EFFECT OF BIOFILM THICKNESS DISTRIBUTION ON SUBSTRATE-INHIBITED KINETICS

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Abstract—The effect of biofilm thickness distribution (BTD) on substrate-inhibited kinetics in anaerobic filters was evaluated. The modeling results show that the kinetic behavior of normal BTD is different from that of uniform biofilm thickness when the substrate utilization rate of some biofilms is limited by diffusion or reaction. However, when all biofilms are either diffusion-limited or reaction-limited, kinetic behaviors of normal BTD and uniform biofilm thickness are identical. In addition, the BTD can reduce the apparent maximum substrate utilization rate and narrow the multiple steady-state region of substrate-inhibited kinetics. In this study, phenol was used as an inhibitory substrate. A general agreement was found between the numerical simulation results using the normal BTD and experimental results for anaerobic filters. On the contrary, numerical simulation using uniform biofilm thickness failed to predict the outcome of the experiments. This indicates that an anaerobic filter is composed of a biofilm with varying thickness and thus, the BTD of a filter is of great importance, especially for treating wastewater containing inhibitory substrates.

Key words—biofilm thickness distribution, substrate-inhibited kinetics, anaerobic filter, phenol, orthogonal collocation, diffusion

NOMENCLATURE

- Specific biofilm surface area (L⁻²)
- Coefficient of first derivative at collocation point
- Coefficient of second derivative at collocation point
- Biot number of mass
- Dimensionless substrate concentration of bulk liquid
- Dimensionless substrate concentration within biofilm
- Dimensionless substrate concentration of influent
- Dimensionless substrate concentration at liquid-biofilm interface
- Diffusivity of substrate within biofilm (L²T⁻¹)
- Volume fraction of biofilms with thickness Lf
- Dimensionless inhibition constant
- Substrate flux at biofilm surface (ML⁻²T⁻¹)
- Maximum specific utilization rate (T⁻¹)
- Mass transfer coefficient (LT⁻¹)
- Inhibition coefficient (ML⁻³)
- Half-saturation coefficient (ML⁻³)
- Biofilm thickness (L)
- Surface-average biofilm thickness (L)
- Dimensionless substrate flux at biofilm surface
- Feed flow rate (LT⁻¹)
- Reaction rate (ML⁻³T⁻¹)
- Dimensionless reaction rate
- Substrate concentration of bulk liquid (ML⁻³)
- Substrate concentration within biofilm (ML⁻³)
- Substrate concentration of influent (ML⁻³)
- Substrate concentration at biofilm surface (ML⁻³)
- Total volume of filter (L³)

INTRODUCTION

Recently, interest in mathematical modeling of substrate-inhibited kinetics of biofilm has significantly increased (Stevens, 1988; Tong et al., Gantzer, 1989). In particular, steady-state multiplicity and stability resulting from non-monotonic kinetics were intensively studied. In most cases, the uniform biofilm thickness was assumed to be capable of characterizing the performance of a fixed-biofilm reactor. Nonetheless, the reactor is truly composed of a biofilm with a varying thickness. Therefore, we may inquire about whether an average biofilm thickness is possible of describing the overall performance of the reactor.

Aris (1957) studied the influences of particle size in a fixed-bed catalytic reactor. He found that the effectiveness factor could be calculated by using mean particle size when diffusion was not limiting for any particle size or when diffusion was limiting for all particle sizes. The mean diameter of a catalytic particle can be defined as $d = \left[ \frac{1}{3} \pi \phi d^3 \right]^{-1}$, where $\phi$
is the volume fraction of the \( k \)th size and \( d_k \) is diameter of the \( k \)th particle. However, if the size range means that diffusion is only limiting for some particles, any mean particle size used for calculating an effectiveness factor would be inaccurate.

For a stirred-batch adsorption system using granular activated carbon, Cooney et al. (1983) indicated that the mass of solute adsorbed at any time would vary with the particle size squared. In other words, one should not use an arithmetic average particle size to describe system behavior when a range of particle sizes exists. In fact, it is very difficult to select a suitable average particle size for characterizing the kinetic behavior of the adsorbent when a range of particle sizes exists. The aforementioned viewpoint was also verified by Rasmuson (1985), who studied the effect of particle distribution on the dynamics in a fixed-bed system. In his simulation studies, the breakthrough of a fixed-bed was delayed because the particle size distribution resulted from the presence of particles which were smaller than the average particle size (i.e. having a larger outer surface area per unit particle volume).

Studies on kinetics involving biofilm thickness distribution (BTD) are rather scarce. Examination of chemical reactor data in the aforementioned literature suggests that the BTD in a biological system may affect its kinetic behavior, especially for treating wastewater containing inhibitory substrates. When a range of biofilm thicknesses coexist, some microbial cells within the biofilm existing with diffusional resistance may have an effectiveness factor greater than unity, others may be inhibited by the excess amount of substrate.

The objectives of this study include: (1) a preliminary analysis regarding the effect of BTD on substrate-inhibited kinetics and (2) verification of the proposed model with the experimental data in anaerobic filters.

**MATHEMATICAL MODEL**

The following assumptions were made for the development of the mathematical model:

1. Microbial cells are homogeneously distributed in the biofilm with uniform activity \( X_r \).
2. Phenol is a growth-limiting nutrient. All other nutrients are present in excess amounts. The substrate-utilization reaction within the biofilm follows the Haldane relationship and can be expressed as

\[
r = \frac{K X_r S_i}{K_i + S_i + S_1^k / K_i}
\]

3. Diffusion of substrate within the biofilm only occurs in the Z direction which is normal to the surface of the biofilm, and Fick’s law follows. A plane coordinate is employed to mathematically represent the dimension of the biofilm.

\[
\frac{d^2 S_k}{dZ^2} = r^k
\]

The boundary conditions for equation (2) are

\[
\frac{dS_k}{dZ} = 0 \text{ at } Z = 0
\]

\[
D_r \frac{dS_k}{dZ} = j_k = K_i(S_b - S_k) \text{ at } Z = L_i
\]

For an anaerobic filter with effluent recycle, the complete mixing in liquid phase can be normally established. Young and Young (1988) concluded that models based on test results from filters using conventional-sized media would be more likely to fit a completely mixed flow regime because of inherent mixing associated with biogas production.

Mass balance of substrate in liquid phase can be expressed as

\[
S_m - S_b = \frac{V_{eq} \sum j_k}{Q \tau} \frac{S_1^k J_k}{L_i}
\]

It must be noted that the superscript \( k \) represents biofilm thickness group \( k \). The quantity of \( \frac{\sum j_k}{L_i} \) is the total outer biofilm surface of thickness group \( k \) per unit reactor volume.

The above equations with dimensionless form can be given by

\[
\frac{d^2 C_k}{dY^2} = (\phi^k)^2 R(C_k^j)
\]

\[
\frac{dC_k}{dY} = 0 \text{ at } Y = 0
\]

\[
\frac{dC_k}{dY} = N_k = Bim^k(C_b - C_k) \text{ at } Y = 1
\]

\[
C_m - C_b = \eta R(C_b)
\]

where

\[
C_t = S_t / \sqrt{K_i K_j}; \quad C_b = S_b / \sqrt{K_i K_j};
\]

\[
C_c = S_c / \sqrt{K_i K_j}; \quad C_m = S_m / \sqrt{K_i K_j};
\]

\[
Y = Z / L_i; \quad (\phi^k)^2 = (K X_r (L_i^k))^2 / (D_r K_i);
\]

\[
R(C_k^j) = C_k^j / (1 + g C_k^j + (C_b^j)^2); \quad g = \sqrt{K_i / K_j};
\]

\[
N_k = j_k L_i^k / (D_r \sqrt{K_i K_j}); \quad Bim^k = K_i L_i^k / D_r
\]

\[
\tau = \frac{V_{eq} K_i}{Q K_i}
\]
MATERIALS AND METHODS

Feed wastewater

The ingredients of feed synthetic wastewater included phenol (625–5000 mg/l), yeast extract (25–200 mg/l), NH₄Cl (250 mg/l), K₂HPO₄ (250 mg/l), KH₂PO₄ (80 mg/l), NaHCO₃ (1000 mg/l), Fe³⁺ (0.5 mg/l), Ni²⁺ (0.1 mg/l), Co²⁺ (0.06 mg/l) and Mo⁶⁺ (0.06 mg/l). The concentrations of COD ranged from 1500 to 12000 mg/l and the pHs ranged from 7.4 to 7.8. Phenol was measured by using the 4-aminoantipyrine colorimetric method (APHA, 1985).

Reactor design and operation

Two acrylic jacketed continuous upflow anaerobic filters (see Fig. 1) were operated with an effluent recycle rate of 60 l/day in order to achieve a nearly completely-mixed condition within filters. The flow regime of these filters was already evaluated in a previous study (Jih et al., 1992). That is, a small Peclet number (Pe) ranging from 0.2 to 0.9 was determined in tracer studies which were operated under the feed flow rates ranging from 7.8 to 57 l/day. Therefore, complete mixing in the liquid phase in this study would be expected. Various pertinent physical characteristics are listed in Table 1. The inner tube was enclosed in an outer jacket through which hot water was circulated for maintaining the temperature of filters at 35 ± 2°C. Both filters had been continuously fed with acetate synthetic wastewater in the previous study for 1 year. Thereafter, these two filters were fed with phenolic synthetic wastewater and operated for a period of 6 months prior to the initiation of further experiments. The Haldane model [equation (1)], solved by using the adaptive stepsize Runge-Kutta method (Press et al., 1986), was used to describe the utilization rate of phenol. Model solutions were then compared with experimental data (i.e. the remaining concentration of soluble phenol vs. time) obtained from batch study. A non-linear least square fitting routine using the Levenberg-Marquardt algorithm was applied for searching for a set of kinetic parameters which would best fit the experimental data.

Numerical methods

The continuity equation for the solid phase [equation (6)] and its boundary conditions [equations (7) and (8)] were transformed by using 15th-order approximation of orthogonal collocation (Villadsen and Michelsen, 1978) in which symmetry polynomials were used as trial functions. Therefore, equations (15) and (16), used for respectively determining the substrate concentration at the biofilm surface and the ith interior point, could be derived:

\[ C_{i,N+1} = \frac{B_{i,C^b} + \sum_{j=1}^{N} A_{N+1,j} C_{i,j}}{B_{i} + A_{N+1,N+1}} \]  

\[ \sum_{j=1}^{N} B_{i,j} C_{i,j} = \frac{B_{i} + \sum_{j=1}^{N} A_{N+1,j} C_{i,j}}{B_{i} + A_{N+1,N+1}} \]  

The effectiveness factor of equation (13) is replaced by

\[ \eta^i = \frac{\sum_{i=1}^{N+1} W_i R(C^b_i)}{\sum_{i=1}^{N+1} W_i R(C^b)} \]  

Both coefficients of \( A_{ij}, B_{ij} \) and the weighting factor of \( W_i \) could be calculated by using the roots of Jacobi polynomials (Villadsen and Michelsen, 1978). Equation (16), a systematic non-linear algebraic equation, was solved using the Newton-Raphson method. The calculated \( C^b_i \) at interior points was then inserted into equation (15) to determine the substrate concentration at biofilm surface (\( C_{i,N+1} \)). In order to calculate the overall effectiveness factor of a filter, the effectiveness factor for each group of biofilm thickness (\( k \)) was first calculated by using equation (17) and then
The K value found in this study is about one half of degrading biomass but also the methanogen and disrupted biofilm does not only contain the phenolic-extracellular polymers. However, model calculations quantity, those generally found in a suspended-growth culture.

The Ki value of 61.8 mg/l was then estimated. After the rough estimation of Kf value (61.8 mg/l), the experimental data were fitted using the Haldane equation again; and the estimated values of Ks for both filter-A and filter-B were 5.26 × 10^{-7} and 2.50 × 10^{-7} m/s, respectively. The derived values of Kf were very low because the superficial velocity of filters used in this study was far below 10^{-4} m/s.

Using the Wilke-Chang correlation (Sherwood et al., 1975), the diffusivity of phenol in water (Dw) calculated at 35°C was 1.23 × 10^{-9} m²/s. The effective diffusivity within the biofilm (Dr) was taken to be 80% of this value (Dr = 9.87 × 10^{-10} m²/s). Saez et al. (1991) selected the ratio of Dr/Dw for phenol to be 80% for model calculations in the anaerobic fluidized-bed reactor. The ratio of Dr/Dw for phenol in the aerobic biofilm ranging from 5 to 88% was estimated by Andrew and Howard (1989).

**Biokinetic parameters**

Phenol utilization in a batch reactor using a disrupted biofilm is shown in Fig. 2. In the course of searching for biokinetic parameters, the Ks value appears to have no sensitivity in the fitting routine. This phenomenon was also found by Suidan et al. (1988). To overcome this problem, the Kf was neglected in the denominator of equation (1) when the value of S was greater than 1000 mg/l (i.e. S >> Kf). The Ks value of 61.8 mg/l was then estimated. After the rough estimation of Ks value (61.8 mg/l), the experimental data were fitted using the Haldane equation again; and the estimated values of K and Ks were 0.337 day^{-1} and 34.7 mg/l, respectively. Suidan et al. obtained a K value of 0.66 day^{-1}, Ks value of 363 mg/l and assumed a Kf value of 0.03 mg/l for the anaerobic mixed-culture. They also reported that the quantity, (Ks/Kf)^1/2, at which the maximum specific utilization rate occurred was below 50 mg/l in their literature review. In our study, the quantity of (Ks/Kf)^1/2 was 46.3 mg/l which was consistent with those generally found in a suspended-growth culture. The K value found in this study is about one half of that determined by Suidan et al. (1988) because the disrupted biofilm does not only contain the phenolic-degrading biomass but also the methanogen and extracellular polymers. However, model calculations in this study were based on total biofilm mass (i.e. not active biomass) accumulated in the filter. A good quality of fit between the experimental data and the Haldane model (i.e. see Fig. 2) implies that all parameter values mentioned above are reliable.

**Mass transfer coefficient and diffusivity of phenol**

The mass transfer coefficient (Kf) was estimated by using the experimental data obtained from anaerobic filters. When Sb was very low, the filter was under the mass transfer limiting condition. The apparent substrate utilization rate would then appear to follow first-order kinetics with respect to Sb, regardless of the functional form of the intrinsic rate expression, that is

\[
\text{rate} = \frac{Q(S_a - S_b)}{V} = aK_fS_b \quad (18)
\]

For Sb < 100 mg/l, the Kf value could be calculated using equation (18). The estimated values of Kf for both filter-A and filter-B were 5.26 × 10^{-7} and 2.50 × 10^{-7} m/s, respectively. The derived values of Kf were very low because the superficial velocity of filters used in this study was far below 10^{-4} m/s.
Kinetic behavior and biofilm thickness

Table 2. Biofilm thickness distributions used for model solution

<table>
<thead>
<tr>
<th>Function</th>
<th>D1</th>
<th>U1</th>
<th>D2</th>
<th>U2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filter</td>
<td>A</td>
<td>A</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>Biofilm thickness</td>
<td>normal distribution</td>
<td>uniform</td>
<td>normal distribution</td>
<td>uniform</td>
</tr>
<tr>
<td>Mean (mm)</td>
<td>4.09</td>
<td>1.68</td>
<td>4.56</td>
<td>1.87</td>
</tr>
<tr>
<td>Coefficient of variation</td>
<td>0.5</td>
<td>0</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>$L_0$ (mm)</td>
<td>1.68</td>
<td>1.68</td>
<td>1.87</td>
<td>1.87</td>
</tr>
</tbody>
</table>

more biofilm until the accumulated biofilm reached maximum. The volume fraction of biofilm was assumed to be near maximum within each test run period. Therefore, the $e_f$ value was assumed to be constant with respect to time in the simulation of the model.

$a$ and $L_0$. The actual specific biofilm surface area ($a$) was reasonably assumed to be the same as that of the packing media. Thus, the surface average biofilm thicknesses of filter-A and filter-B were 1.68 and 1.87 mm, respectively (i.e. $L_0 = e_f/a$).

BTD. Grotenhuis et al. (1991) found that the weight fraction of granular particle size distribution in UASB reactors was close to normal distribution. Based on the data obtained from Grotenhuis et al. (1991) and the assumption of constant granular density, the mean diameter and the coefficient of variation (CV) on a volume fraction basis were calculated to be 1.55–2.13 mm and 0.32–0.5, respectively. In this study, the normal BTD functions ($F^*$) based on volume fraction were selected to make the value of the surface average thickness equal to the uniform biofilm thickness in each filter (see Table 2), and the value of CV was 0.5. The CV value falls into the upper range of 0.32–0.5 for the fixed biofilm (Grotenhuis et al., 1991) because the shape of the void in the filters is very irregular. The relationship between surface average thickness and BTD is

$$L_0 = \frac{e_f}{a} = \left( \frac{\sum F^*}{\sum L^2} \right)^{-1}$$

(19)

The cumulative frequency curves of two normal BTD are shown in Fig. 3. In the numerical solution procedure, each BTD was considered as a discrete distribution and divided into 35 intervals. The distribution region that extended from a 2.5% frequency level on one side to 2.5% frequency level on the other side was considered.

RESULTS AND DISCUSSION

Performance of anaerobic filters

Each filter was continuously operated for 18 test runs, and the feed flow rates ranged from 7 to 30 l/day. The apparent substrate utilization rate vs bulk phenol concentration was plotted, as shown in Fig. 4. At a low phenol concentration, the substrate utilization rate appears to follow first-order kinetics with respect to substrate, regardless of the inhibitory effect of phenol. This was expected since the filter was mass-transfer-limited. As the phenol concentration was increased, the substrate utilization rate increased and tended to approach a maximum rate at a phenol concentration of about 600–800 mg/l which was 15 times greater than the value of $(K_sK_I)^{1/2}$. This disparity was because of the resistances of both external mass transfer and diffusion within the biofilm. However, the substrate utilization rate would be decreased if the phenol concentration was beyond 600–800 mg/l. These kinetic behaviors found in fixed-biofilm studies are intuitively consistent with the concept of substrate-inhibited kinetics.
Model simulation

Model simulation was conducted to describe both uniform biofilm thickness and normal BTD. A comparison of the effect of BTD on substrate utilization rate is shown in Fig. 4. As mentioned earlier, the surface average thickness of normal BTD is made to be equal to the uniform biofilm thickness. Figure 4 shows that the substrate utilization rate of normal BTD (labeled D1 and D2) is less sensitive with respect to $S_n$ than that of uniform biofilm thickness (labeled U1 and U2). In addition, the BTD can reduce the maximum substrate utilization rate. This is due to the existence of thicker and thinner biofilm in the normal BTD. Some biofilms have effectiveness factors greater than unity but others are inhibited in a wider range of substrate concentrations. Therefore, one might expect the responded kinetic curve of normal BTD to be flatter and broader than that of a biofilm of uniform thickness. However, the substrate utilization rates of normal BTD and uniform biofilm thickness are identical, when the bulk phenol concentration is either very low or very high. This fact indicates that the BTD must be accounted for when interpreting the performance of reactors, unless diffusion is limiting for all thicknesses of biofilm or diffusion is not limiting for any thickness of biofilm. This is consistent with the finding of Aris (1957).

Volumetric substrate utilization rate in dimensional form is

$$\frac{Q}{V} (S_n - S_b) = \eta \frac{KX_r S_b}{K + S_b + S_b^2/K} \quad (20)$$

Since the Haldane model is a non-monotonic equation, it must be noted that more than one steady state could exist in a filter when the influent substrate concentrations and feed flow rates are within a certain range. The right-hand side of equation (20) is plotted in Fig. 5, while the left-hand side is simply a straight line (i.e. dotted line) of slope $Q/V$, anchored at the influent concentration $S_m$. This straight line intersects the curve of uniform biofilm thickness (U2) at two points (Fig. 5) corresponding to stable steady state and a third midpoint corresponding to unstable steady state. In Fig. 5, it must be noted that, under the same operating conditions, only one stable steady state prevails in the normal BTD (D2). Figure 4 also shows that the curves of the substrate utilization rates for the uniform biofilm thickness are sharper with respect to $S_n$ than those for the normal BTD. Therefore, the uniform biofilm thickness is intuitively more prone to multiplicity than the normal BTD. Figure 6 shows the region of multiple steady states for a certain hydraulic retention time vs a certain influent phenol concentration. Any point that lies in the area between the two curves (i.e. either the solid- or dashed-line) leads to three steady states. From Fig. 6, it can be seen that the multiple steady state region for the uniform biofilm thickness is broader than that for the normal BTD. Thus, the multiplicity is pronounced for the bioreactor having a narrower BTD.

Simulation results vs experimental data

A comparison of the model and experimental apparent substrate utilization rate for filter-A and filter-B is shown in Fig. 4. There is general agreement between the simulation results for normal BTDs (labeled D1 and D2) and experimental data, but not for uniform biofilm thickness. This is certainly because the anaerobic filter is composed of biofilm with various thickness and the normal BTD functions used in this study are not significantly different from reality. The non-uniform biofilm distribution in a bioreactor was also found by Riemer et al. (1980) and Wang et al. (1987) in a denitrification filter and in an anaerobic expanded-bed reactor, respectively. In both cases, the BTD function was not adopted in their models; instead, they accounted for the degree of biofilm coverage on packing media which might give a reasonable explanation of their experimental results.

It is quite possible that the substrate utilization rate of wastewaters containing inhibitory substrates treated by fixed-biofilm reactors is easily influenced by the BTD. When the bulk substrate concentration is within a certain range, microbial cells in the deep side of a very thick biofilm could result in an effectiveness factor greater than unity because diffusional resistance of substrate transport has occurred within the biofilm.
the biofilm. On the contrary, microbial cells in a thin biofilm could be inhibited by the excess amount of substrate. Under these circumstances, any average biofilm thickness could not be appropriately used for describing the performance of a bioreactor and thus, the concept of BTD must be adopted for modeling.

SUMMARY AND CONCLUSIONS

The apparent substrate utilization rate observed in anaerobic filters can be adequately described by the normal BTD, but not by the uniform biofilm thickness. This indicates that an anaerobic filter is composed of biofilm with various thicknesses and thus, the BTD of a filter is of great concern, especially for treating the wastewater containing inhibitory substrates.

The modeling results show that the kinetic behavior of normal BTD is different from that of uniform biofilm thickness when the substrate utilization rate of some biofilms is limited by diffusion or reaction. However, when bulk substrate concentration is either very low or very high, the kinetic behaviors of normal BTD and uniform biofilm thickness are identical. This simply implies that the BTD must be considered for describing the performance of a fixed-biofilm reactor except for these two limiting bulk substrate concentrations. Furthermore, the BTD can reduce the maximum substrate utilization rate and narrow the multiple steady state region of substrate-inhibited kinetics.

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