88-015

ABSTRACT

FUNDAMENTALS OF BIOFILM PROCESSES

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Presented at AIChE Summer National Meeting
Denver, Colorado August 21-24 1988

Biofilm accumulation and activity are the net result of several physical, chemical, and microbiological processes. A numerical model for the prediction of biofilm accumulation and activity has been developed which incorporates the following processes : substrate-dependent microbial growth, particulate attachment/detachment, molecular diffusion in the biofilm, advective transport in the bulk water, and chemical inhibition effects. The model predicts biofilm accumulation, substrate depletion, suspended cell concentration, and biocide effects on these variables.

Introduction

A biofilm is an accumulation of microbial cells on a surface which is exposed to life-sustaining nutrients. Biofilms are becoming an important research topic because of an increased awareness of both the problems and the potential benefits of biofilm growth. Problems caused by biofilms include reduced energy transfer in heat exchange equipment, increased frictional resistance to fluid flow, and clogging of small channels in porous media. The benefits include potential uses such as "in situ" aquifer reclamation, and the ability to "fix" microbes in biosynthesis reactors to reduce the amount of purification needed for a high quality final product and to allow greater throughput.

Biofilm accumulation (Fig. 1) is the result of many interrelated physical, chemical, and microbiological processes. This paper will focus on fundamental biofilm processes. A numerical model for the prediction of biofilm accumulation and activity has been developed which incorporates the following processes: substrate-dependent microbial growth, particulate attachment/detachment, molecular diffusion in the biofilm, advective transport in the bulk water, and chemical inhibition effects. The model predicts biofilm accumulation, substrate and nutrient depletion, suspended cell concentration, and biocide concentration.

Fundamental Processes

Net biofilm accumulation can be measured relatively easily by a number of different means; however this gives little insight to the causes of microbial accumulation. Examining the fundamental processes provides answers that can be applied to a variety of systems, so that one not only knows the extent of biofilm accumulation, but the mechanisms which caused the observed accumulation.

Cell Growth. Biofilm production at the surface is primarily the result of cell division and polymer formation. Given proper conditions, some bacterial cells can divide quite rapidly. For example, Pseudomonas Aeruginosa can

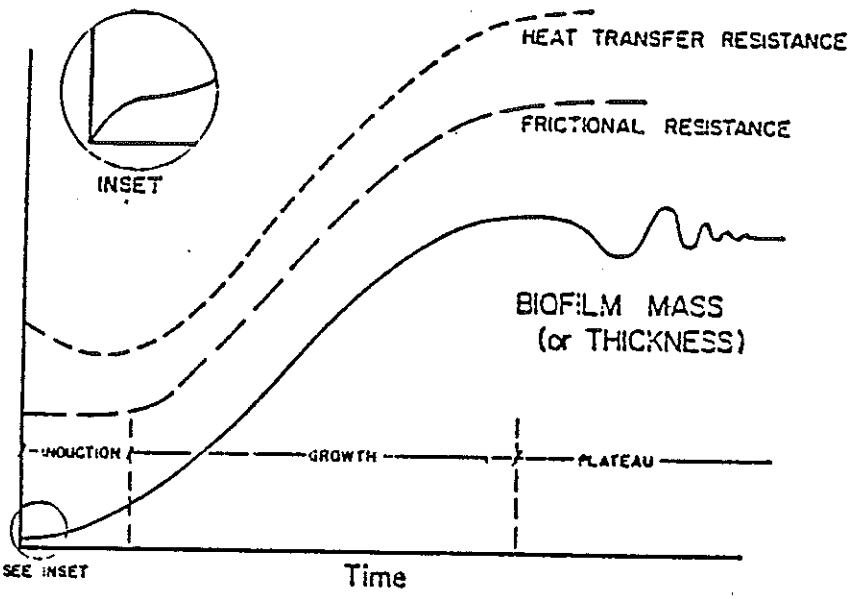

Figure 1. Typical progression of biomass accumulation and resulting energy losses in experimental systems. (Characklis, Bryers, Trulear, Zelver 1980)

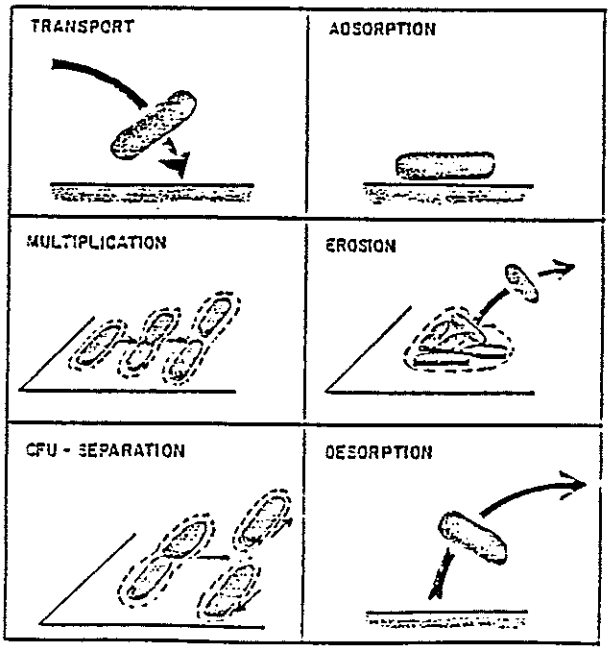

Figure 2. Definition of processes during early colonization of substratum. (Escher 1986)

reproduce itself every two hours or so. Escherichia Coli. can divide every 20 minutes under ideal conditions. However, conditions are not always ideal, therefore the growth rates do not always reach the highest possible value. A relationship between actual cell growth rate, or specific growth rate, and the amount of available substrate was proposed by Monod (1942) to describe dispersed bacterial growth

$$(1)\ \mu = \frac{\mu_{max}\, S}{Ks + S}$$

Trulear and Characklis (1982) showed this type of equation can be valid for use in biofilm systems.

Adsorption/Desorption. Adsorption is defined as the linking of a cell to the substratum (the substratum is the solid surface on which a biofilm forms). Desorption is the breaking of this cell-substratum linkage, with subsequent cell removal (Fig. 2). Research by Escher (1986) suggests a biofilm initially begins to form on an exposed surface by a four-step mechanism.

1) Transport of dispersed cells from the liquid phase to the solid surface (substratum).

2) Reversible cell adsorption to the substratum by a direct substratum-cell interaction.

3) Desorption of some of the adsorbed cells and their re-entrainment in the liquid phase.

4) Irreversible adhesion of non-desorbing cells followed by cell growth.

The rate of irreversible adhesion to the substratum appears to be a function of dispersed cell concentration and fluid shear stress, as well as substratum composition and roughness (Fig. 3). This initial process can be approximated by a first order rate with dispersed cell concentration.

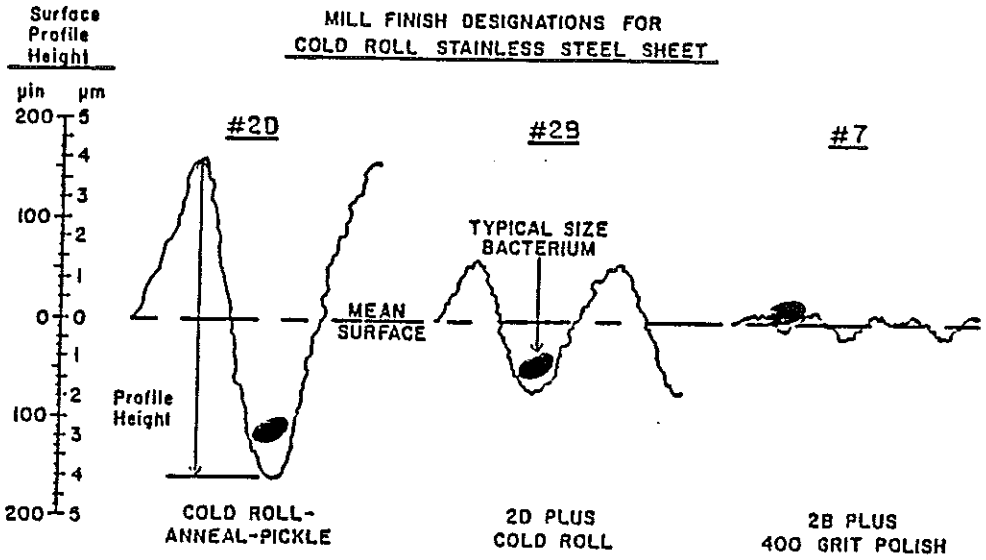

Figure 3. Comparison of typical bacterium size with various mill finish designations for cold roll stainless steel sheet.

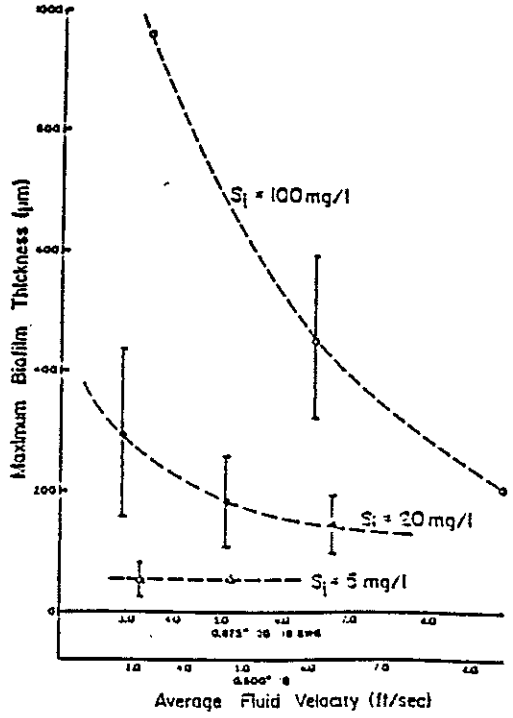

Figure 4. Effect of nutrient loading and fluid shear on maximum biofilm thickness. (Characklis Bryers, Trulear, Zelver 1980)

Attachment. The distinction between attachment and adsorption comes from the condition of the surface to which the cell adheres. Adsorption is adhesion to a clean substratum, whereas attachment is adhesion to other cells already fixed to the surface. These fixed cells produce polymeric fibers which could enhance the attachment rate, since the surface the cell "sees" is a fibrous mat rather than a hard surface. Attachment can apply to agglomerates of cells and is not limited to individual cells as is adsorption. Attachment is also modelled as a first order rate with suspended cell concentration.

Detachment. Detachment is the removal of cellular material from a biofilm. Biofilm detachment can be separated into two catagories.

1) Erosion - the continuous shearing of microscopic portions of the biofilm.

2) Sloughing - the massive removal of large portions of the biofilm at seemingly random locations.

Erosion is generally attributed to shear stresses at the biofilm-fluid interface (Trulear and Characklis 1982), and can significantly affect the biofilm accumulation rate and thickness (Fig. 4). An expression for the erosion rate is given in equation (2) by Trulear and Characklis (1982), with data indicating that the erosion rate increases with increasing shear stress and increasing biofilm thickness.

$$2) \quad Rd = Kd\ \sigma\ Lf\ A\ p$$

Sloughing is usually observed in nutrient-rich environments where biofilm thickness can become quite large. Howell and Atkinson (1976) have proposed a model where biomass decay in the deep, oxygen- and substrate-deficient

biofilm layers causes the detachment of large portions of the biofilm. One effect of sloughing is an increased nutrient transport to the cells which remain on the surface.

Biofilm Model

Mass and momentum transport phenomena play an exceedingly important role in the accumulation and activity of biofilms. In an effort to quantify this role, a numerical model for simulating unsteady state biofilm accumulation in a pipe system has been developed. This model incorporates the biofilm processes given above with mass and momentum transport equations to predict biofilm accumulation, substrate and nutrient depletion, biocide concentration and suspended cell concentration in a turbulent flow pipeline.

Modeling Mass Transport in Biofilm Systems. Biofilms depend on nutrients for survival. These nutrients are replenished from the bulk water by advective transport and molecular diffusion. Biofilm growth is often limited by the rate at which substrate can be transferred from the bulk water, through the laminar sublayer, and into the biofilm. To model the diffusion of substrate into the biofilm, a value for the substrate effective diffusivity must be determined (Fig. 5). A correlation for turbulent eddy diffusivity was calculated from data given in Sherwood, Pigford and Wilke and used in combination with an estimation of effective diffusivity in the turbulent boundary layer given by Notter and Sleicher (1971).

Model Equations. The purpose of this section is not to explicitly derive the partial differential material balance equations, but to show the relationship between the fundamental biofilm processes and the equations in the numerical solution.

A cylindrical coordinate system was chosen to represent a pipe flow biofilm reactor. Beginning with the general equation of continuity for a cylindrical coordinate system (Bird, Stewart, and Lightfoot 1960), and applying the assumptions given in Table 1, one can derive the material

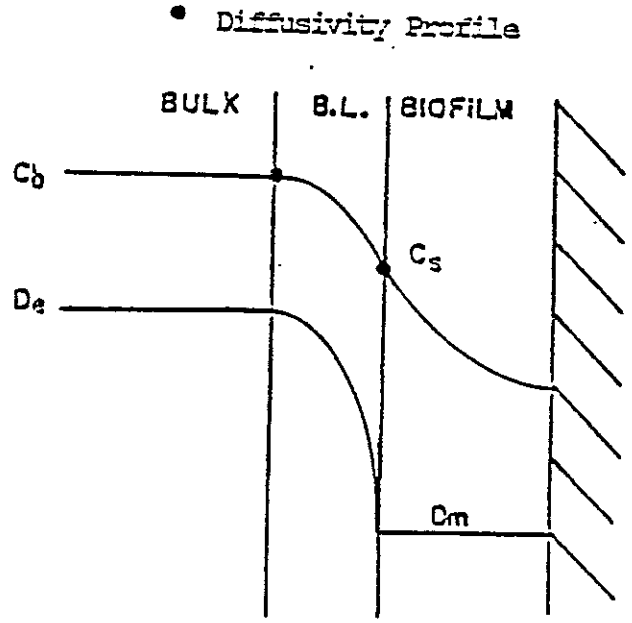

Figure 5. Schematic of the effective diffusivity profile used in the turbulent pipe flow biofilm accumulation model.

balance equation for a solute, given in Table 2. Similarly, applying material balance concepts to each fundamental biofilm process which affects the biofilm results in the equations given in Table 3.

Model Results. The computer model will be used to numerically test hypotheses concerning biofilm-related processes. For example, it can lead to better biofilm sampling and control programs. The series of graphs below (Figs. 6-8) give the substrate concentrations at three radial locations in a turbulent flow pipe as a function of pipe length and time. Figure 6 shows the average substrate concentration in the pipe; this is the substrate concentration that would be measured by a "grab" or "cup" sample . Notice the average substrate concentration at 1000 meters and 168 hours is approximately 3 mg/l. Figure 7 shows the substrate concentration just at the interface between the biofilm and the bulk liquid. At 1000 m and 168 h, the substrate concentration that the surface layer of the biofilm actually "sees" is about 1.6 mg/l. The substrate concentration at the pipe wall (Fig. 8), again at 1000 m and 168 h, is about 0.7 mg/l. This concentration is over four times lower than the concentration that would actually be measured by a grab sample at this location and time. Figure 9 shows the biofilm accumulation down the length of the pipeline for the concentration profiles given in figures 6-8.

How does this relate to biofilm sampling and control? If a comparison of flush-mount and mid-stream biofilm coupons were made (a coupon is a removable piece of solid material used for biofilm analysis), the mid-stream coupon would be exposed to substrate concentrations 2 - 4 times higher than a flush-mount coupon at the same location. At the same time, the flush-mount coupon may be exposed to 2 - 4 times lower biocide concentrations than the corresponding coupon located in mid-stream. This numerical model may be used to develop a more meaningful sampling regime or may improve the interpretation of the previously obtained results.

Solute Material Balance: Assumptions

1) Axial Symmetry $\frac{di}{d\theta} = 0$

2) Time Averaged Flow $v_i = \bar{v}_i$

3) Pseudo-Steady State $\frac{dS}{dt} = 0$

Substrate profiles develop rapidly relative to biofilm growth.

4) Negligible Bulk Flow into Biofilm $v_r = 0$

5) Radial Diffusion >> Axial Diffusion

$$\frac{1}{r}\frac{\partial}{\partial r}\left(D_{sr}\, r\frac{\partial S}{\partial r}\right) >> \frac{\partial}{\partial z}\left(D_{sz}\frac{\partial S}{\partial z}\right)$$

Table 1. Simplifying assumptions used to reduce the general equation of continuity for a cylindrical coordinate system.

Solute Material Balance

$$\frac{1}{r}\frac{\partial}{\partial r}\left(D_s r\frac{\partial S}{\partial r}\right) + R = v_z\frac{\partial S}{\partial z}$$

Diffusion + Consumption = Convection

Substrate: $R = -X\left(\frac{\mu_m S}{(k_s + S)}\left(\frac{1}{Y} + k_g\right) + k'_g\right) - \beta_{\Sigma_S}$

Suspended Cells: $R = \frac{X\,\mu_m\, S}{k_s + S} - \beta_{\Sigma_X}$

Biocide: $R = -(\beta_{\Sigma_X} + \beta_{\Sigma_S})$

where β_{Σ_i} = Biocide Effects on Species i

Table 2. Material balance equations for substrate and suspended cells in the turbulent flow biofilm accumulation model.

Biofilm Material Balance

$$\frac{dX_b}{dt} = \frac{X_b \mu_m S}{(k_s + S)} + k_a X|_{int} - k_d X_b - B_{z_B}$$

Accumulation = Growth + Input − Detach. − Biocide

Suspended Cells: Boundary Condition at Biofilm Interface

$$\frac{1}{r}\frac{\partial}{\partial r}\left(D_{sx} r \frac{\partial X}{\partial r}\right) + \frac{X \mu_m S}{(k_s + S)} - B_{z_X} - k_a X|_{int} + k_d X_b = v_z \frac{\partial X}{\partial z}$$

Diffusion + Growth − Biocide − Attach. + Detach. = Convection

Table 3. Material balance equations for the biofilm compartment showing a rate equation for each fundamental biofilm process, and the interaction between suspended and attached biomass.

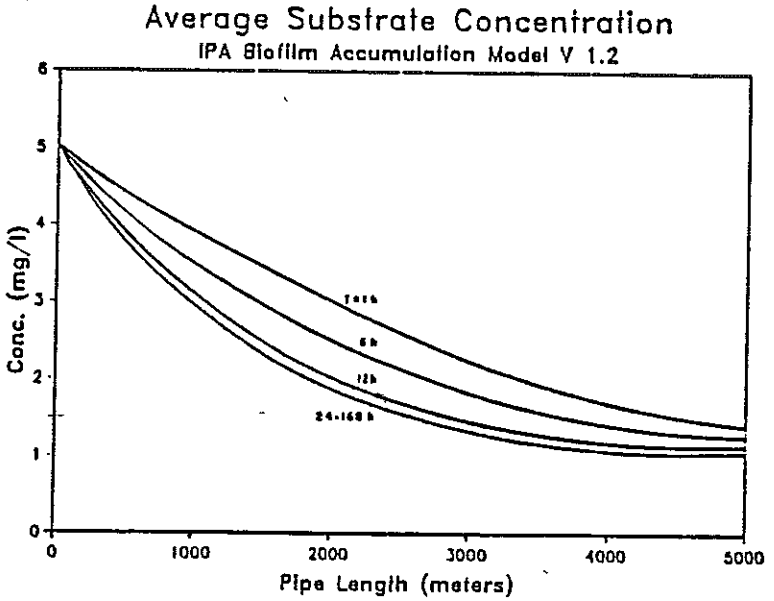

Figure 6. Average substrate concentration with axial distance from the pipe entrance and with time. (Initial biofilm thickness = $6x10^{-6}$ m)

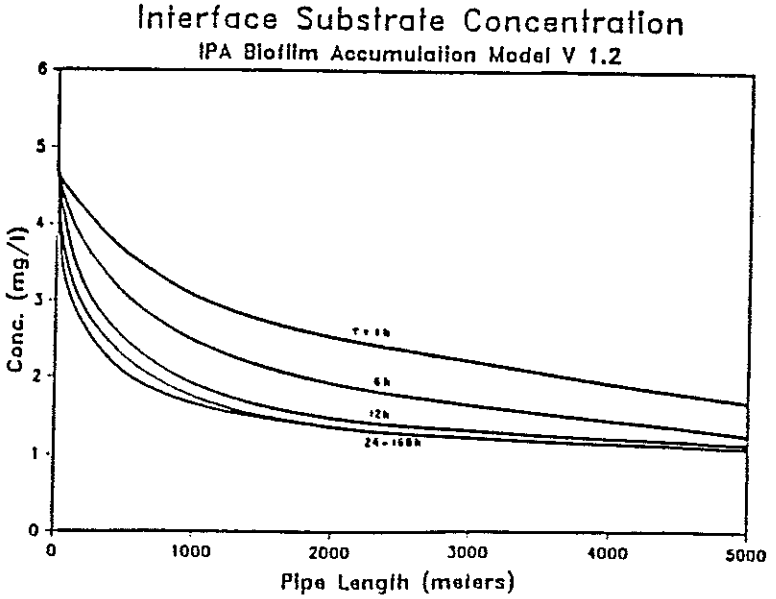

Figure 7. Substrate concentration at the biofilm-bulk liquid interface with axial distance and time.

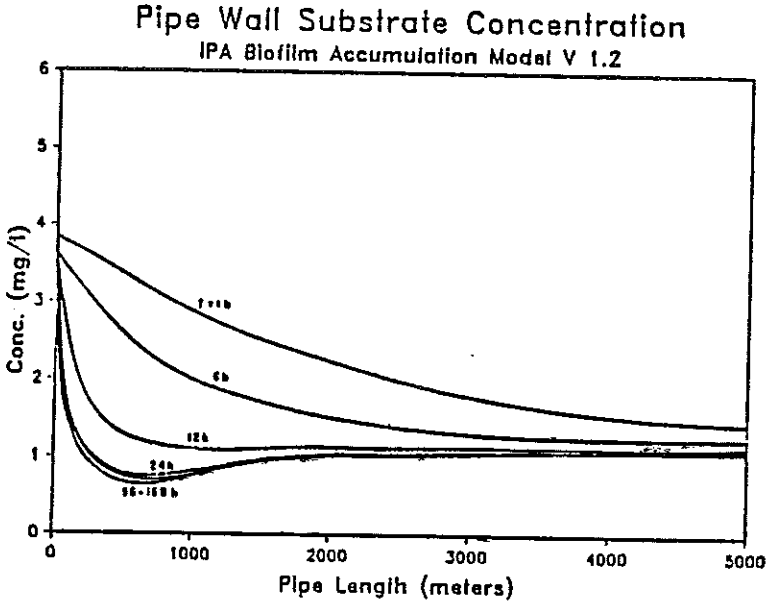

Figure 8. Substrate concentration at the pipe wall below the biofilm with axial distance and time.

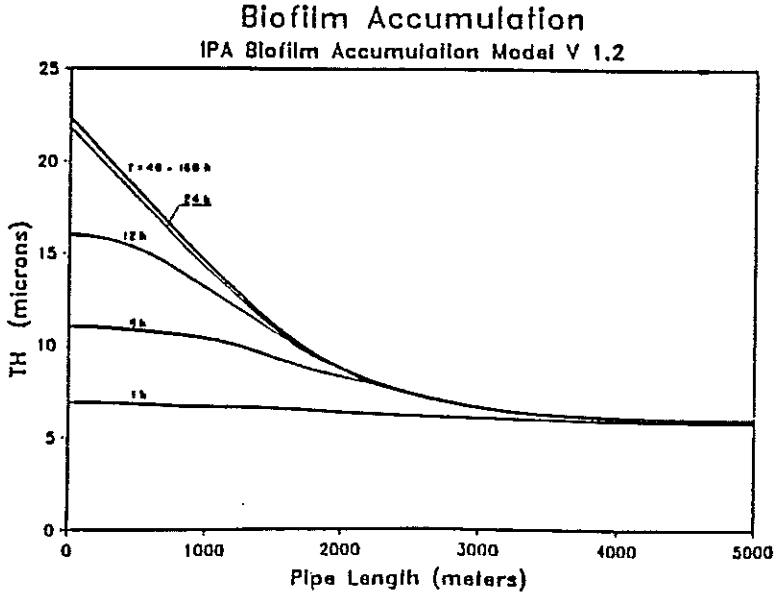

Figure 9. Biofilm thickness with axial distance from the pipe entrance and with time. (Initial biofilm thickness = 6×10^{-6} m)

Nomenclature

A = biofilm area [L^2]

B_{Ei} = Biocide effects on species i

D = Diffusivity [$L^2\ t^{-1}$]

Kd = erosion coefficient [$L^2\ N^{-1}\ t^{-1}$]

Kg = Coefficient for growth associated polymer production [$Ms\ Mx^{-1}$]

Kg'= Coefficient for non-growth polymer production [$Ms\ Mx^{-1}\ t^{-1}$]

Ks = saturation coefficient for Monod kinetics [M L-3]

L = Length units

Lf = biofilm thickness [L]

M = Mass units

N = Force units

Rd = erosion rate [$M\ L^{-3}\ t^{-1}$]

S = substrate concentration [M L-3]

t = Time units

u = Fluid velocity [$L\ t^{-1}$]

X = Biomass concentration [M L-3]

Greek Symbols

μ = specific growth rate [t^{-1}]

μ_{max} = maximum specific growth rate [t^{-1}]

p = biofilm density [$M\ L^{-3}$]

σ = shear stress [$N\ L^{-2}$]

θ = Angular coordinate in a cylindrical system [radians]

References

Bird, R.B., Stewart, W.E., Lightfoot, E.N., Transport Phenomena, John Wiley and Sons, 1960

Characklis, W.G., Bryers, J.D., Trulear, M.G., Zelver,N., "Biofouling Film Development and its Effects on Energy Losses: A Laboratory Study." Condenser Biofouling Control (Garey, J.F. et al., eds.) Ann Arbor Science Publishers, Inc., Ann Arbor, MI, pp 49-76 (1980)

Escher, A.R.," Bacterial Colonization of a Smooth Surface: An Analysis with Image Analyzer." doctoral dissertation, Montana State University, Bozeman, Montana, 1986

Howell, J.A., and Atkinson, B., "Sloughing of Microbial Film in Trickling Filters." Water Research, 10, pp 307, (1976)

Monod, J., "Recherches sur la Croissance des Cultures Bacteriennes." Hermann et cie., Paris, France (1942)

Notter, R.N., Sleicher, C.A., "The Eddy Diffusivity in the Turbulent Boundary Layer Near a Wall." Chemical Engineering Science, 26 pp.161 (1971)

Sherwood, T.K., Pigford, R.L., Wilke, C.R., Mass Transfer, McGraw Hill Book Company, New York

Trulear, M.G., Characklis, W.G., "Dynamics of Biofilm Processes." J. WPCF, 54:9 (1982)