BIOFILM ACCUMULATION & ACTIVITY: A PROCESS ANALYSIS APPROACH

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1. Introduction

"What is the good of that," said Rabbit.
A.A. Milne, The House at Pooh Corner.

Process Analysis refers to the application of systematic methods to recognize, define and clarify problems and to develop methodologies for their solution. Biofilm formation and persistence in both natural and engineered systems is governed by a collage of complex physical, chemical, and biological processes; each process dependent on a unique set of system parameters. Process analysis applied to biofilm formation provides an integrated approach which incorporates microbial physiology, reaction engineering, and transport phenomena to understand, control, and exploit biofilm processes. Application of process analysis allows one to *(a)* interpret the operation of an existing biofilm system, *(b)* design new biofilm reactor systems, and *(c)* understand the complexities of natural biofilm systems. It is increasingly apparent that research into biofilm processes which does not comprise microbial, chemical, and fundamental engineering aspects is incomplete.

Here, we will present the concepts of *process analysis* and the *rate concept* approach to mathematically describing complex reaction systems as exemplified by the formation and persistence of a biofilm. The basis of process analysis lies in the fundamental conservation equations of mass, energy, and momentum. Application of process analysis and the rate concept provides a systematic protocol with which to either interpret an existing reaction system or to design and operate a new system. First, we will define various model types and their relative utility with special attention paid to both structured and unstructured model concepts. Second, those processes contributing to the formation and persistence of a biofilm will be enumerated and the individual rate expressions for these fundamental processes discussed. Errors associated with ignoring the involvement of these individual rate processes in cell adhesion and biofilm formation are also described.

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2. Process Analysis, the Rate Concept, and Mathematical Modeling

It is one of the maxims of civil law, that definitions are hazardous.
Samuel Johnson

Process analysis is simply a method of mathematical describing a complex phenomena as the net result of a number of individual fundamental processes. The *rate concept* more specifically assumes that changes in the state of a system with time can be systematically treated as the summation of the rates of the individual processes acting on the system. As implied, some degree of modeling of the biological system is posed, predictions of the model compared to observation, and the model verified or refuted. Thus, modeling must be, by nature, an evolutionary process in order to provide insight about a system.

"...as we advance into the terra incognita of tomorrow, it is better to have a general and incomplete map, subject to revision and correction, than to have no map at all.
Alvin Toffler, Power Shift, p. xxi

Modeling is basically the scientific process of forming and testing a hypothesis. A model need not be mathematical nor need it exactly replicate reality. A road map is a model of a section of the Earth that allows one to successfully navigate from point A to B without representing every house or structure that actually exists. Models need not (and can not) simulate all details of a system to be of use. Models are simply our inherent tendency to explain unknown phenomena based on perceived principles. A good portion of basic science is predicated on proving or refuting such hypotheses. Mathematical models do provide a rigid structure with which to formulate concepts regarding a complex system. Mathematical models provide a means of verifying the goodness of a model by way of many statistical criteria. Important variables and system parameters can be easily identified and, through the use of dimensionless groups of such variables, mathematical models can expedite experimentation. And should the mathematical model prove successful in correlating observable data from a system at one set of conditions, then one has the ability to extrapolate; predicting the responses of the system under a variety of different conditions.

Though this be madness, yet there is method in't.
Shakespeare, Hamlet, Act II, Sc. 2

Three basic types of models are: *(1)* transport or continuum models, *(2)* population models, and *(3)* empirical or statistical models. Residence time distributions and synchronous growth are examples of population models while statistical regression of data sets is an example of empirical models. Transport models, the type considered here, essentially account for the changes of either mass, energy, or momentum in a continuum or defined control volume.

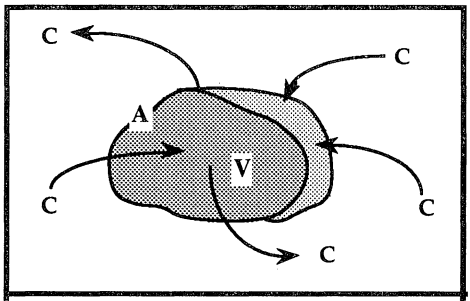


Figure 1. Continuum Approach to the Conservation of Mass

Consider the control volume illustrated in Figure 1. The control volume can exchange mass, energy, and momentum with its surroundings as indicated by the arrows. The conservation equation for mass equates the rate of accumulation within the control volume to the net rate of input into the control volume plus the summation of all process rates producing or consuming mass in the volume. A verbal form of the conservation equation for the mass of a component C can be expressed as follows:

Net rate of accumulation of C in control volume = **+ rate of transport of C into the volume across area A**
- rate of transport of C out of the volume across area A
+ Σ (all transformation processes generating or consuming C within the volume V)

The net accumulation rate and the rates of transport into and out of the volume are termed *rates of change* or *system rates* in that they represent the observed change in the measured component. System rates are extensive quantities and are by definition system-specific and can not be correlated to fundamental parameters (*e.g.*, temperature, velocity) describing the system. Unfortunately, all too often many try.

Transport rates comprise *bulk transport* (movement of component due to the flow of the bulk liquid), *interfacial or interphase transport* (transport across the interface between two phases), and *intraphase transport* (transport within one phase due to a gradient - *e.g.*, diffusion). One common criterion regarding a transport rate is that changes in a component's concentration (in the case of mass transport) are not due to a molecular change in the component.

Conversely, process rates describe transformations that occur due to either chemical, biochemical, or biological reactions that either produce or consume the component. Process rates are fundamental intensive quantities in that they can be correlated to system parameters such as temperature, pressure, concentration, and velocity. Process rates and their correlation to such fundamental parameters are independent of the system in which they occur.

Process or transformation rates comprise two fundamental concepts: *stoichiometry* and *reaction rate kinetics*. Stoichiometry provides the relative basic molar quantities of the various reactants and products within a balanced reaction. Stoichiometry for a given set of components comprising a reaction establish the thermodynamics of the system. For example, consider the chemical oxidation of glucose,

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O \qquad \mathbf{r} \qquad (1)$$

which states that 1 mole of glucose reacts with 6 moles of oxygen to form 6 moles of carbon dioxide and water. Mathematically, a reaction stoichiometry for N number of reaction components M can be expressed as,

$$\sum_{i=1}^{N} \mathbf{a}_i \mathbf{M}_i = 0 \tag{2}$$

where a_i is the stoichoimetric coefficient of the i-th component and M_i is component i. The convention is that stoichiometric coefficients, $\mathbf{a}_i$, for reactants have negative values while products have positive coefficients - *e.g.*, in Eqn. (1), the coefficients for glucose, oxygen, carbon dioxide, and water are -1, -6, +6, and +6, respectively. The term **r** in Eqn. (1) is the rate of reaction and has units [moles of reaction/volume-time]. Stoichiometry allows one to relate the rates of appearance of all components to each other as follows,

$$\mathbf{r} = \frac{\mathbf{r}_1}{\mathbf{a}_1} = \frac{\mathbf{r}_2}{\mathbf{a}_2} = \ldots = \frac{\mathbf{r}_i}{\mathbf{a}_i} \tag{3}$$

where r_1 = the rate of appearance of component 1, and so on.

The other portion of the process rate is the reaction rate itself. While stoichiometry relates to the molar ratios of components in a transformation, kinetics refers to how fast the transformation occurs. If a reaction rate is *homogeneous*, it is said to occur uniformly throughout the reaction volume V and is expressed as the number of moles reacted per unit volume per time. *Heterogeneous* reactions occur at the interface between two phases and do not occur uniformly throughout the reaction volume; heterogeneous reaction rates are more commonly expressed on a per unit surface area or per unit weight of reacting surface. Rate expressions are mathematical functions that relate the reaction rate to basic fundamental parameters, - *e.g.*,

$$\mathbf{r} = f(\text{temperature, concentration, etc.})$$

Typically, chemical reactions are described assuming this function can be written as the product of a number of separate functions,

$$\mathbf{r} = f_1(\text{temperature}) f_2(M_1, M_2, \ldots M_i)$$

For example, in chemical kinetics, the most common approach is the power-law expression where,

$$\mathbf{r} = k\,(M_1)^w\,(M_2)^x \ldots (M_i)^z \tag{4}$$

and k is a function of temperature only. The exponents in Eqn. (4) are referred to as the *orders* of the components in the reaction where in this example,

$$\mathbf{r} = k\,C^1 D^{0.5} E^{-2} \tag{5}$$

the reaction rate is first order in component C and half order in component D. The reaction rate **r** varies with the concentration of E to the -2 power.

It is critical to further discussions that the distinction between a process rate and a system rate be clear. Consider in Figure 2, a mass balance on component C that reacts in a reaction vol-

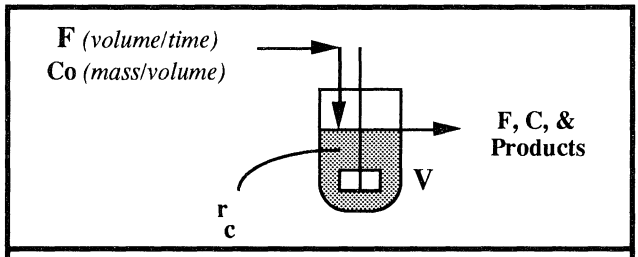


Figure 2. Mass Balance for C over an Ideal Continuously Fed Stirred Tank Reactor

ume V receiving a continuous supply of reactant C at an inlet concentration C_0. A homogeneous reaction $a_C C \rightarrow a_D D$ occurs in the reactor and its rate expression can be assumed as, $r_C = a_C k C^x$. It is assumed that mixing in the volume is sufficient to eliminate any concentration gradients. A mass balance on the reactor, illustrated in Figure 2, can be stated verbally as follows. . .

The Time Rate of Change of the Mass of Component C	=	**Net Transport Rate of Component C Into the Reactor**	+	**All Reaction Rates Producing or Component C**

or mathematically,

$$VdC/dt = F(C_0 - C) + r_C V \qquad (6)$$

Note that the first term in Eqn. (6) describes the rate of change of C in the system. The second term describes the rate component C is transported into and from the system. Both terms are system rates - *i.e.*, observable rates. The third term in the equation pertains to those process rates which describe how the reaction rate r changes as a function of the concentration C; if this rate expression truly represents the chemical or biological reaction, then it is valid irrespective of the system in which the reaction occurs. If the reaction rate, r_C, were a heterogeneous reaction rate written on a *per unit surface area* basis then the last term of Eqn. (6) would read instead, $r_C A$.

In the remaining sections of this paper we will present the various process rates that contribute to the net rate of change of biofilm mass or active cell concentration on a substratum. Those processes governing biofilm formation and persistence will be reviewed and rate expressions for each process discussed. Differences between structured and unstructured models of biofilm formation are presented detailed elsewhere in this volume by Bryers in the chapter on mixed population biofilms.

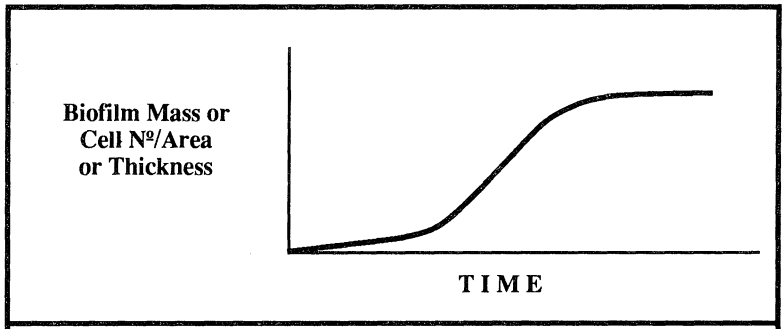


Figure 3. Biofilm net accumulation as a function of time.

3. Net Biofilm Accumulation

Figure 3 illustrates, irrespective of the analytical measure, the observed surface concentration of biofilm at a substratum as a function of time. Unfortunately, this type of data allows an estimate of only the rate of biofilm accumulating at the surface (*i.e.*, the slope of the curve); such a rate is a *system rate* or *rate of change*. Unfortunately, all too often, rates from such data are used to predict the stoichiometry and kinetics of the fundamental processes that contribute to the observed accumulation. In this section, we will describe the individual processes that collectively affect biofilm formation and provide the most current rate expression that most accurately describes that process.

Biofilm accumulation (Figure 4) is considered the net result of the

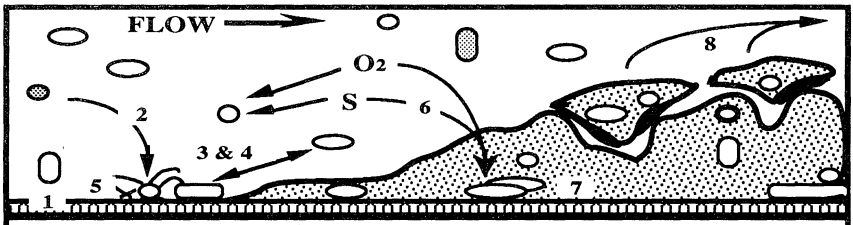


Figure 4. Fundamental processes governing biofilm formation. Definition of individual processes are given in the text below.

following physical, chemical and biological processes:

1. Biasing or pre-conditioning of the substratum either by macromolecules present in the bulk liquid or intentionally, as in the case of pre-coating endoprosthetic biomaterials with adhesion molecules (*e.g.*, fibronectin, vitronectin, fibrinogen, von Willebrand's factor).

2. Transport of planktonic cells from the bulk liquid to the substratum.

3. Reversible adsorption of cells at the substratum for a finite time.

4. Desorption is the release of reversibly adsorbed cells due fluid shear forces.

5. Irreversible adsorption is where cells remain permanent adsorbed to the surface.

6. Substrate metabolism by the attached cells.

7. Cell growth, replication, and extracellular polymer production.

8. Detachment of biofilm material can be either continuous, as in the case of erosion brought on by shear forces, or random, as in the case of biofilm sloughing.

If all of these processes occur in series, then the slowest rate process in the sequence will exert the greatest influence and limit the overall accumulation of biofilm. This slowest process is the *rate-limiting step* in that the overall accumulation of biofilm can go no faster than this slowest rate. Identifying the rate limiting step in a collage of processes such as biofilm formation is critical to data interpretation and potential scale-up. For example, if the goal is to derive the kinetics of cell adhesion but the system is *rate-limited* by the step of cell transport to the substratum, then any resultant kinetic rate expression for cell adhesion would be erroneous. Process analysis methods provide the protocol to analyze complex phenomenon as the summation of individual rates.

3.1 DEPOSITION PROCESSES

Process rates 1-5 are often grouped in mathematical models as single process termed "deposition". However, correlating "deposition rates" to system parameters, such as velocity or planktonic cell concentrations, is not sound *process analysis*.

3.1.1. *Pre-conditioning of the Substratum.* In the case of intentional pre-treatment of a surface, (*i.e.*, adhesion molecules for biomaterials), there is no rate to considered; the substratum essentially enters the system with a biased surface chemistry. However, in cases where a "clean" substratum material is exposed to an aqueous environment, transport of dissolved organic molecules or macromolecules in laminar flow is basically by molecular diffusion; in turbulent flow, transport must also consider eddy diffusion effects. Once at the surface, adsorption of macromolecules occurs almost instantaneously, immediately changing the surface chemistry of the exposed material. Loeb & Neihof (1975) and Depalma *et al.* (1979) have measured adsorption rates of organic molecules to various solid substrata in seawater and Bryers (1980) has observed analogous adsorption rates in a freshwater laboratory system. The net rate of adsorption in these studies can be described as...

$$r_{adsorption} = k_a S_i[1 - S_i/k_s] \qquad (7)$$

where $r_{adsorption}$ = the rate of net adsorption ($ML^{-2}t^{-1}$); k_a = adsorption rate constant, (Lt^{-1}); k_s = surface saturation coefficient, (ML^{-2}); and S_i = areal concentration of adsorbed species, (ML^{-2}). While experiments indicate the maximum amount of adsorbed material may not exceed 0.10 μm in thickness, the surface properties resulting from adsorption of an organic film can significantly bias subsequent microbial events.

3.1.2. *Cellular Adsorption and Desorption.* Observations have indicated that cells can adsorb to a surface for a period of time and then may desorb from the substratum, returning to the bulk liquid. If cells spend sufficient time at the surface, they can become permanently bound, perhaps due to extracellular polymer production, and can only be removed by rather aggressive physical or chemical means. One can conceive of this overall process much like two reaction steps in series, with the reversible adsorption process first followed by an irreversible step pertaining to permanent adsorption,

$$\underset{\text{Suspended Cells}}{X} \leftrightarrow \underset{\text{Reversibly Adsorbed Cells}}{X_{rev}} \rightarrow \underset{\text{Irreversibly Adsorbed Cells}}{X_{irr}} \qquad (8)$$

Observation of the total amount of cells at a substratum at any one time comprises both reversibly and permanently adsorbed cells - *i.e.*, $X_t = X_{rev} + X_{irr}$.

The rigorous approach of Escher (1986) derives material balances, over the surface for each cell type, that include the rate of initial adsorption, the rate of desorption of reversibly attached cells, and the rate of transition from reversibly to permanently attached cells. For the experiments with *Pseudomonas aeruginosa* shown in Figure 5, Escher was able to estimate the individual rate dependencies for the three processes in question.

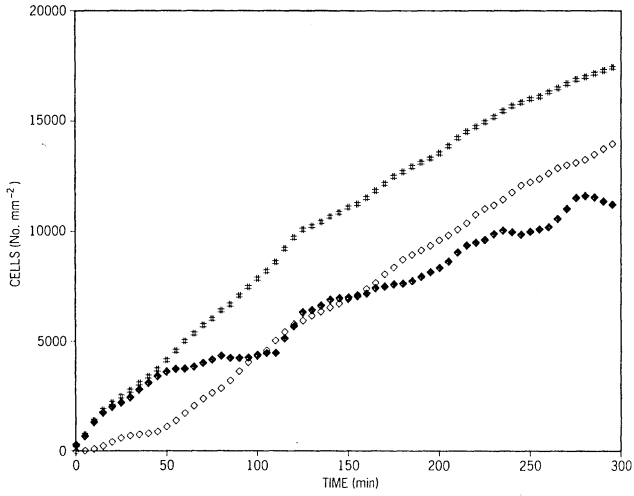


Figure 5. Progression of *Pseudomonas aeruginosa* colonization on smooth glass under laminar flow at a shear stress of 0.5 N m^{-2}. (#) sorption-related processes, desorption (◊), and (◆) net accumulation. Escher (1986).

The rate of cell adsorption (cell-$L^{-2}t^{-1}$) to the surface can be described as a first order dependency on the suspended cell concentration:

$$r_{X\text{-}ads} = k_{X\text{-}ads}X \qquad (9)$$

where $k_{X\text{-}ads}$ = cell adsorption rate constant (Lt^{-1}) and X = the suspended cell concentration (cell-L^{-3}). The adsorption rate constant, $k_{X\text{-}ads}$, is a strong function of the cell size, density difference between the cell and the bulk fluid, and the prevailing flow regime (laminar vs. turbulent). The rate of reversibly attached cell desorption, $r_{X\text{-}des}$, was found to be a discontinuous function; prior to a critical time, t_r, the rate was zero and after t_r, $r_{X\text{-}des}$, was proportional to $r_{X\text{-}ads}$. The rate of transition from reversibly to irreversibly attached cells is also a discontinuous function of time where the rate is zero prior to time t_r, and is a zero order function of the surface concentration of reversibly attached cells. From Escher's experiments, results indicate that the numbers of reversible attached cells per area is independent of time.

Another common tack is to define the actual net adsorption process, from X to X_{irr}, as a fraction of the maximum amount of cells that impacts the surface, using the concept of a *sticking efficiency*, α. The sticking efficiency is the ratio of the number of cells irreversibly attached to a substratum to the total number of cells transported to the target surface. It is often thought of as the probability that a cell, once transported to the substratum, will adsorb. Bowen *et al.* (1978) and Beal (1972) derive continuity equations describing the transport of suspended particles from a flowing fluid to the surfaces of a surrounding conduit that accounts for both convective and molecular transport mechanisms. From their work, the maximum flux of particles transported to the surface, L distance from the inlet, of a rectangular flow cell, with gap height 2h, can be written as

$$J \text{ (cells-L}^{-2}t^{-1}) = k_{tr} X \mathcal{D}_X / h \tag{10}$$

where k_{tr} = particle transport coefficient = $[(2/9)K^{0.33}/\Gamma(4/3)]$ and $K = (1/Pe)(8L/3h)$ where Pe = Peclet number = $4vh/\mathcal{D}_X$, and v = average velocity of the fluid, Γ = the gamma function ($\Gamma(4/3) = 0.89338$), $\mathcal{D}_X$ = the diffusivity of cells in the liquid. For non-motile cells, the Brownian diffusion coefficient can be estimated from the Stokes-Einstein equation,

$$\mathcal{D}_X = k_b T / 3\pi\mu d_c \tag{11}$$

where k_b = Boltzmann constant (1.38×10^{-23} J/°K), T = absolute temperature, μ = absolute viscosity, d_c = cell diameter. To compensate for cell motility, Jang and Yen (1985) propose the following equation,

$$\mathcal{D}_X = v_m d_r / 3(1 - \cos\sigma) \tag{12}$$

where v_m = velocity of motility, d_r = free length of a random run, and σ = the angle turned by the motile cell.

Rates of net adsorption are thus modeled as,

$$r_{X\text{-}dep} = \alpha J \tag{13}$$

3.1.3 *Attachment.* Attachment is the capture of particles from the fluid phase by the biofilm. This process differs from adsorption in that once cells are transported from the liquid the target surface is now a developed biofilm. The biofilm poses a different type of substratum

in that its surface morphology is generally more irregular (perhaps filamentous), very porous, more compliant, and gelatinous. Models of the rates of cell attachment simply adjust the value of the sticking efficiency α to reflect the "stickier" surface. However, recent experimental results indicate that the increased capture of cells by a biofilm-covered substratum may depend on far more subtle mechanisms than changes in mere surface morphology.

Banks (1989) and Banks & Bryers (1992) report rates of cell deposition increased when cells were exposed to biofilm surfaces versus clean glass

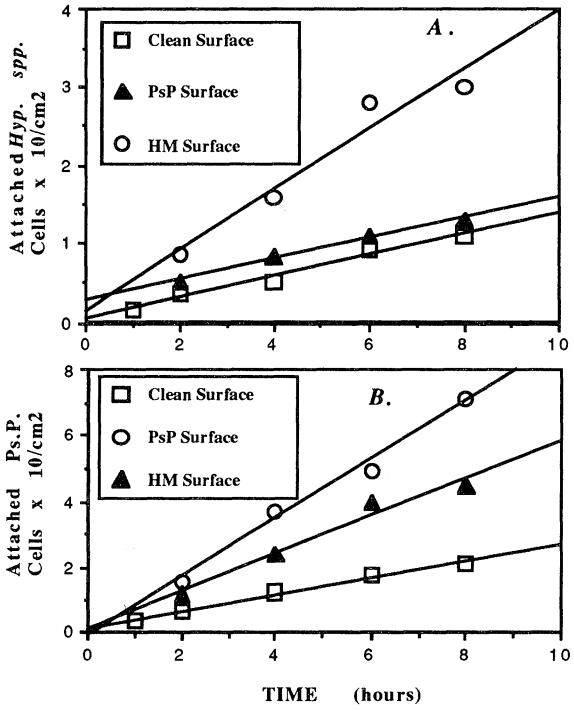


Figure 6. **(A)** Attachment of *Hyphomicrobium spp.* to various surfaces. Suspended cell concentration = 4.5E+07 cells/mL. **(B)** Attachment of *Pseudomonas putida* to various surfaces. Suspended cell concentration = 1.5E+08 cells/mL.

(Figures 6 A and B). Two species were investigated, *Pseudomonas putida* and *Hyphomicrobium ZV620* as to their deposition rates, under laminar flow conditions, onto both clean glass and biofilm-covered surfaces. *Pseudomonas putida* cells attached to a *Pseudomonas putida* biofilm at a constant rate that was zero order in attached cell concentration (0.088 cell $cm^{-2}hr^{-1}$) that was 3.6x higher than to clean glass. *Pseudomonas putida* cells attached to a *Hyphomicrobium* biofilm at a zero order in attached cell concentration (0.058 cell $cm^{-2}hr^{-1}$) that was 2.4x higher than to clean glass. *Hyphomicrobium* illustrated an attachment rate (0.041 cell $cm^{-2}hr^{-1}$) to a

Hyphomicrobium biofilm at a zero dependency on attached cells that was 3.3x higher than to clean glass. The attachment rate of *Hyphomicrobium spp.* cells to a *Pseudomonas putida* biofilm (0.016 cell $cm^{-2}hr^{-1}$) was only 1.3x that to clean glass. The attachment rate of *Hyphomicrobium cells to a Hyphomicrobium* biofilm was about half of the same rate of *Pseudomonas* cells attaching to a *Pseudomonas* biofilm, even though suspended *Pseudomonas* cell concentrations were three times higher. Results insinuate that species-dependent enhancement of cell attachment may occur by specific rather than non-specific adhesion mechanisms (Hammer & Lauffenburger, 1987).

3.2 TRANSFORMATION PROCESSES

Transformations are processes in which molecular rearrangements occur *i.e.*, reactions. Once a cell adsorbs to a surface or becomes attached to a biofilm, it will continue its metabolic processes in response to its immediate environment. Four fundamental rate processes can be identified: cellular growth and replication, product formation, endogenous decay or maintenance, and cell death and lysis. Historically, it has been easier to measure per reactor area observed transformation rates in the bulk liquid (*e.g.*, electron donor removal rate, electron acceptor uptake rate, total biofilm mass accumulation) than to measure growth, replication, and death of cells in a biofilm. This led to the development of a number of unstructured models that paid little attention to the various biofilm component parts (viable cells, total cells, extracellular polymer) or that ignore the time course of biofilm development altogether. These latter, steady-state models (Harremoës, 1971; Tanaka & Dunn, 1982) were useful at the time in estimating the flux of growth rate limiting substrate into a biofilm of fixed thickness, density, and reactivity, taking into account both internal molecular diffusion and biological reaction.

The major transformation carried out by cells in the biofilm is the metabolism of both the electron donor and acceptor to produce soluble by-products, extracellular polymers, carbon dioxide, and water. Depending on the microbial population in question and the ambient concentration of electron donor and acceptor, a biofilm can be either aerobic, anoxic (denitrifying), anaerobic (sulfate reducing bacteria, methane formers), or fermentative. In simple unstructured models, a single biological "growth rate of the biofilm", $r_B(M_X\text{-}L^{-2}t^{-1})$, relates cell growth to substrate uptake rate, r_S $(M_S\text{-}L^{-2}t^{-1})$, using a single yield coefficient, Y $(M_X\text{-}M_S^{-1})$,

$$r_S = r_B/Y \tag{14}$$

where growth rate of biomass is assumed to follow a saturation kinetic dependency on substrate concentration,

$$r_B = \mu^*SB/(K_S + S) \tag{15}$$

Said models do not take into account multiple species participating in different biological reactions, competition for the same substrate(s), or spatial gradients in substrate or cell concentration in the biofilm. For example, in the case of an anaerobic methane producing culture, one could add structure to a biofilm model by considering the various different populations involved (particulate degraders, acetic acid formers, methanogens) each with their own individual growth rate expression similar to Eqn. (15).

Analysis of biofilm bacterial metabolic rates are frequently complicated by the effects of significant mass transfer resistances in both the liquid phase and within the developing biofilm. The steady-state models (Harremoës; 1977, 1978) are useful to estimate the observed flux of growth rate limiting substrate (M_S-$L^{-2}t^{-1}$) into a biofilm of fixed thickness, density, and reactivity, taking into account both external and internal molecular diffusion coupled with a simultaneous biological reaction rate. Based on identical models for one dimensional heterogeneous catalysis, these models assume a constant biofilm concentration B (tacitly implying a spatially uniform reactivity) and diffusion path (biofilm thickness) which allows one to predict (a) the concentration profile of limiting substrate (and by stoichiometry all other nutrients) with biofilm depth and (b) the maximum substrate uptake or flux to the biofilm.

The ratio of the reaction rate observed under practical system conditions (possibly controlled by mass transfer effects) to the true instrinsic reaction rate evaluated at system conditions assuming no mass transfer effects is defined as the *effectiveness factor* for the solid (or biofilm) in question. Models by Harremoës (1977, 1978) and Vos *et al.* (1990) of immobilized cell or biofilm kinetic studies define the effectiveness factor as a function of a dimensionless group of pertinent observable system parameters. Such models define a reacting geometry (one dimensional slab, cylinder, or sphere) with uniform distribution of the biological catalyst through out the reacting volume.

For a one dimensional biofilm of thickness L and uniform areal biomass concentration B, metabolizing a growth limiting substrate S, according to a biological reaction r_g, the second order differential equation describing the concentration of S in the biofilm as a function of position is . . .

$$\mathcal{D}_e\,(d^2S(z)/dz^2) = r_g \tag{16}$$

where $\mathcal{D}_e$ = the effective diffusivity of S in the biofilm. Solution to Eqn. (16) for S as a function of the spatial coordinate z, depends upon specific system boundary conditions and the particular reaction rate dependency on local substrate concentration (*i.e.*, first or second order, or saturation kinetics). For the case of saturation kinetics, one can define the following dimensionless variables . . .

$$\kappa = S_B/K_S \quad S^* = S/S_B \quad z^* = z/L \tag{17}$$

thus rewriting Eqn. (16) as,

$$d^2S^*/d(z^*)^2 = \theta^2\, S^*/(1 + \kappa S^*) \tag{18}$$

where

$$\theta = \text{Thiele Modulus} = \left\{A_S\mu^*X_iL^2/Y_iK_S\mathcal{D}_e\right\}^{0.5} \tag{19}$$

and A_S is the surface area to volume ratio, μ^* is the maximum growth constant for the microorganism, X_i is the areal concentration of microorganisms, and K_S is the saturation constant in the growth rate expression.

Boundary conditions for the dimensionless Eqn. (18) are correspondingly,

$$S^* = 1 \quad \text{at} \quad z^* = 1$$
$$dS^*/dz^* = 0 \quad \text{at} \quad z^* = 0$$

Once the specific substrate profile is determined, the effectiveness factor can be derived according to its definition . . .

$$\eta = \frac{\text{rate observed with mass transfer}}{\text{true reaction rate without mass transfer effects}},$$

$$\eta = r_{obs}/r_{true} = \left[-\mathcal{D}_e \, dS/dz\right]_{|z=L} / \left\{-A_S \mu^* X_i L^2 S_B/(S_B + K_S)\right\} \tag{20}$$

or in dimensionless terms =

$$\eta = [(1 + \kappa)/\theta^2] \, dS^*/dz^*_{|z^*=1} \tag{21}$$

The most convenient means of depicting the influence of operating parameters on the effectiveness factor was presented by Pitcher (1975) using a modified Thiele modulus defined as,

$$\theta_P = [\theta\kappa/(1 + \kappa)] \; [2\kappa - 2ln(1 + \kappa)]^{0.5} \tag{22}$$

Classically, in heterogeneous enzyme catalysis or in steady-state biofilm models, the above derivation holds since the concentration of surface-bound catalyst (*i.e.*, enzyme or biofilm bacteria) and the diffusion path (*i.e.*, immobilized enzyme support or biofilm thickness) are assumed constant in time. Also, the reaction rate constant μ^* is assumed to pertain to one substrate conversion process.

Modifications have been made in this approach to estimate the *unsteady-state* flux of multiple growth rate limiting substrates in several biological reactions in series. Tanaka & Dunn (1982) derive four unsteady state substrate balances (ammonia, nitrite, nitrate, and oxygen) for autotrophic nitrification. This system of equations assumes a constant biofilm thickness and that the concentration of the two bacterial species involved is constant in both time and space in order to solve the set of coupled partial differential equations. A similar, steady-state model of denitrification of nitrate to nitrite then to nitrogen was presented in Boaventura & Rodrigues (1988) and Droste and Kennedy (1986). Here two separate effectiveness factors and corresponding Thiele moduli are defined for nitrate and nitrite conversion but no distinction between bacterial species is made; in essence, a uniform biofilm density is assumed constant. Unfortunately, no work has considered evaluation of an effectiveness factor for either the case of an unsteady-state biofilm thickness or time dependent concentration profiles of different bacterial species in the biofilm.

With estimates of the appropriate diffusion coefficients, a Thiele modulus and its corresponding effectiveness factor can be evaluated as biofilm thickness changes in order modify Eqn. (15) accordingly, *i.e.*,

$$r_B = \eta \; \mu^* SB/(K_S + S) \tag{23}$$

In an unstructured model, biofilm surface concentration, B, is a lumped parameter representing both biofilm bound viable cells, X_B, and extracellular polymers, EP. Extracellular polysaccharides and lipoproteins comprise the EP product pool that establishes the gelatinous matrix of the biofilm. Product formation, especially EP, is critically important in interpreting biomass yield in a biofilm system. Neglecting the amount of exogenous substrate transformed by a cell into EP will lead to an overestimation of cellular yields. Consequently, in a structured model of the biofilm formation, material balances on X_B, EP, and the limiting substrate, S would be necessary as reported by Robinson *et al.* (1984) and Turakhia and Characklis (1988). Product formation rate, r_{EP} ($M_{EP}M_X^{-1}t^{-1}$), is frequently modeled as two production terms; one growth associated production rate and one non-growth associated, traditionally term a Leudeking-Piret equation:

$$r_{EP} = k_g r_B + k_{ng} \tag{24}$$

where k_g = growth associated EP coefficient ($M_PM_X^{-1}$) and k_{ng} = non-growth associated EP coefficient ($M_{EP}M_X^{-1}t^{-1}$). Here, r_B must be modified, substituting the active biomass component X_B in place of B.

Microorganisms persist in environments that are low in essential nutrients and carbon sources. Most studies of microbial growth under oligotrophic environments observe decreasing cellular yields with decreasing growth rate. As either the limiting exogenous substrate drops below a certain value or the growth rate is low, cells turn to endogenous sources of energy - *i.e.*, maintenance. Should exogenous substrate deprevation persist for a prolonged period, the cell may cease to be metabolically active and no longer replicate; eventually the cell membrane deteriorates and lysis of the cell occurs. Collectively, these processes all detract from the net production of viable cell mass in the culture appearing as a decrease in the observed yield.

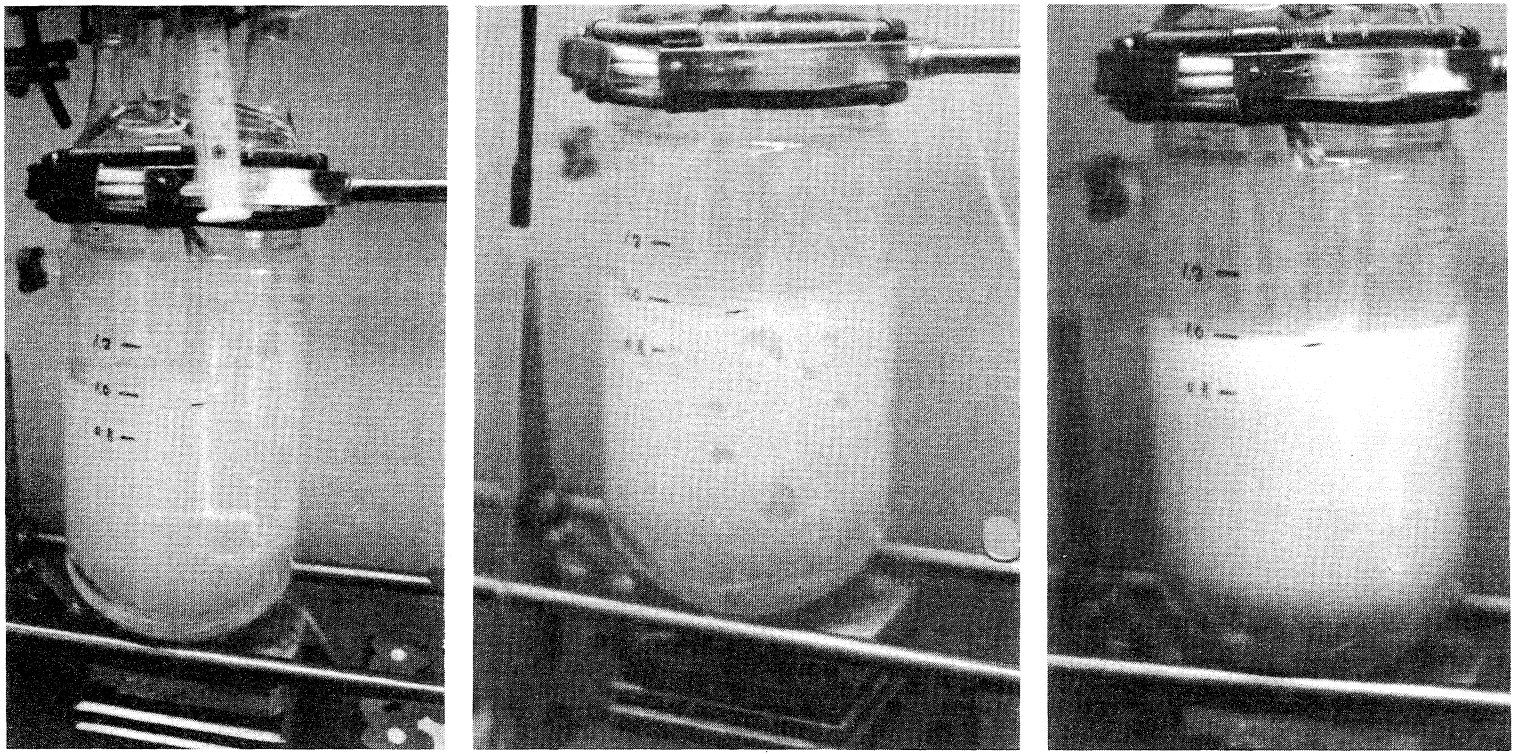
In a modeling context, the processes of maintenance, death, lysis have traditionally been lumped into a single "decay" rate that is a first order function of the amount of cell mass (either suspended or in the biofilm). Only a few researchers (Mason *et al.* 1986a,b; Banks & Bryers, 1990) have actually developed a tested structured models of microbial growth that account for the individual processes comprising decay. The most direct way to account for decay in a biofilm cell growth term is to redefine Eqn. (15) as a **net** cell growth term where,

$$r_B = [\mu^*SB/(K_S + S)] - [k_{decay}B] \tag{25}$$

where k_{decay} = endogenous decay rate constant (t^{-1}).

3.3 BIOFILM REMOVAL PROCESSES

Biofilm removal processes involve the actual loss of material from the biofilm; either cells or cell:biofilm matrix debris. Desorption (Section 3.1.2) is the release of cells from the substratum and occurs from the onset of adsorption. Biofilm detachment or removal represents loss of biomass from the biofilm and arise by way of three distinctively different mechanisms: erosion, sloughing, or abrasion.



A. Initial Biofilm Formation B. Isolated Sloughing of Biofilm C. Entire Sloughing of Biofilm

Figure 7. Sequence of biofilm formation and eventual sloughing in an anaerobic continuous bacterial culture.

3.3.1 *Abrasion.* Abrasion is the loss of biofilm due to repeat collisions between substratum particles as seen in fluidized bed reactors or in heat exchangers using cooling water of high solids content. To date, there does not exist a rate expression to describe the rate of biofilm removal by abrasion.

3.3.2. *Erosion.* Erosion is the continuous loss of biomass or cell prodigy from the upper layers of a biofilm. The rate of biofilm removal by erosion is modeled as a first order function of either biofilm amount, $r_{ero} = k_{ero}B$, where the erosion removal rate constant, k_{ero}, is a strong function of the prevailing hydraulic shear. Trulear and Characklis (1982) observed that biofilm erosion rates increased with fluid shear and biofilm amount. Applegate and Bryers (1991) reported that the growth conditions of the biofilm strongly affected biofilm removal processes. Stoichiometric limitations of carbon substrate affected biofilms that contained less EP, bound less calcium ion, and exhibited a higher erosion rate (maximum removal rate = 1.05 gm biomass carbon m^{-2} day^{-1}) than oxygen-limited biofilms that accumulated more EP, bound more calcium ions, developed rigid morphology that was very resistant to shear (maximum removal rate = 0.4 gm biomass carbon m^{-2} day^{-1}) prior to sloughing.

3.3.3. *Sloughing.* The catastrophic, apparently random, loss of large pieces or entire sections biofilm is known as sloughing. In attempting to attain a steady-state biofilm amount, the entire sloughing of a biofilm, Figure 7, can come as quite an embarrassment. All too often, random sloughing of a biofilm terminates an experiment which has had the result of yielding very little quantitative data regarding the causes of sloughing. A number of situation specific causes of sloughing have been identified: bubble formation in either anaerobic methane producing biofilm, nitrogen bubble formation in denitrifying biofilm, and artificially induced sloughing by way of calcium chelation by EGTA addition. Applegate and Bryers (1991) reported that the growth conditions of the biofilm strongly affected the biofilm removal processes of erosion and sloughing. The "fluffier" biofilm produced under carbon limited growth exhibited high erosion rates but never sloughed even when subjected to over 300 hours of nutrient starvation. Conversely, rigid biofilms cultivated under oxygen limitations showed little to no erosion but the onset of a catastrophic slough was predictable and repeatable.

4. Summary

This paper presents concepts of process analysis and mathematical modeling as applied to biofilm processes. Examples of both structured and unstructured mathematical analysis of biofilm formation and activity are given. Distinctions between homogeneous and heterogeneous reaction systems are made and errors associated with ignoring mass transfer limitations of biofilm reactions are presented. Our overall goal was to present the utility and limits of mathematical modeling of biological systems and to provide a methodology with which to systematically interpret complex biological processes such as biofilm formation.

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