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THEORETICAL AND EXPERIMENTAL ANALYSIS OF A *PSEUDOMONAS AERUGINOSA* BIOFILM

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Biofilms are biologically active matrices of cells and noncellular material accumulated on solid surfaces. Predicting the rate and extent of biofilm processes would be useful in ecosystem analysis, design and operation of industrial equipment subject to fouling, wastewater treatment plant design and operation, and determining the feasibility of biofilm reactors for biotechnological applications. The major impetus for modelling monopopulation biofilms in our laboratory was the need to elucidate the fundamental processes contributing to biofilm accumulation and persistence without the confounding factors introduced by population dynamics and interactions.

SYSTEM AND PROCESS DESCRIPTION

The subject biofilm consists of two components: Pseudomonas aeruginosa (PA) cells and their extracellular polymeric substances (EPS). The biofilm was observed to be relatively compact with an average thickness ≤ 40 μm in most cases. The soluble substrate, glucose, was the rate-limiting nutrient and sole carbon and energy source. Substrate was supplied continuously to the biofilm in the overlying turbulent water phase of a RotoTorque reactor, a continuous stirred tank reactor described elsewhere (Bakke et al, 1984). Results from chemostat (Robinson et al, 1984) and RotoTorque (Bakke et al, 1984) experiments with P. aeruginosa have produced estimates of stoichiometric and rate coefficients for fundamental processes (Table 1). The components considered in this model were bulk water substrate concentration, S, bulk water cellular concentration, X, bulk water EPS concentration, P, biofilm cellular density, X_b, and biofilm EPS density, P_b. The net transport into the reactor is a result of bulk water flow through the reactor. Processes considered by the model include 1) advective transport of substrate, cells, and products into and out of the bulk water, 2) advective and diffusive transport of substrate into the biofilm, 3) attachment and detachment (erosion) of cells and polymeric products between the bulk water and the substratum and 4) cellular reproduction and product formation in the bulk water and in the biofilm. The model predicts progression of substrate removal, cellular accumulation, and extracellular polymer accumulation in the bulk water and in the biofilm.

Table 1. Kinetic and stoichiometric coefficients for aerobic glucose metabolism by P. aeruginosa in a chemostat (Robinson et al, 1984).

K_S	= 2.0 g glucose carbon/m^3
μ_{max}	= 0.4 h^{-1}
$Y_{X/S}$	= 0.34 g cell carbon/g glucose carbon
$Y_{P/S}$	= 0.56 g EPS carbon/g glucose carbon
k	= 0.27 g EPS carbon/g cell carbon
k'	= 0.035 g EPS carbon/g cell carbon h

STOICHIOMETRY

Carbon, hydrogen, and nitrogen content of the PA biofilm is 53.1% C, 6.1% H, and 12.4% N of volatile solids so biofilm elemental composition is $CH_{1.4}O_{0.4}N_{0.2}$ (Turakhia and Characklis, 1988). Cellular mass yield ($Y_{X/S}$) was between 0.30 - 0.34 g cell C/glucose C. EPS mass yield ($Y_{P/S}$) was approximately 0.56 g EPS C/g glucose C. Observed biomass (cells plus EPS) yield was approximately 0.61 g biomass C/g glucose C. Turakhia (1986) established the oxygen requirements as 1.1 g oxygen/g glucose C. Thus, the stoichiometry of the aerobic degradation of glucose by PA is described by the following:

$$C_6H_{12}O_6 + 2.5\ O_2 + 0.7\ NH_3 \longrightarrow 3.5\ CH_{1.4}O_{0.4}N_{0.2} + 2.5\ CO_2 + 4.6\ H_2O \quad [1]$$

KINETICS

Specific growth rate was presumed to be a saturation function of reactor substrate concentration:

$$\mu = \mu_{max} / (K_S + S) \quad [2]$$

If significant diffusional resistance exists, the specific growth rate expression must be modified. Substrate concentration decreases with biofilm depth due to transformation processes. The average substrate concentration in the biofilm is, therefore, less than S, which results in a specific substrate consumption rate lower in the biofilm than in the bulk liquid phase. Atkinson (1974) derived an effectiveness factor, η_D, based on the Thiele modulus, to describe the influence of diffusional resistance in a biofilm. $\eta_D = 1$ if no diffusional resistance exists (e.g., as may occur in the bulk liquid). Trulear (1983) used the relationships derived by Atkinson and determined that $\eta_D \geq 0.9$ for a PA biofilm. Turakhia (1986) also observed insignificant diffusional resistance in PA biofilms.

Specific EPS formation rate is a linear function of specific growth rate:

$$q_p = k\ \mu + k' \quad [3]$$

Trulear (1983) observed a negligible amount of soluble organic products in his aerobic experiments with PA.

Specific detachment (erosion) rate from the biofilm is presumed independent of substrate load, growth rate, and dilution rate. Data suggest that specific detachment rate is a function of biofilm mass. The model assumes that detachment rate is first order with respect to biofilm cellular concentration:

$$q_{dX} = k_{dX}\ X_f \quad [4]$$

and specific EPS detachment rate is first order with respect to EPS mass:

$$q_{dP} = k_{dP}\ P_f \quad [5]$$

Detachment (erosion) rate may also be related to the hydrodynamic shear force at the biofilm-water interface, τ (Bakke *et al*, 1984):

$$q_d = \alpha\ v^{7/4} \quad [6]$$

where α is a coefficient characteristic of the turbulent flow in the RotoTorque. This equation adequately describes the data of Trulear and Characklis (1982) for total (cells and EPS) biofilm detachment rate, q_d, for a mixed culture.

Intuitively, cellular detachment rate should depend on biofilm cell concentration. However, limited observations for a PA biofilm (Bakke *et al.*, 1984) indicate that q_{dX} is independent of X_f. It is likely that specific cellular detachment rate does depend on substrate loading since detachment rate equals growth rate at steady state. Then in those systems where substrate loading rate is proportional to velocity (plug flow reactor with no recycle), the observed dependence of detachment on velocity may be due to growth processes.

MATERIAL BALANCES

Complete unsteady state material balances and constitutive equations for this biofilm system are derived by Bakke *et al* (1984). At steady state, all derivatives with respect to

time are zero. In addition, since RotoTorque experiments were conducted with a dilution rate significantly greater than μ_{max} ($D = 6\ h^{-1}$), growth and product formation in the bulk water phase were negligible.

Substrate carbon

$$D\ (S_i - S)/(X_f\ A) = \mu \left[1/Y_{X/S} + k/Y_{P/S}\right] + k'Y_{P/S} \quad [7]$$

where $D\ (S_i - S)/(X_f\ A)$ is specific substrate removal rate in the biofilm, q_S. Yield coefficients represent process (as opposed to observed) stoichiometries for PA and are presumed constant. Then, Eq. 7 predicts a linear relation between q_S and μ. Experimental results indicate that q_S is a linear function of μ (Figure 1) in a chemostat with PA. The results from a PA biofilm are consistent with the chemostat data and have been reproduced by Siebel (1987). The results indicate that the energy metabolism of PA in a biofilm state is the same as in the planktonic state.

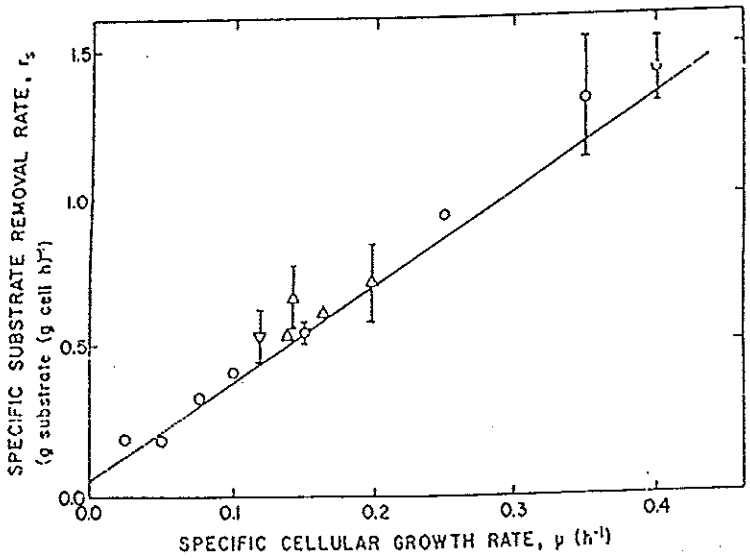

Fig. 1. Relationship between steady state specific substrate removal rate and specific growth rate of P. aeruginosa in a chemostat (o) and in a biofilm (▲, $D = 6h^{-1}$; ▼, $D = 3\ h^{-1}$) (Bakke et al., 1984).

Suspended cell carbon

$$X = X_f\ q_{dX}\ A/(D\ V) \quad [8]$$

Suspended product carbon

$$P = P_f\ q_{dP}\ A/\ (D\ V) \quad [9]$$

Biofilm cell carbon

$$\mu = q_{dX} \quad [10]$$

Biofilm polymer carbon

$$q_p\ X_f = q_{dP}\ P_f \quad [11]$$

Thus, production rate (cells and EPS) equal detachment rate at steady state.

SPATIAL DISTRIBUTION OF BIOFILM COMPONENTS

A homogeneous distribution of cells and EPS in both the bulk liquid and the biofilm phase is assumed. Transmission electron micrographs of a PA biofilm at steady state, suggests a cell distribution somewhere between uniform and random. Biofilm thickness is also presumed to be

uniform. Bakke (1986), however, has observed significant changes in PA biofilm morphology with time in a laminar flow system. In the early stages of biofilm accumulation, the variation in biofilm thickness was relatively small. As the biofilm matured, "channels" formed in the biofilm and the variation in film thickness became significant. A continuous increase in the variation in biofilm thickness was observed in these experiments and reflects changing biofilm morphology. The increasing "roughness" of the biofilm undoubtedly influences mass transport at the biofilm-water interface. The morphology differences in turbulent flow may also be significant.

DENSITY OF BIOFILM COMPONENTS

Constant cellular and EPS volumetric densities are assumed for the biofilm. Consider the following relationship between biofilm thickness and biofilm density:

$$X_f = p_f L_f \quad [12]$$

where p_f = biofilm volumetric density (M L^{-3}), L_f = biofilm thickness (L), X_f = biofilm areal density (M L^{-2}). Bakke (1986) has shown (in laminar flow) that thickness of a PA biofilm reaches a steady state very rapidly while cell density continues to increase (Figure 2). Then, the time derivative of X_f must be modified as follows using Eq. 12:

$$d X_f/dt = L_f (d p_f/dt) + p_f (d L_f/dt) \quad [13]$$

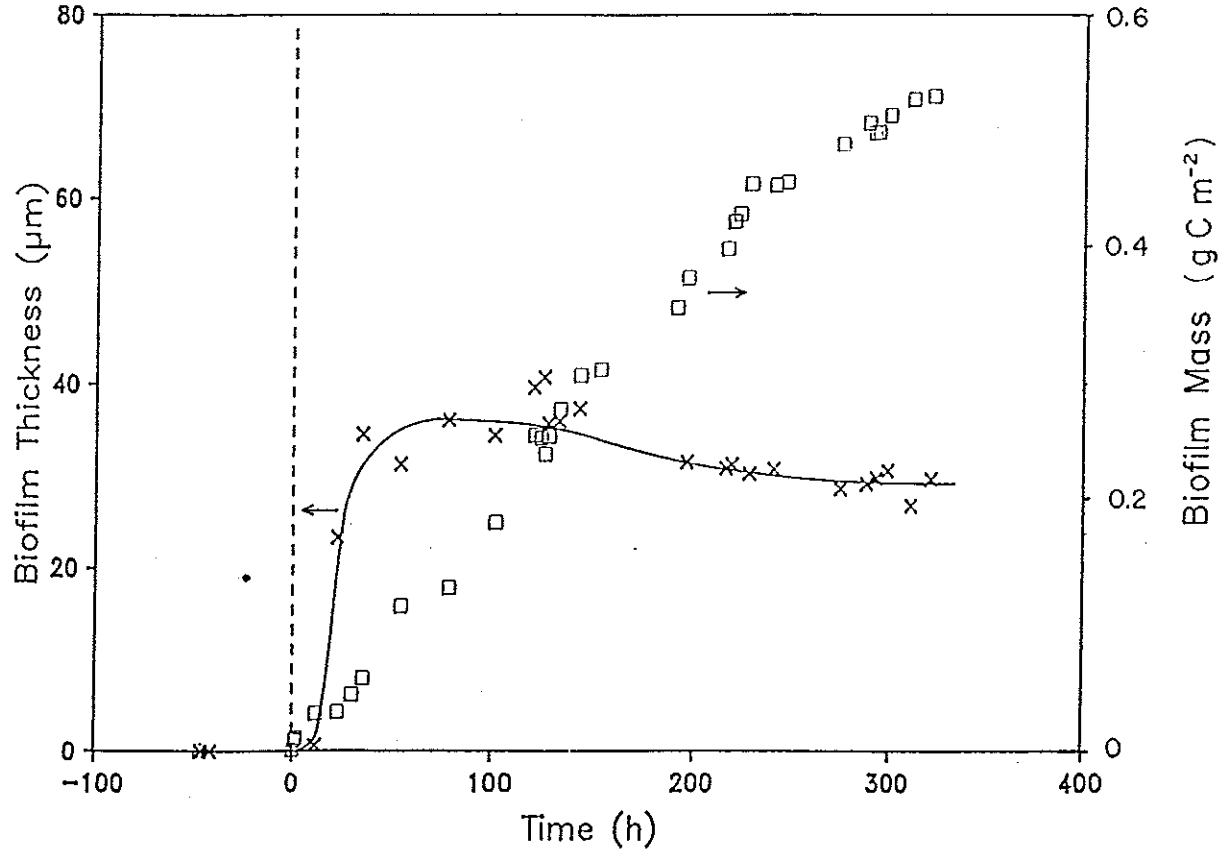

Fig. 2. The progression of biofilm thickness and biofilm cellular density in a rectangular duct reactor with laminar flow (Bakke, 1986).

Then using Figure 2 as an illustration, Eq. 13 is valid through approximately 40 h of the biofilm progression. After 40 h, Eq. 13 reduces to the following:

$$L_f (d p_f/dt) = \mu p_f L_f - q_d \quad [14]$$

In undefined mixed populations, density in the biofilms has been observed to increase with increasing biofilm depth.

COMPARISON OF MODEL AND EXPERIMENT

The kinetic and stoichiometric coefficients used in the computer simulation were derived from experimental apparatus different from the experiments conducted in the RotoTorque reactors (Bakke et al., 1984). Adsorption coefficients were determined by Nelson et al., (1985) in a turbulent CFSTR. Trulear (1983) and Robinson et al. (1984) determined growth and product formation coefficients in chemostat experiments. Detachment coefficients were derived from steady state biofilm data (Trulear, 1983; Bakke et al., 1984). Thus, coefficients were obtained from a distinct set of experimental systems and were used to predict biofilm behavior in the RT system. Experimentally observed biofilm cell and EPS concentrations are compared to model predictions in Figure 3. Based on the model, the influence of system variables on progression and steady state behavior in bioreactors can be predicted.

Operation of bioreactors can be controlled by regulating cell, substrate, and hydraulic loading rates through regulation of S_i and/or D. The influence of these parameters on biofilm cell progression is illustrated in Figures 4 and 5. Biofilm cell numbers are presented on a logarithmic scale to increase the range of S_i and D presented and to emphasize the subtle effect of the initial events in biofilm accumulation. Polymer and suspended cell concentration progressions have similar shape to that for X_f.

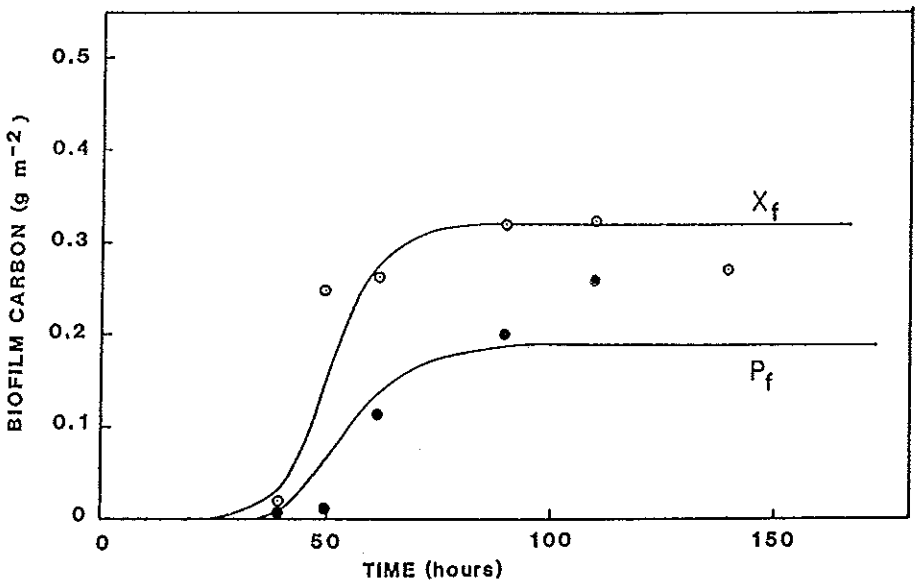

Fig. 3. Predicted and observed progression of P. aeruginosa biofilm cell (X_f) and biofilm EPS (P_f) carbon in a turbulent RotoTorque reactor (Trulear, 1983).

Influent substrate concentration, S_i, influences both the rate and extent of biofilm accumulation (Figure 4). Rate of biofilm cell accumulation increases with increasing S_i at constant X_i and D. Substrate loading rate is proportional to S_i for constant D. Dilution rate also affects both rate and extent of biofilm accumulation (Figure 5). The range of substrate loading rates simulated are the same in Figures 4 and 5. The resulting plateau biofilm accumulation is the same for a given loading rate in both illustrations. The progression, on the other hand, is significantly influenced by the nature of the loading rate. For example, the biofilm cell progression for $S_i = 1$ in Figure 4 requires four times longer to reach "plateau" than the progression for $D = 1$ in Figure 5, even though substrate loading is the same. The initial phase of the biofilm accumulation is not influenced as much by changing D as by changing S_i.

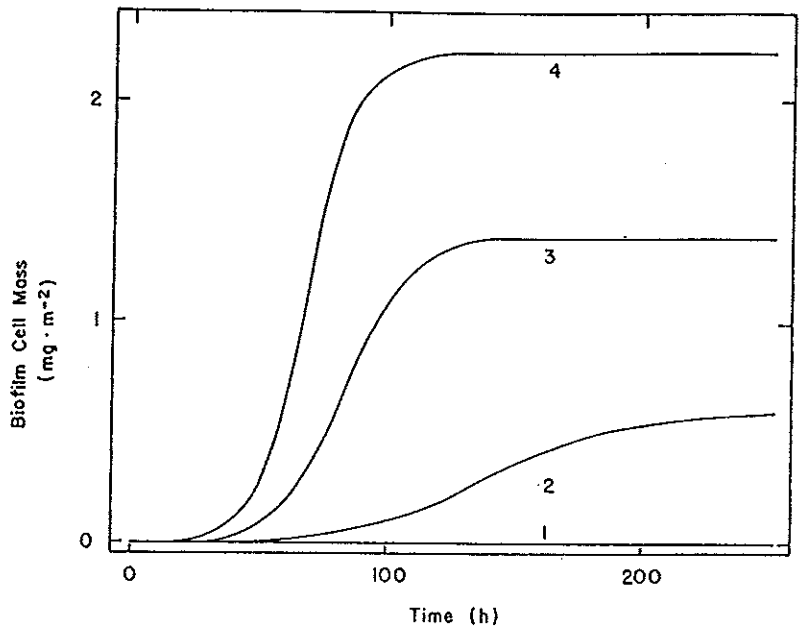

Fig. 4. Predicted progression for biofilm areal cell concentration as a function of influent substrate concentration (S_i in g m^{-3}). Dilution rate, D, was 1 h^{-1}.

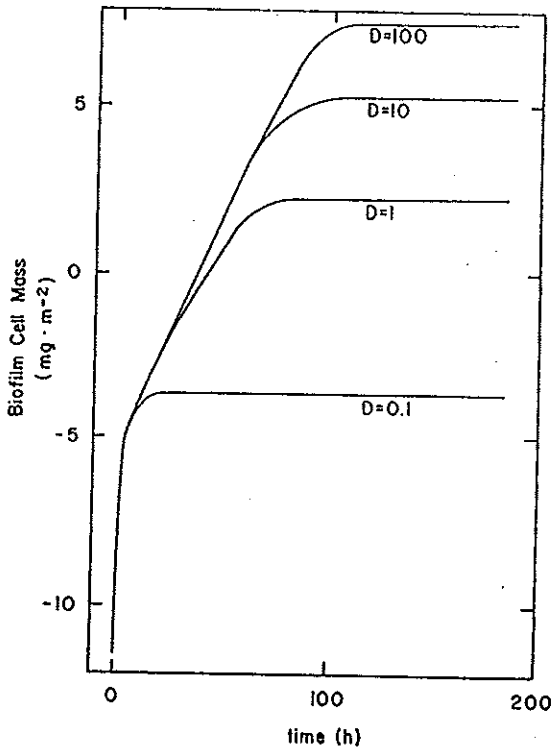

Fig. 5. Predicted progression for biofilm areal cell concentration as a function of dilution rate (D). Influent substrate concentration, S_1, was 1 g m^{-3}.

SUMMARY

A mathematical model for accumulation and activity of a PA biofilm in a CFSTR (RotoTorque) has been presented which is based on conservation principles, transport phenomena, and fundamental kinetic and stoichiometric relationships. The validity of the model was tested by comparing theoretical predictions to experimental data. Experimental data from chemostat experiments were used successfully to predict progression of several variables in a biofilm reactor. Model predictions were presented in terms of the effects of a few important variables on biofilm accumulation and activity.

There is a need to consider spatial and temporal variations within the biofilm if further progress is to be made. For example, biofilm density changes with time and undoubtedly influences biofilm activity and biofilm effects on other transport processes. Density may also change with biofilm depth. PA biofilm thickness is not uniform especially after significant accumulation. With other microbial species such as Klebsiella pneumoniae (Siebel, 1987), biofilm thickness is extremely variable. The influence of a "rough" biofilm on fluid frictional resistance and mass transfer may be important. Diffusional limitations within the PA biofilm were never significant. Is this characteristic of monopopulation biofilms? Indications are that other monopopulation biofilms can exhibit significant diffusional resistance. For example, Siebel (1987) experimented with Klebsiella pneumoniae and suggests that resistance to oxygen diffusion is important to the activity of these biofilms.

Efforts to simulate biofilm accumulation and activity in various reactor geometries and environments must continue. The models described in this chapter simulate monopopulation biofilms. Environmental engineers have been modelling biofilms as if they consisted of single populations for many years so relevant technological applications of monopopulation biofilms exist. The major impetus for modelling monopopulation biofilms, however, is the need to elucidate the fundamental processes contributing to biofilm accumulation and persistence without the confounding factors introduced by population dynamics and interactions.

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