

Biofilm Development: A Process Analysis

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Abstract. This paper will provide a framework for understanding the process of biofilm development in the context of stoichiometry and kinetics. Biofilm development is described in terms of selected fundamental rate processes and environmental parameters which influence their rate and extent. The properties of the biofilm and its microenvironment lead to topics of microbial ecology within the biofilm and the physiology of the organisms immobilized within it. These topics will be discussed in terms of unstructured models for the microbial processes.

BIOFILMS

Microbial cells attach firmly to almost any surface submerged in an aquatic environment. The immobilized cells grow, reproduce, and produce exopolymers which frequently extend from the cell forming a tangled matrix of fibers which provide structure to an assemblage which is termed a biofilm. Biofilms sometimes provide a relative, even coverage of the wetted surface and at other times are quite "patchy." Biofilms can consist of a monolayer of cells or can be as much as 30-40 cm thick as observed in algal mats. In the case of thick biofilms such as the algal mats, the biofilm can contain both aerobic and anaerobic environments due to diffusion limitations within the reacting mat. Both aerobes and anaerobes, (e.g., sulfate-reducers) produce significant quantities of exopolymers. Consequently, the term biofilm does not necessarily reflect a surface accumulation which is uniform in time and/or space.

In the simplest case, biofilms are composed of microbial cells and their

products such as exopolymers. Such a biofilm generally is a very adsorptive and porous ($\geq 95\%$ water) structure. As a result, biofilms observed in many natural environments consist of a large fraction of adsorbed and entrapped materials such as solutes and inorganic particles (e.g., clay, silt). Many such deposits are found to contain less than 20% volatile mass, suggesting that the deposit organic content is a minor component, an observation which may be far from the truth. Organic materials have a specific gravity of approximately 1.05 (cells and carbohydrates), whereas the inorganic materials may have a specific gravity of as much as 2.5. As a result, the organic material is a more significant volume fraction than mass fraction of the biofilm. In any case, the organic matrix may be necessary to bind the inorganic components into a coherent deposit. Consequently, the term biofilm may reflect a surface accumulation which is composed of a significant fraction of inorganic or other abiotic substances held together by a biotic organic matrix.

Biofilms serve beneficial purposes in the natural environment as well as in some modulated or engineered biological systems. For example, biofilms are responsible for removal of soluble and particulate "contaminants" from natural streams and in wastewater treatment plants (fixed film biological systems such as trickling filters, rotating biological contactors, and fluidized beds). Biofilms or mats in natural waters frequently determine water quality by influencing dissolved oxygen levels and serve as a sink for many toxic and/or hazardous materials. These same mats may play a significant role in the cycling of the elements. Biofilm reactors are used in some common fermentation processes (e.g., "quick" vinegar process) and may find considerably more application in the near future (Atkinson, this volume).

Most recent attention focused on biofilms, however, reflects their nuisance roles. Fouling refers to the undesirable formation of inorganic and/or organic deposits on surfaces, which results in unsatisfactory equipment performance or reduces equipment lifetime. Biofilms cause fouling of industrial equipment such as heat exchangers, pipelines, and ship hulls, resulting in reduced heat transfer, increased fluid frictional resistance, and/or increased corrosion. Fouling is of commercial concern in the manufacture of miniaturized electronics, toilet bowl cleaners, rolled steel, and paper. In the medical or dental areas, biofilms are responsible for health problems.

PROCESS ANALYSIS

Process analysis, in the context of this paper, refers to the application

of thermodynamic and kinetic principles to the recognition and definition of various processes contributing to biofilm accumulation, microbial activity within the biofilm, and biofilm persistence in a given environment. Specifically, the use of mass and energy balances have been most useful in assessing the importance of various fundamental processes contributing to the accumulation and activity of a biofilm. The process analysis generally requires a) mathematical specification of the problem for the given physical situation, b) development of a mathematical model, c) experimental testing of the model, and d) synthesis and systematic presentation of results to ensure full understanding. The process denotes an actual operation(s) or treatment(s) of materials as contrasted with the model which is a mathematical description of the process.

The basis for the process analysis rests in the conservation equations: mass and energy conservation derived from thermodynamics and momentum conservation from Newton's laws of motion. The most important results from a process analysis of a reacting system are expressions which quantitatively describe the rate and stoichiometry of the fundamental reaction processes contributing to the overall process. These expressions, ideally, are useful, regardless of the physical factors imposed on the system (e.g., geometry, flow rates).

In contrast, much of the previous emphasis on biofilm processes has concentrated on biological and chemical aspects of mechanism without resorting to rate and stoichiometric analyses. But the physical, chemical, and biological processes of interest in biofilm development are completed in a certain period of time. For biofilm development, a specified change in fouling of a heat exchanger may signal the shutdown of manufacturing operations and the beginning of cleaning operations. The rate and extent of biofilm development on a tooth surface is reduced by brushing the tooth twice a day. The time required for any specified change is inversely proportional to the rate at which the process occurs. Thus, the rate must play a most important role in the process analysis.

In most, if not all, reported research on biofilms, certain observed or measured quantities are reported: net biofilm accumulation and/or substrate (or oxygen) removal. Substrate removal is frequently determined by removing the biofilm from its environment and conducting a batch test with pulsed substrate (e.g., heterotrophic potential test). Such *ex situ* kinetic determinations are relatively useless since their accuracy cannot be assessed, nor are they necessarily representative of processes occurring in the sampled environment. Another difficulty with these

observed quantities is that they reflect the contribution of several quantities of more fundamental significance. For example, net biofilm accumulation may be the sum of the following processes: a) transport of cells to the substratum, b) attachment of cells to the substratum, c) growth and other metabolic processes within the biofilm, and d) detachment of portions of the biofilm. If, then, the overall process consists of a number of processes in series, the slowest step of the sequence exerts the greatest influence and controls the overall process rate. This step is called the "rate-determining step" or "rate-controlling step."

Which process controls the rate of net biofilm accumulation? Obviously, the rate-limiting step will depend on the environment at the substratum-liquid interface. Parameters such as concentration of nutrients, fluid shear stress, and cell density will determine which fundamental process will control the rate of net biofilm accumulation. For example, the rate-limiting process for biofilm accumulation may be different in a tube enclosing a turbulent flow as compared to a glass slide immersed in a quiescent fluid, even if the rates of accumulation are the same.

FUNDAMENTAL PROCESSES CONTRIBUTING TO BIOFILM DEVELOPMENT

In this discussion, biofilm development will be considered to be the net result of the following physical, chemical, and biological processes:

1. Transport of organic molecules and microbial cells to the wetted surface.
2. Adsorption of organic molecules to the wetted surface resulting in a "conditioned" surface.
3. Attachment of microbial cells to the "conditioned" surface.
4. Metabolism by the attached microbial cells resulting in more attached cells and associated material.
5. Detachment of portions of the biofilm.

These processes can be further classified into transport and transformation processes. Transport processes describe the transfer of energy or material between the environment and the system in question or the transfer of energy or material from the system boundary through the system. For example, if the system is defined as the biofilm, 1 and 2 above are transport processes describing transfer of materials between the environment and the system. Transformation processes are those processes resulting in molecular transformations of matter, i.e., chemical or biochemical reactions. Transformation processes are described by rate equations or, more specifically, constitutive or kinetic equations of the

following form:

$$r = r(c_1, \dots, c_n), \quad (1)$$

in which $c_1 \dots c_n$ are concentrations of the various reacting components.

Of the fundamental processes listed above, all can be classified as transport processes except for metabolism by the attached cells and the production of more attached cells and associated material which refers to transformation processes. These transformation processes can be broken down further into the following processes: a) microbial growth and reproduction, b) product formation (e.g., exopolymers), c) maintenance, and d) decay. Note that these are unstructured processes in that specific intracellular transformations need not be considered. Otherwise, our models would be considerably more complex. In a system experiencing rapid environmental fluctuations, homeostasis (balanced growth) is not possible and "unstructured" models will not suffice to describe the system in phenomenological terms. In fact, batch reactors sometimes suffer from the disadvantage of creating these transient conditions which make modelling more difficult.

The categorization of these processes as fundamental is somewhat relative. However, at this time, I have not been able to further subdivide these processes while maintaining my dependence on observable quantities for the analysis. Another criterion for a fundamental process, in this paper, is that accepted mathematical descriptions for the process exist.

CONSERVATION OF MASS

Generally, in systems such as biofilm "reactors," transport and transformation processes are combined in a general type of model based on the conservation of mass. The equations in such a model have the following form:

$$\text{In} - \text{Out} + \text{Conversion} = \text{Accumulation} \quad (2)$$

or

$$\text{transport} + \text{transformation} = \text{Accumulation} \quad (3)$$

In batch "reactors," the transport term is zero since there is no exchange with the environment.

The conservation equation is especially useful since it permits the in

situ determination of the rates, especially the transformation rates, in a natural or experimental system. The conservation equation also incorporates the thermodynamic (e.g., biofilm mass and specific gravity) and constitutive (e.g., reaction and transport coefficients of the biofilm) properties of the system.

TRANSPORT PROCESSES

Transport processes, as indicated in the previous section, include transport of organic molecules and microbial cells to the substratum, cell attachment to the substratum, and detachment of cells from the substratum. These processes are closely related, especially during early events in the accumulation of a biofilm. Transport processes discussed in this section will be limited to those relevant to early events in the establishment of a biofilm. Other transport processes, such as diffusion of reactants within the biofilm, are significant at a later stage in biofilm development and are discussed by Atkinson (this volume).

Significant effort has been invested in studying the mechanisms by which cells accumulate at the substratum. For example, substratum surface and bulk properties, microbial cell surface properties, exopolymers, and type or extent of the "conditioning" film are variables that have been considered as influencing attachment of cells to the substratum.

However, less effort has been used to determine the rate and extent of attachment and the influence of physical factors in the environment. In order to explore such matters, consider the following events which represent a composite of the observations of many investigators for the past forty to fifty years:

1. Organic molecules are transported from the bulk fluid and adsorbed to the substratum.
2. A fraction of the suspended microbial cells are transported to the conditioned substratum. In a quiescent environment, the predominant mechanism for transport may be sedimentation or motility of the organism. In laminar flow, the primary transport mechanism may be diffusion (Brownian motion), whereas in turbulent flow convective (that related to fluid motion) mechanisms may dominate.
3. A fraction of the cells that strike the substratum attach to the substratum for some finite time, "the critical residence time," and then detach. This process will be termed reversible attachment. Detachment may result from fluid shear forces, but other detachment mechanisms are certainly plausible.
4. A fraction of the reversibly attached cells remain attached beyond

the "critical residence time." These cells are irreversibly attached.

Transport and Adsorption of Organic Molecules

Transport and adsorption of organic molecules to the surface is very rapid as compared to transport of microbial cells. As a consequence, microbial cell attachment is essentially always to a "conditioned" surface. Therefore, the rate of "conditioning" is instantaneous as compared to other rate processes. Hence, the term substratum in this paper will specifically refer to a "conditioned" substratum. Debates continue regarding the necessity of the conditioning film for attachment of microbial cells.

Transport of Microbial Cells

As far as this author knows, no one has measured the rate of transport of suspended microbial cells to a surface. In fact, little is known about transport rates of any particles in aqueous systems. The quantity which is inevitably measured is net rate of accumulation, i.e., the number of cells that are transported to the surface and which irreversibly adhere. The number of cells that strike the surface is unknown. The ratio of the number of adhesions to the number of strikes can be considered as the adhesion efficiency and could serve as an assay for the adhesiveness of a microbial cell under a variety of environmental conditions.

Few reports exist regarding mechanisms of cell transport in various liquid flow regimes. Certainly, the dominant mechanism for cell transport is different in turbulent flow than it is in a quiescent system. In fact, flow may significantly influence adhesion efficiency. For example, experimental data for net accumulation rate as compared to calculated transport rates, assuming the mechanism for transport was known, are presented in Table 1. Fletcher (3) measured net accumulation rate under quiescent conditions in which sedimentation could have been the dominant transport mechanism. Under these conditions, detachment is negligible. Powell and Slater (6) conducted their experiments in laminar flow where cell transport is presumably controlled by diffusion. Using these assumptions, transport rates of cells to the substratum were calculated (Table 1). Several observations are striking:

1. Calculated rate of transport is significantly greater in the quiescent system. Microscopic observations in our laboratory confirm the dramatic difference in the striking rate between these two flow regimes (Nelson, unpublished results). In laminar flow, the transport rates increase with increasing shear stress. The rate of transport is greater in the high shear stress laminar experiment than in the low shear

TABLE 1 - A comparison between measured net cell accumulation rate at a surface and the calculated cell transport rate to the surface.

Reference	Fluid Shear Stress (N m ⁻²)	Rate of Transport (cells m ⁻² s ⁻¹ X 10 ⁻⁴)	Rate of Accumulation (cells m ⁻² s ⁻¹ X 10 ⁻⁴)	Cell Concentration cells m ⁻³ X 10 ⁻¹³	Presumed Transport Mechanism
Fletcher (3)	0	5000	4170	10.0	sedimentation
Powell and Slater (5)	0.80*	472	3	1.4	diffusion
	0.09**	167	31	1.4	diffusion

* Reynolds number = 11

** Reynolds number = 1.2

stress experiment. The apparent difference in transport rates between quiescent and laminar flow may be attributed to a change in the dominant transport mechanism.

2. Net rate of accumulation is significantly greater in the quiescent system. Net rate of accumulation is greater in the laminar flow with lower shear stress than in the higher shear stress experiment. One apparent explanation for these results is that detachment rate increases with increasing shear stress (6).
3. The calculated adhesion efficiency in the various experiments is proportional to shear stress (Fig. 1).

Transport is also influenced by the macroscopic geometry of the experimental system. Figure 2 is a composite of several environments that are encountered in a power plant condenser system. The fluid forces in the various environments are quite different and will influence transport rates significantly. The resulting microenvironments, e.g., crevices, will also determine the type (e.g., aerobic or anaerobic) of microbial activity in the biofilm.

Attachment of Cells to the Substratum

Reversible attachment occurs after the cell is transported to the substratum. The cell may be held at the surface by relatively weak bonds such as electrostatic interactions. If the cell does not detach after a certain time period, the cell is irreversibly attached. Does the cell require a finite or critical residence in the reversible state to irreversibly adhere

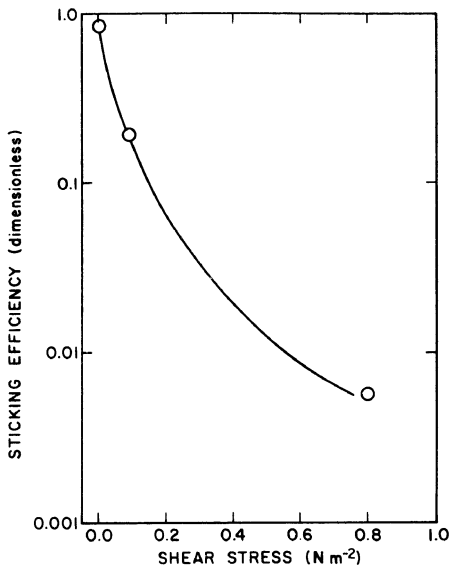


FIG. 1 - Influence of fluid shear stress on adhesion efficiency of bacterial cells (data from (3, 6)).

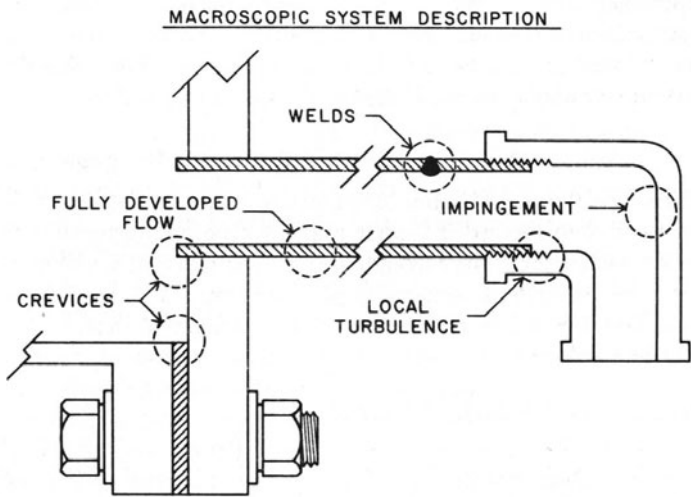


FIG. 2 - Schematic composite diagram of various geometries existing within a condenser/heat exchanger.

or is irreversible attachment spontaneous? Is exopolymer production a necessary prerequisite for irreversible attachment? If so, is it a specific polymeric substance? If exopolymer production is necessary, then a finite time will be required for its synthesis and critical residence time appears to be a viable concept. Other explanations have been offered (see Robb, this volume).

The extent of attachment provides other tantalizing questions. Fletcher (3) observed approximately 45% coverage of the substratum surface at the saturation point in a quiescent system. Powell and Slater (6) observed 1-5% coverage at saturation in laminar flow. Experiments in our laboratory (Nelson, unpublished results) in turbulent flow resulted in approximately 0.01% coverage at saturation and uniform distribution of cells across the surface. The uniform distribution and low saturation coverage suggest that there exists a "zone of influence" around the attached cells possibly defined by exopolymers or other chemosensory mechanisms. In any case, saturation coverage appears to be strongly influenced by flow regime. Kjelleberg (personal communication) reports less than 5% saturation coverage in all samples he has observed.

The physiological state of the organism influences the rate and, possibly, the extent of attachment (3). Bryers and Characklis (2) observed that attachment rate was directly proportional to growth rate in a mixed culture system when feeding the biofilm reactor from a chemostat. Nelson (unpublished results) has observed a decrease in attachment rate with increasing specific growth rate for a *Pseudomonas* species (pure culture) in a similar experimental system.

The surface roughness of the substratum may be a significant factor which has been overlooked in experimental studies purporting to simulate a specific environment. Microroughness of various roll finishes in stainless steel tubes is described schematically in Fig. 3. The figure clearly emphasizes the probable role of microroughness in both transport and attachment of microbial cells. Convective transport rates near the microrough surface will be greater than at a smooth surface. Once the cell arrives at the surface, attachment rates will probably be higher for several reasons. Two reasons which support the observations that net cell accumulation rate is greater on rougher surfaces are: a) detachment due to shear forces will be reduced since the cells are shielded from the bulk fluid flow, and b) more substratum surface area may be available for contact with the cell.

MICROSCOPIC SYSTEM DESCRIPTION

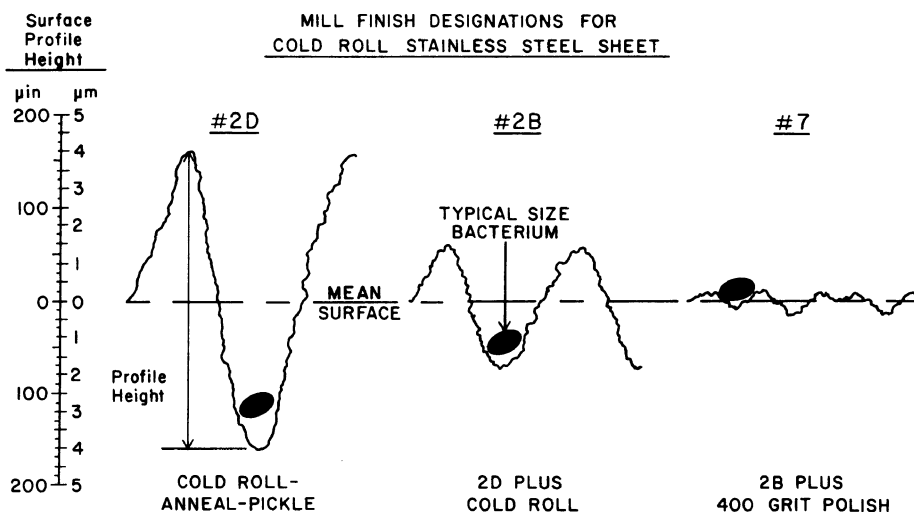


FIG. 3 - Schematic diagram of microroughness in stainless steel tubing as compared to a microbial cell.

Detachment from the Substratum

During early events in biofilm formation, hydrodynamic influences control detachment (5, 7). Detachment increases with increasing fluid shear stress at the substratum surface. Macro- and microroughness may significantly influence detachment since cells attached in cavities will be sheltered from severe hydrodynamic shear.

Shear forces along a conduit are exerted parallel to the surface. Hence, a cell detached from the surface due to shear may be transported close to the surface (in the viscous or laminar sublayer) for a significant distance, i.e., it may bounce or roll. This behavior will result in numerous collisions with the surface and, hence, more opportunities for reattachment. In turbulent flow, a significant lift force is exerted which may transport the detached cell quickly into the bulk liquid. The lift forces, however, are several orders of magnitude smaller than the shear forces.

Detachment may also result from a chemical treatment (e.g., chelants, oxidants). Little fundamental information is available on kinetics of detachment, despite their potential usefulness as comparative criteria in testing chemical control treatments.

TRANSFORMATION PROCESSES

Transformation processes refer to those processes in which molecular rearrangements occur, i.e., reactions. After a significant number of cells attach to a substratum, microbial transformation or metabolic processes will become significant if nutrients are available and "colonization" will ensue (1).

Studies of transformation processes within biofilms have generally relied on a relatively unstructured approach to analysis of the biomass component. Unstructured models characterize the biotic component only in terms of biomass with little attention given to the various biofilm components, physiological state of the organisms, microbial species, or exopolymer content. Processes in unstructured models are limited to those that can be evaluated without considering activities within the biofilm, i.e., a more macroscopic approach.

Fundamental and Observed Processes

Recent studies (Robinson et al., submitted; Bakke et al., submitted) have provided more information on biofilm structure by using pure cultures (*Pseudomonas aeruginosa*), measuring exopolymer content, and culturing cells in a chemostat. To obtain these results, four fundamental rate processes were identified: a) growth, b) product formation, c) maintenance and/or endogenous decay, and d) death and/or lysis. Any or all of these processes may be occurring in a biofilm at any time. Growth refers to cell growth and multiplication. The cells also form products, some of which are retained in the biofilm (e.g., exopolymers) and some of which diffuse out into the bulk fluid. The cells also have to maintain their internal structure, another energy-consuming process. If nutrients are depleted or toxic substances are present, death and/or lysis ensues.

The rates of the fundamental microbial processes are difficult to measure directly and are generally inferred from more easily observed rate processes. The more familiar observed rate processes include a) substrate consumption, b) electron acceptor consumption, c) biomass production, and d) product formation. The relationship between fundamental and observed process rates is presented in Table 2. The stoichiometry of the process is qualitatively represented by each row in the matrix

TABLE 2 - A matrix representation for the fundamental microbial rate processes.

FUNDAMENTAL PROCESS		STOICHIOMETRY						
		REACTANTS			=	PRODUCTS		
Process	Rate Coeffi- cient	Sub- strate s	Nu- trient z	Electron Acceptor e	Bio- mass x	Pro- duct Pe	Metabo- lite Pi	lite a
Growth	μ	-	-	-	+	+	(+)	+
Maintenance exogenous	m	-	-	-		+		+
endogenous	k_e		+	-	-	+	-	+
Product Formation	k_p	-	-	-		+	+	+
Death	k_d	(+)	(+)		-	+		
OBSERVED RATE		q_s	q_z	q_e	μ_n	q_p		q_a

q = specific production or removal rate (t^{-1})

μ = specific growth rate (t^{-1})

x = biomass concentration (ML^{-3})

Pe = extracellular microbial product concentration (ML^{-3})

Pi = intracellular microbial product concentration (ML^{-3})

s = substrate concentration (ML^{-3})

z = nutrient concentration (ML^{-3})

e = electron acceptor concentration (ML^{-3})

μ_n = net specific biomass production rate (t^{-1})

(- refers to reactants and + refers to products). The columns of the matrix indicate the fundamental rate processes that may contribute to the observed rates (last row in the matrix). For example, substrate removal (column 1) is the net result of growth, maintenance, and product formation.

Several investigators ((2, 8), Robinson et al., submitted; Bakke et al., submitted) have used similar experimental techniques in a laboratory biofilm reactor to quantify the rate and stoichiometry of the fundamental processes within a biofilm. The reactor is a continuous flow stirred tank reactor which provides a relatively uniform surface environment for microbial attachment and biofilm activity. Their results suggest the following:

1. The growth rate of cells (*Ps. aeruginosa*) in the biofilm can be estimated from their growth rate in chemostats when substrate concentration in the microenvironment of the cell is equal in both conditions. Results

of experiments in a chemostat and in a biofilm reactor are compared in Fig. 4. Results indicate that specific substrate removal rate is the same for *Ps. aeruginosa* at the same growth rate in a chemostat and in the biofilm. The linear slope suggests constant energy metabolism in both microbial environments. Maximum specific growth rate, saturation coefficient, and yield are the same in a chemostat and in the biofilm. One important restriction requires that no significant diffusional resistances exist in the biofilm, which was the case in this study. Another concern requires that the substratum surface not release any components which influence metabolism. In these studies, acrylic plastic was the substratum.

2. Exopolymer formation rate and yield by biofilm cells (*Ps. aeruginosa*) was essentially the same for dispersed cells. However, exopolymer accumulation rate in the biofilm was quite high. Therefore, exopolymer may be the dominant component in the biofilm (up to 90% of the biofilm organic carbon in this study).
3. Maintenance requirements are essentially negligible until the biofilm becomes very thick. Even then, the results of exopolymer formation or anaerobic metabolism deep within the biofilm may be mistaken for maintenance energy requirements if sufficient measurements are lacking.

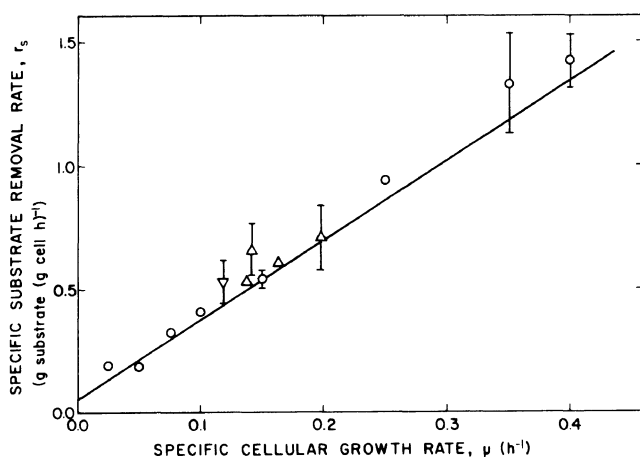


FIG. 4 - Relationship between steady state specific substrate removal rate and specific growth rate of *Ps. aeruginosa* in a chemostat ($\circ$) and a biofilm (Δ , $D = 6\text{h}^{-1}$; ∇ , $D = 3\text{h}^{-1}$). Definition of specific growth rate in a biofilm is expressed by Eq. 11 (see Table 3).

One of the important conclusions from these results is that chemostat-derived rate and stoichiometric coefficients may be used for modelling biofilm processes in some cases. However, important questions still remain. Accumulation rate also depends on detachment rate and few useful rate expressions are available for detachment. The results do indicate that substrate removal rate alone is not a sufficient criterion for comparing the activity of attached and dispersed cells since substrate removal is the net result of several fundamental processes. The measurements described above were accomplished *in situ*. Removing the cells from the surface or removing the surface from its environment obviates any relevance in subsequent measurements which purportedly describe the activity of attached cells.

Mass Transfer and Diffusion

Analyses of biofilm process rates and stoichiometries are frequently complicated by significant mass transfer resistances in the liquid or diffusional resistances within the biofilm. Trulear and Characklis (9) have observed that substrate removal rate increases in proportion to biofilm thickness up to a critical thickness beyond which removal rate remained constant. The critical or "active" thickness was observed to increase with increasing substrate concentration. This behavior has been observed by others and attributed to diffusional resistance within the biofilm. Once the biofilm thickness exceeds the depth of substrate penetration into the biofilm, the removal rate is unaffected by further biofilm accumulation.

The biofilm rate processes may also be controlled by mass transfer limitations in the bulk fluid phase (10). For example, substrate removal rate is dependent on fluid velocity past the biofilm. At low fluid velocities, a relatively thick mass transfer boundary layer can cause a fluid phase mass transfer resistance which decreases substrate concentration at the fluid-biofilm interface and, thereby, decreases substrate removal rate. Two factors may result in low mass transfer rates from the bulk fluid to the biofilm: a) low fluid velocities, and b) dilute liquid phase concentrations of the material being transported. Much biofilm research has been conducted at relatively low flows or in quiescent conditions. Mass transfer may be the rate-controlling step in the overall process in these studies and, without further analysis, may be confused with the rates of more fundamental processes such as growth rates, adsorption rates, etc. In highly turbulent systems, mass transfer in the liquid phase is rarely a significant factor.

Summary of Biofilm Transformation Processes

The microbial processes occurring in a biofilm are more complex than suggested by the four fundamental processes defined above. However, this classification has been useful in determining, to some extent, the flow of substrate energy through the biofilm. Mathematical description of the kinetic expressions has also been accomplished (Table 3). Further structuring of biofilm processes may await more sophisticated methods for observing the processes within the biofilm (as opposed to the influence of the processes on the overlying liquid phase) and more specific identification of the products being formed.

Bakke and Characklis (submitted) have observed a remarkable biofilm phenomenon which demands more attention. The supply of growth substrate was increased stepwise to a biofilm with the following results: a) biofilm material was immediately detached, b) biofilm cell numbers remained constant, and c) specific substrate removal rate and product formation rate increased instantaneously. These observations cannot be described with unstructured models but suggest that the biofilm organisms may slough their exopolymers in response to the "shock." In addition, the attached cells seem to possess a "reaction potential" which is expressed in response to instantaneous increase in substrate loading. The experiments clearly indicate the need to observe microbial physiology while the organisms are attached in their growth environment (in situ) and lead to questions regarding the use of other techniques, such as heterotrophic potential, for determining biofilm activity. More effort must be expended to understand these transient biofilm phenomena because of their relevance to natural and technological phenomena and their potential for providing keys to the understanding of mechanisms.

DETACHMENT OF BIOFILM

Detachment of microbial cells and related biofilm material occurs from the moment of initial attachment (see above). However, the macroscopic observation of biofilm detachment is easier as the biofilm becomes thicker.

Detachment phenomena can be arbitrarily categorized as shearing or sloughing. Shearing refers to continuous removal of small portions of the biofilm which is highly dependent on fluid dynamic conditions. Under these circumstances, rate of detachment increases with increasing biofilm thickness and fluid shear stress at the biofilm-fluid interface (9). Sloughing refers to a random, massive removal of biofilm generally attributed to nutrient or oxygen depletion deep within the biofilm (4) or some dramatic change in the immediate environment of the biofilm (see previous

TABLE 3 - System of equations describing biofilm processes in a continuous flow stirred tank reactor with a sterile substrate feed.

Compound	Net Rate of Accumulation	Net Rate of Transport Out of Reactor	Net Rate of Transformation	Units	Equation No.
Bulk Liquid Substrate Concentration, s	$\frac{ds}{dt} =$	$D (s_i - s)$	$-(\frac{\mu}{Y_{x/s}} + \frac{r_p}{Y_{p/s}}) X_b \frac{A}{V}$	$ML^{-3}t^{-1}$	(4)
Suspended Cellular Mass, x	$\frac{dx}{dt} =$	$-Dx$	$+r_{dx} x_b \frac{A}{V}$	$ML^{-3}t^{-1}$	(5)
Biofilm Cellular Areal Density, x_b	$\frac{dx_b}{dt} =$		$\mu x_b - r_{dx} x_b$	$ML^{-2}t^{-1}$	(6)
Suspended Exopolymer Mass, p	$\frac{dp}{dt} =$	$-Dp$	$+r_{dp} p_b \frac{A}{V}$	$ML^{-3}t^{-1}$	(7)
Biofilm Exopolymer Areal Density, p_b	$\frac{dp_b}{dt} =$		$r_p x_b - r_{dp} p_b$	$ML^{-2}t^{-1}$	(8)

At steady state, Eq. 4 becomes

$$r_s^* = \frac{VD(s_i - s)}{x_b A} = \left(\frac{\mu}{Y_{x/s}} + \frac{r_p}{Y_{p/s}} \right) = \text{specific substrate removal rate} \quad (9)$$

Eq. 5 becomes

$$r_{dx} = \frac{D x V}{A x_b} \quad (10)$$

Combining Eqs. 10 and 6 at steady state

$$\mu = \frac{D x V}{A x_b} = \text{specific growth rate in the biofilm} \quad (11)$$

s = substrate concentration (ML^{-3})
 x = cellular concentration in liquid (ML^{-3})
 p = exopolymer concentration in liquid (ML^{-3})
 x_b = cellular concentration in biofilm (ML^{-2})
 p_b = cellular concentration in biofilm (ML^{-2})
 V = liquid volume (L^3)
 A = wetted surface area (L^2)
 D = dilution rate (t^{-1})

μ = specific growth rate (t^{-1})
 r_{dx} = cellular detachment rate (t^{-1})
 r_{dp} = exopolymer detachment rate (t^{-1})
 r_p = exopolymer formation rate (t^{-1})
 $Y_{x/s}$ = cellular yield
 $Y_{p/s}$ = product yield

section). Sloughing is more frequently witnessed with thicker biofilms developed in nutrient-rich environments. Shearing is probably occurring under the same conditions under which sloughing is occurring but no such direct measurements have been attempted.

Hydrodynamic Influences

Truhear and Characklis (9) have observed that biofilm detachment increases

with both fluid shear stress and biofilm mass. Both Powell and Slater (5) and Timperley (7) conducted studies to determine the influence of fluid dynamics on detachment. Both investigators observed an increase in detachment with an increase in Reynolds number. Timperley also considered different tube sizes and, within that context, concluded that mean fluid velocity was more significant in determining cleaning effectiveness than Reynolds number.

As fluid velocity increases, the viscous sublayer thickness decreases. Consequently, the region near the tube wall subject to relatively low shear forces (i.e., the viscous sublayer) is reduced. As a result, there may be some upper limit to the effectiveness of any cleaning operation based on fluid shear stress. The viscous sublayer may provide a valuable a priori criterion for predicting the maximum effectiveness (the minimum thickness attainable) of any cleaning technique dependent on fluid dynamic forces.

Detachment processes must also be significant in the processes of cell turnover (individual cell residence time) in the biofilm. As a biofilm develops, succession in species is observed. Truhear (8) developed a biofilm of *Pseudomonas aeruginosa* in conditions of relatively high shear stress and then challenged it with *Sphaerotilus natans*. The *Sphaerotilus* quickly became the dominant species within the biofilm. Detachment, influenced strongly by fluid shear stress, may serve to "wash out" organisms from the biofilm. Growth processes may also dilute out the slower growing organisms.

Chemical Treatment

Detachment may occur for reasons other than hydrodynamic forces. Bakke and Characklis (submitted) have observed massive detachment when substrate loading to the biofilm was instantaneously doubled. One hypothesis suggests that cell membrane potential plays a key role in the phenomena. Turakhia et al. (10) have observed dramatically increased detachment upon the addition of a calcium-specific chelant (EGTA), suggesting the importance of calcium to the cohesiveness of the biofilm. Many other chemical treatments have been used to detach biofilm material with varying success, including: a) oxidizing biocides (e.g., chlorine), b) uv radiation, c) surfactants, and d) non-oxidizing biocides.

Summary of Detachment Processes

Detachment processes must play a major role in the ecology of the biofilm. Detachment from and absorption into the biofilm of microorganisms

provides the means for interaction between dispersed (planktonic) organisms and the biofilm. Detachment of biofilm is the major objective of many antifouling additives used in manufacturing processes.

Very little is known regarding the kinetics of detachment and the factors affecting the removal. Such kinetic expressions would be useful for modelling purposes and for serving as comparative criteria in testing of antifouling treatments.

SUMMARY

Biofilm processes have been discussed in terms of the more fundamental physical, chemical, and biological processes which contribute to biomass accumulation at a surface. The purpose was twofold: a) to present a framework for analysis of the rate of biofilm development and extent of biofilm development, and b) to stimulate fundamental investigations on topics related to biofilm processes.

The purpose of the analysis and the investigations is a predictive "model" for biofilm accumulation and activity.

Biofilms are emerging as a most critical factor affecting natural aquatic systems, water distribution systems, wastewater treatment, heat exchangers, fuel consumption by ships, and even human diseases. More attention must be directed to their behavior. Some topics that require more attention include the following:

1. Physical, chemical, and biological properties and structure of biofilms as a function of water quality and hydrodynamic regime.
2. Mathematical models relating process rates to bulk water concentrations, surface characteristics, and microbial species.
3. Population dynamics within the biofilm and its relationship to microbial populations in the bulk water.
4. Methods to enhance biofilm accumulation and activity in terms of substrate removal (wastewater treatment) or product formation (fermentation).
5. Structure, properties, and function of exopolymers in biofilms. What are the properties of special relevance to adhesion?
6. Is the surface "sensed" by the cell? If so, how? Sensing may lead to the synthesis of exopolymers. Is sensing the initial step in synthesis of adhesive?
7. Does cross-feeding take place in the biofilm? If it does, which organisms are producers and which are consumers?
8. Does the close proximity of the cells and their extended intimate

- contact facilitate genetic exchange?
9. Little is known about biofilm activity toward particulate substrates found in natural and technological applications. Most studies are accomplished with soluble substrates.
 10. Do biofilms serve as a sink for heavy metals and xenobiotic compounds in natural waters (surface or groundwaters)?

Biofilms play important roles, beneficial and detrimental, in many natural and technological processes. Development of methods to effectively inhibit or exploit biofilm processes will present a considerable challenge but satisfying rewards in the ensuing years.

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