Modeling and Simulation of Competition Between Two Microorganisms for a Single Inhibitory Substrate in a Biofilm Reactor

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Abstract: A simple biofilm model was developed to simulate the competition between two microorganisms for a common inhibitory substrate. The following assumptions were made for the simulations: (1) the biofilm has a uniform thickness and is composed of 5 segments, (2) growth of two microorganisms A and B which utilize the common substrate is expressed by the Haldane kinetics with a spatial limitation term and is independent of the other microorganism in the biofilm reactor, and (3) diffusion of the substrate, movement of the microorganisms, and continuous loss of the biomass by shearing are expressed by Fick's Law-type equations. The qualitative behavior of the biofilm reactor is characterized by five regions, I–V, depending on the operation conditions, the substrate concentration in feed, and the dilution rate. In region I, both microorganisms are washed out of the biofilm reactor. In region II, microorganism B is washed out, and in region III, microorganism A is washed out of the biofilm. In region IV, both microorganisms coexist with one another. In region V, both microorganisms coexist with a sustained oscillatory behavior. Convergence to regions I–V depends on the initial conditions. In regions II–V, washout of either or both microorganisms is also observed with initial conditions too far away. *Biotechnol Bioeng* **66**: 258–264, 1999.
Keywords: biofilm; inhibition; stability; oscillations; multispecies model; multiple steady-states

INTRODUCTION

Wastewater containing toxic chemicals is often treated directly at its source by microbial cultures consisting of a single or a few kinds of microorganisms in small-scale bioreactors. The performance of the reactors largely depends on the microbial kinetics, therefore, fundamental knowledge of culture dynamics is needed, which can help to understand, optimize, and control those reactors. Enrichment or inoculation of naturally occurring or genetically engineered microorganisms with specialized capabilities to the reactors is a recent great concern (Fujita and Ike, 1994; Limbergen et al., 1998; Stephenson and Stephenson, 1992; Soda et al., 1998). For successful wastewater treatment using such reactors, an effective strategy for maintaining the populations of the specialized microorganisms in the reactor is required.

Aris and Humphery (1977) studied the qualitative behavior of two microorganisms competing for an inhibitory substrate in a completely mixed reactor. They described three steady-states in the reactor depending on the relative disposition of the two growth-rate curves of the microorganisms, the dilution rate, and the substrate concentration in the feed: complete wash-out, growth of one microorganism and wash-out of the other, and coexistence of both microorganisms. However, the coexistence is only possible at the very point where the dilution rate exactly equals the crossing point of the two growth-rate curves.

Wastewater treatment processes successfully utilizing biofilms such as trickling filters, rotating biological contactors, and submerged filters, are regarded as more robust than well-mixed processes (Rittmann, 1982). This is often explained by protection of microorganisms in inner layers of the biofilm from the harsh outer environment. There are, however, few quantitative studies of the population dynamics in biofilms using adequate mathematical models and simulations (Furumai and Rittmann, 1994; Rittmann and Manem, 1992; Wanner and Gujer, 1986; Wanner and Reichert, 1996). Those models were mainly used for evaluation of the spatial distribution of microorganisms with different nutritional requirements, such as heterotrophic and autotrophic microorganisms. In this study, a simple numerical model of a biofilm reactor is developed. Using the model, competition between two microorganisms utilizing a common inhibitory substrate in a biofilm is simulated and the difference of population dynamics in the biofilm from that in a completely mixed reactor is discussed.

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MODEL SYSTEM AND GOVERNING EQUATIONS

Modeling of biofilms is so complicated that it is necessary to make some assumptions (Wanner and Gujer, 1986). In this study, the following fundamental assumptions were made: (1) the biofilm has a uniform thickness and is composed of fixed segments; (2) growth of two microorganisms A and B which utilize a common substrate is expressed by Haldane kinetics with a spatial limitation term and is independent of other microorganisms in the biofilm reactor; (3) diffusion of the substrate, movement of the microorganisms, and continuous loss of the biomass by shearing are expressed by Fick's Law-type equations.

Figure 1 illustrates an idealized planar biofilm with a uniform thickness L_f (m). The biofilm is divided into N segments for simulation purposes and each has a thickness of $\Delta Z = L_f/N$ (m). Wastewater containing the substrate is fed to the reactor at a constant-feed rate and a concentration S_f (mg/L). The bulk liquid in the reactor is mixed throughout the tank and the substrate diffuses into the biofilm. The substrate is transported from the bulk liquid having a concentration $S[0]$ (mg/L) to the surface of the biofilm having a concentration $S[1]$ (mg/L). A diffusion layer of a thickness L_l (m) is used to represent the external mass-transport resistance.

Mass Balances in Bulk Liquid

The exchange of microbial cells between biofilm and bulk liquid is a very important process in biofilm systems. However, modeling of simultaneous attachment and detachment to/from biofilms and flocs is complicated and has not been fully studied (Soda et al., 1999). Here, the mass balances in the bulk liquid for the substrate and microorganisms A and B with a continuous flow are simply described as following:

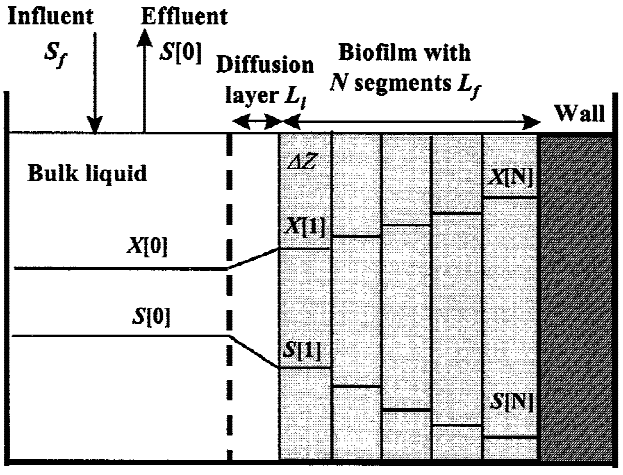

Figure 1. Completely mixed tank with idealized biofilm consisting of N segments with fixed thickness.

$$\frac{dS[0]}{dt} = D(S_f - S[0]) - aD_S\frac{S[0]-S[1]}{\Delta Z} - \frac{\mu_A[0]X_A[0]}{Y_A} - \frac{\mu_B[0]X_B[0]}{Y_B} \quad (1)$$

$$\frac{dX_A[0]}{dt} = -DX_A[0] - aD_{XA}\frac{X_A[0]-X_A[1]}{\Delta Z} + (\mu_A[0] - b_A)X_A[0] \quad (2)$$

$$\frac{dX_B[0]}{dt} = -DX_B[0] - aD_{XB}\frac{X_B[0]-X_B[1]}{\Delta Z} + (\mu_B[0] - b_B)X_B[0] \quad (3)$$

where S is substrate concentration (mg/L). X_A and X_B are biomass of microorganisms A and B (mg/L), respectively. Each number in the brackets refers to the bulk liquid or a segment illustrated in Figure 1. D_X, b, Y, and μ are diffusion coefficient of microorganisms (m^2/d), biomass decay coefficient (d^{-1}), yield coefficient (−), and net specific-growth rate (d^{-1}). Subscripts A and B refer to microorganisms A and B. D, D_S, a, and t are dilution rate (d^{-1}), diffusion coefficient of substrate (m^2/d), specific area perpendicular to the flux (m^{-1}), and time (d), respectively.

Mass Balances in Biofilm

Reactions within the biofilm are described by diffusion reaction equations. The mass balances of the surface segment are described as following:

$$\frac{dS[1]}{dt} = D_S\frac{S[0]-S[1]}{L_l\Delta Z} - D_S\frac{S[1]-S[2]}{\Delta Z^2} - \frac{\mu_A[1]X_A[1]}{Y_A} - \frac{\mu_B[1]X_B[1]}{Y_B} \quad (4)$$

$$\frac{dX_A[1]}{dt} = D_{XA}\frac{X_A[0]-X_A[1]}{L_l\Delta Z} - D_{XA}\frac{X_A[1]-X_A[2]}{\Delta Z^2} + (\mu_A[1] - b_A)X_A[1] \quad (5)$$

$$\frac{dX_B[1]}{dt} = D_{XB}\frac{X_B[0]-X_B[1]}{L_l\Delta Z} - D_{XB}\frac{X_B[1]-X_B[2]}{\Delta Z^2} + (\mu_B[1] - b_B)X_B[1] \quad (6)$$

Component mass balances are written for each segment ($i = 2, \ldots, N\text{-}1$), where:

$$\frac{dS[i]}{dt} = D_S\frac{S[i-1]-2S[i]+S[i+1]}{\Delta Z^2} - \frac{\mu_A[i]X_A[i]}{Y_A} - \frac{\mu_B[i]X_B[i]}{Y_B} \quad (7)$$

$$\frac{dX_A[i]}{dt} = D_{XA}\frac{X_A[i-1]-2X_A[i]+X_A[i+1]}{\Delta Z^2} + (\mu_A[i] - b_A)X_A[i] \quad (8)$$

$$\frac{dX_B[i]}{dt} = D_{XB}\frac{X_B[i-1]-2X_B[i]+X_B[i+1]}{\Delta Z^2} + (\mu_B[i] - b_B)X_B[i] \quad (9)$$

The mass balances of the boundary segment on the support wall are described by following equations:

$$\frac{dS[N]}{dt} = D_S \frac{S[N-1] - S[N]}{\Delta Z^2} - \frac{\mu_A[N]X_A[N]}{Y_A} - \frac{\mu_B[N]X_B[N]}{Y_B} \tag{10}$$

$$\frac{dX_A[N]}{dt} = D_{XA} \frac{X_A[N-1] - X_A[N]}{\Delta Z^2} + (\mu_A[N] - b_B)X_A[N] \tag{11}$$

$$\frac{dX_B[N]}{dt} = D_{XB} \frac{X_B[N-1] - X_B[N]}{\Delta Z^2} + (\mu_B[N] - b_B)X_B[N] \tag{12}$$

The "diffusion" coefficients of microorganisms, D_{XA} and D_{XB}, represent displacement by cell division and by shearing off at the film boundary contacting the bulk liquid.

Growth Kinetics of Microorganisms

The inhibitory influence of high-substrate concentration was described by Haldane kinetics. The two types of microorganisms compete for substrate but in the biofilm they also have to compete for the limited space available. Therefore, growth of the microorganisms was described by Haldane kinetics with a spatial-limitation term which was originally proposed as cell-inhibition kinetics by Han and Levenspiel (1988).

$$\mu_A[i] = \frac{\mu_{mA} S[i]}{K_{SA} + S[i] + \dfrac{S[i]^2}{K_{IA}}} \left(1 - \frac{X_A[i] + X_B[i]}{X_m}\right) \tag{13}$$

$$\mu_B[i] = \frac{\mu_{mB} S[i]}{K_{SB} + S[i] + \dfrac{S[i]^2}{K_{IB}}} \left(1 - \frac{X_A[i] + X_B[i]}{X_m}\right) \tag{14}$$

where K_I, K_S, and μ_m are inhibition constant (mg/L), half-saturation constant (mg/L), and maximum specific-growth rate (d^{-1}). X_m (mg/L) is the maximum capacity of total biomass of microorganisms A and B in a segment.

The formulation of the spatial-limitation term used here is the most simple one possible with non-restricted growth at 0-biomass concentration and zero growth at maximal biomass concentration X_m.

PARAMETER VALUES AND NUMERICAL METHODS

Basically, four relative dispositions of the growth-rate curves [Eqs. (13) and (14)] of the two microorganisms are possible. The growth-rate curves of the four cases which are calculated with X_m set to be infinite are shown in Figure 2.

In case 1, microorganism A grows faster than microorganism B at any substrate concentration. In case 2, the stable branches of the growth curves cross each other. Microorganism A can grow faster than microorganism B at substrate concentrations higher than the intersection point. In case 3, the unstable branches of the growth curves cross each other. Here, microorganism A can grow faster than microorganism B at substrate concentrations lower than the intersection point. In case 4, the stable branch of the growth-rate curve of microorganism A crosses the unstable branch of the growth-rate curve of microorganism B. Microorganism A can now grow faster than microorganism B at substrate concentrations higher than the intersection point.

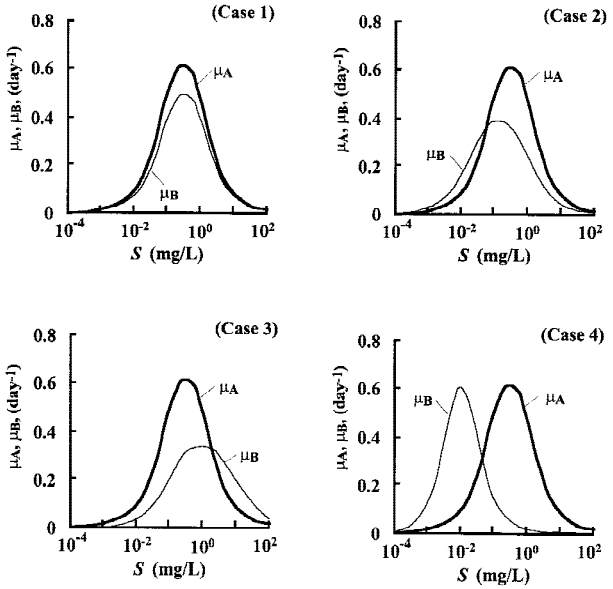

Figure 2. Growth-rate curves of microorganisms A and B [Eqs. (13) and (14)] without the spatial-capacity limitation (X_m = symbol 165 \f "Symbol" \s 12∞). Growth parameters in cases 1–4 are listed in Table I.

The four relative dispositions of the growth-rate curves are determined by combination of the six parameters, μ_{mA}, μ_{mB}, K_{SA}, K_{SB}, K_{IA}, and K_{IB}. For simulations of cases 1–4, arbitrary parameters listed in Table I were assumed. It was also assumed that the biofilm consisted of five segments ($N = 5$), and physicochemical parameters of the biofilm are listed in Table II.

The diffusion coefficient of substrates such as acetate, ammonium, and oxygen, in biofilms is often assumed to be $10^{-5} - 10^{-4}$ m^2/d for simulation studies (Rittmann and Manem, 1992; Wanner and Gujer, 1986). The diffusion coefficient of microorganisms in biofilms has not been fully

Table I. Bacterial model parameter values used in cases 1–4 of Figure 2.

	Microorganism A	Microorganism B			
	Case 1–4	Case 1	Case 2	Case 3	Case 4
μ_m ($^{-1}$)	1.0	0.8	0.4	0.5	1.8
K_S (mg/L)	0.1	0.1	0.1	0.02	0.01
K_I (mg/L)	1.0	1.0	10	1.0	0.01
b (d^{-1})	0.05	0.05			
Y (−)	0.5	0.5			
D_X (m^2/d)	5×10^{-8}	5×10^{-8}			

Table II. Physicochemical parameters of the biofilm.

a (m^{-1})	2000	L_f (m)	1.0×10^{-3}
D_S (m^2/d)	5×10^{-5}	X_m (mg/L)	400
L_l (m)	1.0×10^{-4}	ΔZ (m)	2.0×10^{-3}

studied but should be much smaller than the diffusion coefficients of microorganisms in pure water. The random motility coefficient of microorganisms in the liquid phase is evaluated to within the magnitude of $10^{-7} - 10^{-5}$ m^2/d (Ford et al., 1991). Diffusion coefficient of heterotrophs, autotrophs, and inert biomass in a biofilm was assumed 5×10^{-9} m^2/d for a simulation study (Wanner and Reichert, 1996). In this study, the diffusion coefficients in the biofilm, D_{XA} and D_{XB}, are assumed to be 1.0×10^{-8} m^2/d. The biomass concentration in biofilms was estimated or assumed to be 5–100 g/L (McCarty et al., 1981; Rittmann and Manem, 1992; Zhang and Bishop, 1994). Here, the maximum biomass capacity of microorganisms A and B in the biofilm containing other microorganisms, X_m, was assumed to be only 400 mg/L corresponding to a loose biofilm.

Simulations were carried out at various values of the operational parameters, D and S_f, using the Rosenbrock method from the simulation program MADONNA for Windows (Hannon and Ruth, 1997).

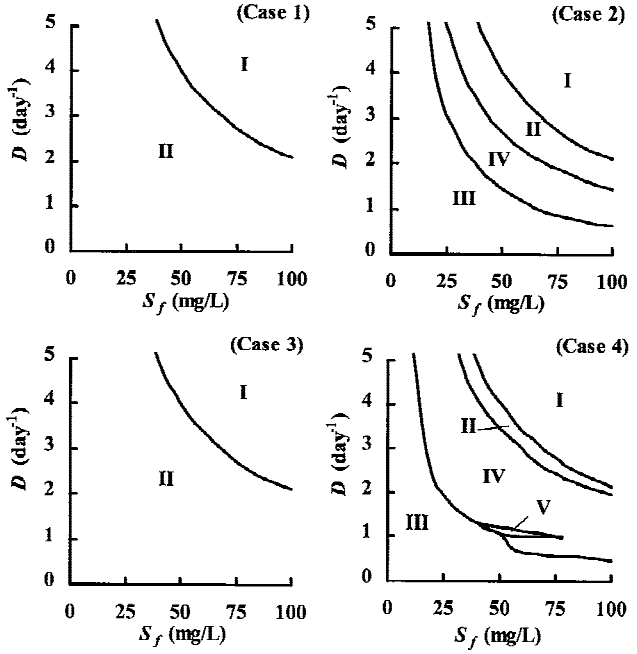

Figure 3. Operating diagrams for the biofilm reactor in cases 1–4. Region I: complete wash-out of both microorganisms, region II: wash-out of microorganism B, region III: wash-out of microorganism A, region IV: stable coexistence, and region V: oscillatory coexistence. In regions II–V, wash-out of either or both microorganisms is also observed depending on the initial conditions.

RESULTS

Operational Diagrams of the Biofilm Reactor

The nature of solutions of the equations depends clearly on the assumed model parameters and imposed operational conditions. This dependence is summarized in operating diagrams (Fig. 3).

The microorganisms can survive in the reactor at higher dilution rates than their maximum specific-growth rates because of the biofilm. The operating plane ($S_f - D$) is divided into five regions, I–V. In region I, the only stable steady-state is wash-out of both microorganisms from the biofilm reactor. However, in the other four regions, there exists stable steady- or oscillatory-states in which at least one microorganism can survive depending also on the initial conditions. In region II, microorganism B is washed out from the biofilm but the population of microorganism A establishes itself. In region III, microorganism A is washed out but the population of microorganism B survives. In region IV, both microorganisms coexist stably with one another. Interestingly, in region V, both microorganisms coexist with a sustained oscillatory behavior. Convergence to regions II–V is additionally dependent on the initial conditions. Wash-out of either or both microorganisms is also observed if the initial populations of microorganisms A or B are too small compared to the initial substrate concentrations, in other words, multiple steady-states are observed.

In cases 1 and 3, the diagrams consist of only two regions, I and II. In case 2, the diagram consists of four regions, I–IV. In case 4, the diagram consists of five regions, I–V. These results suggest that for coexistence of both microorganisms it is necessary that one stable branch of the growth-rate curve crosses the other growth rate curve. It is important to note that in regions IV and V microorganism B cannot survive in the biofilm without microorganism A. This is explained by the fact that microorganism A decreases the substrate concentrations enough to allow microorganism B, which is more sensitive to the substrate inhibition, to survive in the biofilm.

Oscillatory Coexistence of the Microorganisms in the Biofilm

The competition in case 4 is an interesting case because both stable steady (region IV) and oscillatory coexistence (region V) are observed. For an example of the oscillations, $X_A[1]$, $X_B[1]$, and $S[1]$ at $D = 1.2$ d^{-1} and $S_f = 50$ mg/L in region V are shown in Figure 4. In this situation, the period of the oscillations is about 18 d. The trajectories in X_A, X_B, and S space are shown in Figure 5. The oscillation in the bulk liquid and in all the segments of the biofilm occurs at the same period. A plot of μ_A, and μ_B versus S indicates that the oscillations occur at the intersection of the unstable branch of the growth-rate curve of microorganism B and the stable branch of microorganism A (Fig. 6). In this situation, microorganism A is always on the stable branch, whereas microorganism B is on the stable branch only in the inner segments 4 and 5 of the biofilm. Growth of microorganism

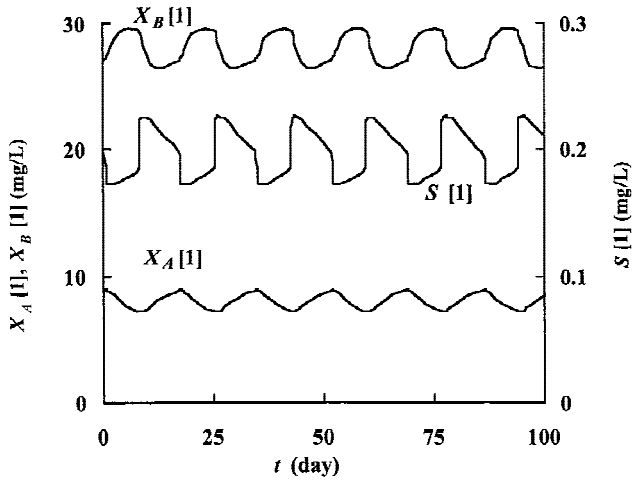

Figure 4. Time course of $X_A[1]$, $X_B[1]$, and $S[1]$ at $D = 1.2\ d^{-1}$ and $S_f = 50$ mg/L in region V of case 4.

A can suppress the wash-out of microorganisms B, however, large enough perturbation of the substrate concentration causes wash-out of microorganism B.

Effect of the Operational Parameters on the Populations

Steady-states at $D = 3.0\ d^{-1}$ with various S_f values in case 4 are shown in Figure 7. Top and bottom panels in the figure show substrate and biomass profiles of the biofilm reactor, respectively. Substrate consumption rates, $\mu_A X_A/Y_A$ and $\mu_B X_B/Y_B$, which indicate microbial activities in the bulk liquid and the segments are also shown in the middle panels. Substrate concentrations and the total biomass of the microorganisms in the reactor increase with increasing substrate concentration in the feed. Thus, drastic changes in the substrate-consumption rate and the biomass of the specific microorganisms in the segments are observed. At S_f values lower than 17 mg/L (region III), microorganism A is washed out but microorganism B can survive in the biofilm depending on the initial conditions. From 17 to 58 mg/L of the S_f value (region IV), the microorganisms can coexist with one another, and an increase in the S_f value brings an advantage for the survival of microorganism A. At S_f values higher than 58 mg/L (region II), microorganism B is washed out and only microorganism A can survive in the biofilm. At S_f values higher than 69 mg/L (region I), both microorganisms are completely washed out. The total biomass concentration in inner layers is always higher than that in outer

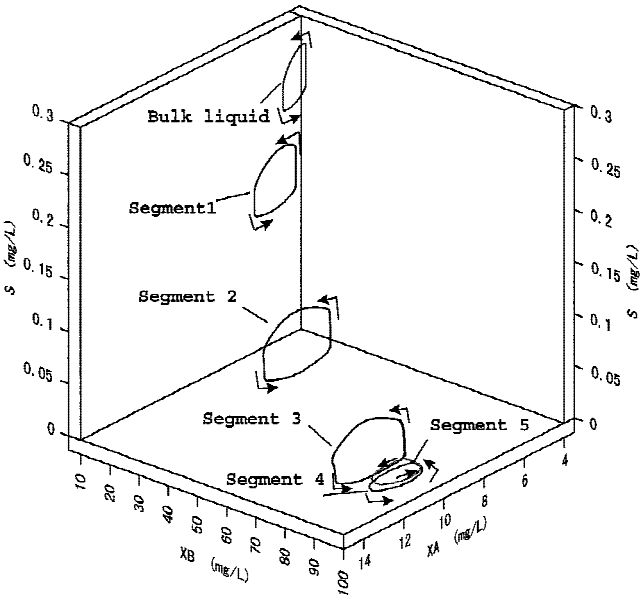

Figure 5. Trajectories in X_A, X_B, and S space at $D = 1.2\ d^{-1}$ and $S_f = 50$ mg/L in region V of case 4. Indices as specified in Figure 1.

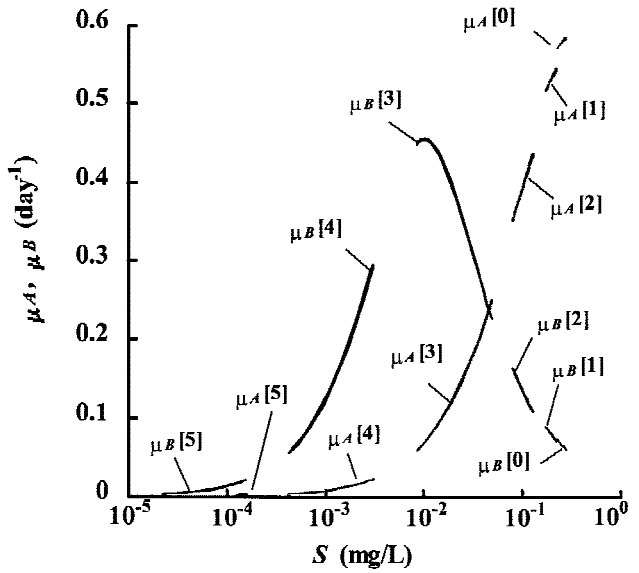

Figure 6. Specific-growth rate (μ_A and μ_B) and substrate (S) variations in bulk liquid [0] and biofilm segments ([1]–[5]) versus S at $D = 1.2\ d^{-1}$ and $S_f = 50$ mg/L in region V of case 4. Indices as specified in Figure 1.

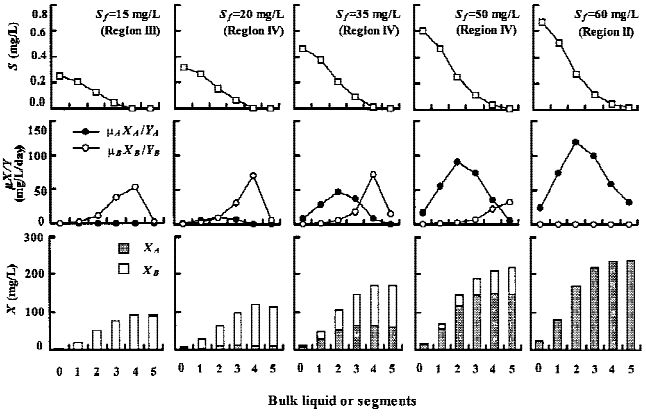

Figure 7. Effect of the substrate concentration in influent on populations of microorganisms A and B in the biofilm at $D = 3.0\ d^{-1}$ in case 4. Indices as specified in Figure 1.

layers in all simulations. It is interesting that the highest substrate consumption rate is observed in the inner segments 2, 3, or 4, but not in the outer segment 1 nor in the inner segment 5. This is because the outer segments suffer from higher sheering stress, while the substrate is essentially depleted in the innermost segments.

Figure 8 shows the result of simulations at $S_f = 80$ mg/L with various D values in case 4. At D values lower than 0.58 d^{-1} (region III), microorganism A is washed out but microorganism B can survive in the biofilm depending on the initial conditions. From 0.58 to 2.3 d^{-1} of the D value (region IV), the microorganisms can coexist stably, and an increase in the D value brings an advantage for microorganism A. At S_f values higher than 2.3 d^{-1} (region II), microorganism B is washed out and only microorganism A can survive. At D values higher than 2.6 mg/L (region I), both microorganisms are completely washed out from the biofilm reactor. Substrate concentrations, the total biomass, and the population ratio of microorganism A in the biofilm segments increase with the D value. However, the total biomass in the bulk liquid is little affected by the change of the D value. This is because an increase in the dilution rate means not only an increase in the substrate loading on the microorganisms, but also an increase of biomass seeding from the biofilm to the bulk liquid represented by the diffusion-type terms in Eqs. (2) and (3).

DISCUSSION

Some biofilm models have been already developed and are classified into 10 different types in view of the microbial-spatial distribution in biofilms (Wanner and Gujer, 1986). The model developed in this study will belong to the monolayer- (monosegment-) series type (McCarty et al., 1981) or variable-type models (Kissel et al., 1984), and competition of two microorganisms for a single substrate expressed by these models has not been studied as far as we know. The assumptions used in this model are not always fully valid, because an increase in the thickness of the biofilm due to the growth of the microorganisms A and B is not considered. Thus, little quantitative information on exchange of microorganisms between biofilms and bulk liquid and diffusion of microorganisms in biofilms are available. It is also not fully justified whether the spatial-limitation term is applicable to population dynamics of actual biofilm microorganisms. However, in many practical cases, a stationary biofilm thickness will be obtained, and in such cases, the model presented here seems adequate to understand the fundamental phenomena.

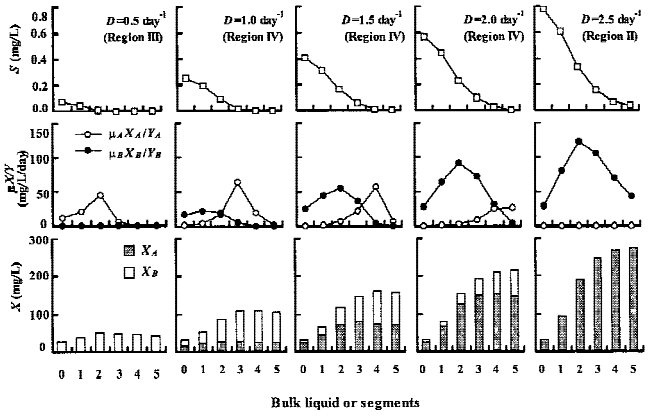

Figure 8. Effect of the dilution rate on populations of microorganisms A and B in the biofilm at $S_f = 80$ mg/L in case 4. Indices as specified in Figure 1.

The competition of two microorganisms in the biofilm is summarized in Table III. The multiple-steady states are characteristic of the Haldane kinetics with single and multiple microorganisms (Aris and Humphery, 1977). Only four sets of the parameters (cases 1–4) were used, however, it is enough to demonstrate the fact that behavior of microorganisms in the biofilm is very different from that in a completely mixed reactor. The coexistence of microorganisms in the completely mixed reactor is observed only at one fixed-dilution rate, but the practical impossibility of maintaining that very dilution rate would result in exclusion of one or the other of the microorganisms, if this situation were studied experimentally. It is difficult for microorganisms to coexist in a completely mixed environment without any secondary rate-limiting substrate (Taylor and Williams, 1975) or autoinhibitory metabolite (Freitas and Fredrickson, 1978). It is a novel finding that the microbial populations in the biofilm show an oscillatory behavior in a wide range of operational conditions. Oscillations generally require a specific ratio of feed-forward and feed-back influences within the system. It is well-known that populations expressed by the Lotka-Volterra model (which describes a predator–prey relationship) exhibit stable steady-states, unstable steady-states, and oscillatory behavior (Lotka, 1920). Important examples for the oscillatory behavior described in the literature also include single microbial systems, e.g., *Saccharomyces cerevisiae*, where this type of control is occurring within the metabolic pathway during storage and remobilization of glycogen as described in a simple model (Heinzle et al., 1982). The coexistence and oscillatory behavior of the microorganisms observed in this study without any predator and without any metabolite prove nothing about any specific biofilm, however, it was confirmed that biofilms can play a significant role in the dynamics of microbial populations, particularly in the survival of species in a competitive environment.

Table III. Summary of behavior of the microorganisms in the biofilm.

	Multiple steady-state	Coexistence	Oscillation (observed)
Case 1	+	−	−
Case 2	+	+	−
Case 3	+	−	−
Case 4	+	+	+

NOMENCLATURE

a	specific area perpendicular to the flux, related to bulk liquid volume (m^{-1})
b	biomass decay rate (d^{-1})
D	dilution rate (d^{-1})
D_S	diffusion coefficient of substrate (m^2/d)
D_X	diffusion coefficient of microorganisms (m^2/d)
K_I	inhibition constant (mg/L)
K_S	saturation constant (mg/L)
L_l	thickness of diffusion layer (m)
L_f	thickness of biofilm (m)
N	number of segments in biofilm (−)
$S[i]$	substrate concentration in element i (mg/L)
S_f	substrate concentration in feed (mg/L)
$X[i]$	biomass (mg/L)
X_m	spatial capacity of total biomass of microorganisms A and B in a segment (mg/L)
Y	yield coefficient (−)
ΔZ	thickness of each segment (m)
μ	maximum specific growth rate (day^{-1})

Subscripts

A	refers to microorganism A
B	refers to microorganism B

Numbers in brackets

0	refers to bulk liquid
1–5	refers to segments 1–5

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