a biofilm, a material balance is required. The general mass balance equation for biomass in a biofilm is:

$$Accumulation = Input - Output + Transformation \quad (1)$$

In the induction period, *before* a monolayer of biofilm has formed, biomass accumulation can be modelled as follows:

$$\underset{rate}{Accum.} = \underset{rate}{Adsorption} - \underset{rate}{Desorption} + \underset{rate}{Growth} \quad (2)$$

After a monolayer of biofilm has accumulated, the processes of adsorption and desorption become negligible as compared to growth and detachment (Escher and Characklis, 1990), so that the following equation applies:

$$\underset{rate}{Accum.} = \underset{rate}{Attachment} - \underset{rate}{Detachment} + \underset{rate}{Growth} \quad (3)$$

The processes in Eqs. 2 and 3 are described in the following sections.

Adsorption

Adsorption of the Conditioning Film. Immediately upon immersion of a clean surface, organic molecules adsorb to the clean surface. This organic layer is called the conditioning film. Conditioning films are mainly composed of polymers such as glycoproteins (Baier and Weiss, 1975; Baier, 1980) and are not static, but are subject to a high turnover rate (Brash and Samak, 1978). Bryers (1980) measured 1.5×10^{-2} g m^{-2} of chemical oxygen demand (COD) of organic compounds in a conditioning film on glass in a lab system. Little and Zsolnay (1985) have performed similar experiments with stainless steel in seawater and after 0.25 hours obtained up to 0.8×10^{-3} g m^{-2} of adsorbed organic matter. Deposition of proteins has been shown to affect adsorption of bacteria. Meadows (1971) found that albumin inhibited adsorption, while casein and gelatin enhanced microbial adsorption rates. Fletcher (1976) showed a precoating

of a mixture of albumin, gelatin, fibrinogen, and pepsin inhibited the adsorption of a pseudomonad on polystyrene. Although deposition of a conditioning film has been shown to occur before the adsorption of cells to the substratum (Marshall, 1979), it has not been shown to be a *prerequisite* for cell adsorption.

Adsorption of Cells to Surface. The first step in the development of a biofilm is the adsorption of a cell to a solid surface. Adsorption is defined as the interphase accumulation of cells from the bulk liquid directly on the substratum. Processes important to the adsorption phenomenon are shown in Figure 3.

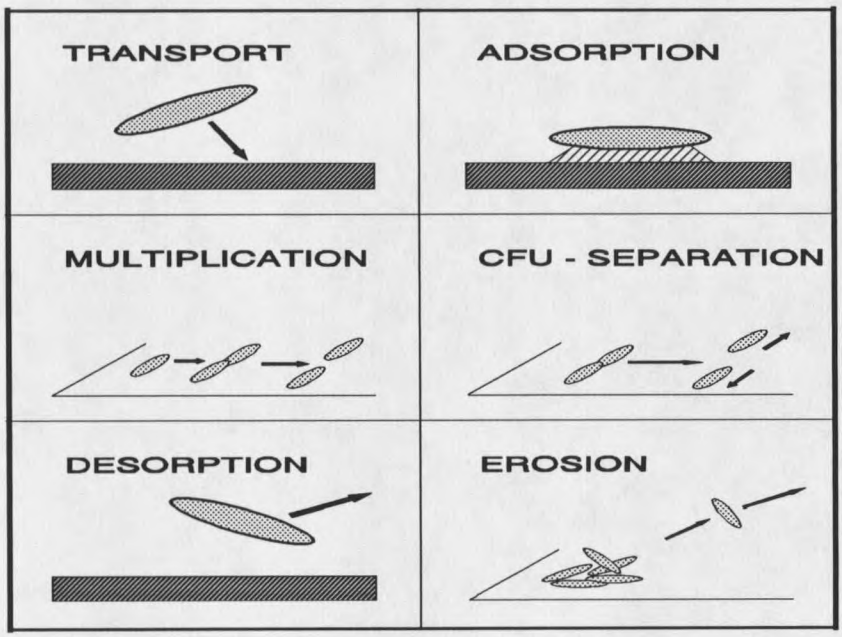

Figure 3. Diagram of the processes fundamental to the initial microbial colonization of a substratum.

CFU-separation, shown in Figure 3, is not important to the adsorption phenomenon itself, but in adsorption studies, cells which have separated on the substratum must be distinguished from cells adsorbing from the bulk liquid. Adsorption plays a major role in biofilm accumulation only in the initial stages of colonization. Subsequently,

growth of cells is the dominant process responsible for the accumulation of a biofilm. Much research has been done on the topic of adsorption; however, only recently have factors such as momentum transport been included in these studies. Shear stress has been shown to play an important role in the sticking efficiency of cells to a substratum (Hermanowicz et al., 1989; Powell and Slater, 1983; Escher and Characklis, 1990). The sticking efficiency is defined as

$$Sticking\ Efficiency = \frac{number\ of\ cells\ adsorbed\ to\ the\ substratum}{number\ of\ cells\ transported\ to\ the\ substratum} \qquad (4)$$

The sticking efficiency is the ratio of the rate of cell adsorption to the rate of transport of cells to the surface, and is, therefore, affected by the fluid flow regime under which the adsorption occurs. In turbulent flow, turbulent bursts from the bulk fluid penetrate to the wall (Cleaver and Yates, 1975 and 1976) and these bursts may contribute significantly to the transport of cells to the surface in turbulent flow (Escher and Characklis, 1990). Other processes which contribute to the transport of cells to the surface in both turbulent and laminar flow are cell motility (Jang and Yen, 1985), Brownian motion, and gravity.

Adsorption is affected by substratum properties (Mueller, 1990), with different materials experiencing varied rates of cellular adsorption under similar flow conditions. Roughness is also believed to strongly influence the adsorption properties of cells in flowing systems. For quiescent conditions, Van Haecke et al. (1990) concluded surface roughness had little effect on the adsorption kinetics of *Pseudomonas aeruginosa* on stainless steel. Under quiescent conditions, the cell surface hydrophobicity was the major parameter affecting adsorption, and measurable adsorption occurred within 30 seconds (8.33×10^{-3}h). The physiological state of the

cells can play an important role in the adsorption rate. Cell adsorption per unit area was found to be linear with specific growth rate history (Nelson et al., 1985) in a turbulent flow system. As the specific growth rate was increased, a linear decrease in adsorbed colony forming units was observed. Dawson (1981) has shown that starved *Vibrio* were more hydrophobic than normal cells, and formed polysaccharide-rich tubules which were believed to enhance adsorption. Extracellular polymeric substances (EPS) may assist in the binding of microbes to a substratum and, thus, influence adsorption. Electron microscopy (Fletcher and Floodgate, 1973; Marshall and Cruickshank, 1973) has shown the presence of EPS adsorbed to a substratum.

Reversible / Irreversible Adsorption. Most research has focused on irreversibly adsorbed cells, where only the cells which have "permanently" adsorbed to the substratum are included in the analysis. However, a few studies (Zobell, 1943; Marshall et al., 1971; Powell and Slater, 1983; Escher, 1986) show that some cells adsorb reversibly. Some cells are adsorbed to the substratum for a finite period of time, then desorb and are reentrained in the bulk liquid. Factors influencing irreversible adsorption are most likely those which also influence reversible adsorption.

Attachment

Attachment is defined as cells from the bulk liquid sticking to an *existing* biofilm. Attachment of cells could play an important role in the displacement of one cell species by another in an existing biofilm. Although methods have been developed and tested to determine the rate of attachment of suspended cells (Gunawan, 1991) and 1 micron ($1x10^{-6}$m) latex beads (Drury, 1992) to an existing biofilm, very little published quantitative information is available.