Diffusion Coefficients of Phenol and Oxygen in a Biofilm of *Pseudomonas putida*

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A pure culture of Pseudomonas putida was grown as a film on carbon particles in a differential fluidized bed biofilm reactor, a new experimental system for the application of diffusion-reaction models. In the active biofilm, effective diffusion coefficients of the essential substrates — phenol and oxygen — were simultaneously calculated. The multisubstrate biokinetic expression used in the model solution was derived by nonlinear regression analysis of the data of continuous system fermenter experiments. The determined biokinetic equations were utilized to solve the diffusion-reaction model for effective diffusion coefficients in the active biofilm. The ratio of the evaluated effective diffusion coefficient through active biofilm to that of water varied between 17–44% and 9–24% for phenol and oxygen, respectively, for different biofilm densities. Results of the study showed a fair agreement with the literature at low biofilm densities.

Introduction

Although great attention has been paid to the effective diffusion coefficient in the modeling of biofilm reactors, there is surprisingly little agreement about how the presence of biofilm influences the diffusivity. This is due to the fact that the character of a biofilm depends upon the type of organism growing in it and to the many different techniques that have been used to evaluate the diffusion coefficient (Matson and Characklis, 1976).

To assess the effective diffusion coefficient of a substrate in a biofilm, three approaches have been considered in the literature: (1) evaluation of intrinsic reaction kinetics from a separate experimental study and fitting the model under consideration to the experimental data (LaMotta, 1976); (2) evaluation of internal mass-transfer rate by measuring concentration gradient through an active biofilm and coupling it with the flux of the substrate into the biofilm (Zbigniew et al., 1991); (3) evaluation of internal mass-transfer rate from the flux across an inactive biofilm (usually killed by chemicals) in a special environment (Fan et al., 1990; Zbigniew et al., 1991).

In the first method, the effective diffusion coefficient has been used as the fitting parameter in reactor models (Andrews and Tien, 1981). Many authors evaluated different effective diffusion-coefficient magnitudes for the same substrate by this method (Andrews and Tien, 1981; Mulcahy et al., 1981; Ngian and Lin, 1976; Wang, 1981), which illustrated serious drawbacks from assumed models. This difference was likely due to the reactor hydrodynamics, which was not exactly the same with those predicted by the models. The second approach is a direct method for measuring effective diffusion coefficient in active biofilms, but measuring a concentration profile within a biofilm requires special probes to detect targeted molecules. This microelectrode technique was employed for the first time by Bungay and Harold (1971), and recently by Zbigniew et al. (1991) for the determination of effective diffusion coefficient of oxygen in the microbial film. This method is able to avoid the negative effects of nonideal reactor conditions on the measurement of effective diffusion coefficients; however, there is difficulty in finding appropriate probes for different substrate molecules. The third method uses an "inactive" biofilm placed in a special chamber, and the concentration gradient is measured at steady state or after a pulse input given to the chamber. In this method one must be certain that the biofilm is completely killed so that no reaction occurs; however, this condition cancels the natural actions in the living biofilm such as release of gaseous products or symbiotic relationships of active microorganism

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species. It is also possible that the process of deactivating or reforming a biofilm may alter the natural infrastructure of the biofilm.

The given methods in the literature have been generally employed to determine the effective diffusion coefficient for a single limiting substrate. However, none of them has dealt with the case of simultaneous diffusion when more than one substrate limit the growth (Fan et al., 1990; Ngian and Lin, 1976). A multisubstrate limited growth is not unusual (Bailey and Ollis, 1986); in the case of higher biofilm densities, the reaction rate increases such that even the substrates abundant in the bulk phase may become growth limiting inside the biofilm. Moreover, only a limited amount of research investigated the effects of biofilm density on effective diffusion coefficient (Beyenal and Tanyolaç, 1994; Fan et al., 1990), and most researchers used reformed or dead biofilms having a fixed biofilm density for the estimation of diffusion coefficient of a single substrate (Fan et al., 1990; Onuma et al., 1985). However, biofilm density changes during the propagation of the biofilm and naturally mass-transfer resistance dominates at higher density values of the biofilm (Şeker et al., 1995; Denac et al., 1988).

In the modeling of biofilm reactors, the diffusion-reaction model has been widely applied to describe substrate utilization in biofilms (Tang and Fan, 1987; Grady, 1983; Ngian and Lin, 1976). This method has been used in this study with a new experimental technique. In the work, a pure culture of *Pseudomonas putida* was grown as a film on carbon particles in a differential—gradientless—fluidized-bed biofilm reactor (DFBBR). Pseudo-steady-state conditions were established within this reactor, and thus the stable substrate concentrations and flux values were obtained within definite time intervals along with homogeneous biofilm thickness and density. The growth kinetics of the culture was studied in a continuous bioreactor and a multisubstrate growth model was developed. This kinetic model was used to describe limiting substrate utilization in the biofilm, and the limiting substrates were determined to be phenol and oxygen for the culture. Then the effective diffusion coefficients of these substrates in the biofilm were calculated simultaneously with a diffusion-reaction model. Gradientless properties of the differential fluidized-bed reactor enabled convenient and reliable operational and boundary conditions for the model.

Material and Methods

Microorganism and growth medium

The biofilm attached onto carbon support particles was a pure culture of *Pseudomonas putida* strain No. P71 (from Tiermedizin und Tierhygine Universität Hohenheim-Germany). The culture was capable of utilizing phenol to satisfy growth and energy requirements. In our experience, the biofilm grew more rapidly and remained stable on carbon particles (Bacmu) than any other support material such as glass, sand, or polymeric beads (Şeker et al., 1995; Beyenal and Tanyolaç, 1994).

Artificial growth medium constituents were determined by reviewing the literature studies (Livingston and Chase, 1989) for fluidized-bed experiments with the following composition in kg/m^3: Phenol (Merck, Germany), 0.5; K_2HPO_4 (BDH, England), 0.420; KH_2PO_4 (Riedel-de Haen, Germany), 0.375; $(NH_4)_2SO_4$ (Merck, Germany), 0.244; NaCl (Merck, Germany), 0.015; $CaCl_2 \cdot 2H_2O$ (Merck, Germany), 0.0198; $MgSO_4 \cdot 7H_2O$ (Merck, Germany), 0.0614; $FeCl_2 \cdot 4H_2O$ (Merck, Germany), 0.0032, respectively. The medium was prepared with fresh distilled water. To prevent precipitation of Ca^{+2} and Mg^{+2} ions in the growth medium, a stock solution of $CaCl_2 \cdot 2H_2O$, $MgSO_4 \cdot 7H_2O$, and NaCl salts was prepared and added in sufficient quantities to the solution of other medium compounds. Potassium monophosphate was used as a buffer and ferrous chloride was used to prevent precipitation during autoclaving.

Growth kinetics of the culture

Growth kinetics data of the culture were obtained using continuous fermenter experiments. Optimum growth conditions for *Pseudomonas putida* were reported in the literature as 30°C and pH 6.8 (Hill and Robinson, 1975; Yang and Humphrey, 1975). However, in this study room temperature was chosen as the working temperature since most of the wastewater treatment systems operate at around 25°C.

All continuous system runs were carried out in a temperature, pH, dissolved oxygen, and agitation-rate-controlled New Brunswick (BioFlo II) fermenter with a working volume of 5 L. Sensitivity ranges of control units for dissolved oxygen, pH, and agitation were 0.1%, 0.01 unit, and ± 1 rpm, respectively. pH was controlled at 6.8 by adding sterilized 0.1 N NaOH solution intermittently. For setting up the continuous system, a batch culture was initiated by the inoculation of 500 mL inoculum culture (10%, v/v) into the fermenter containing the growth medium. Agitation rate applied was 400 rpm, so that microorganism would grow without flocculation. When entered into exponential growth phase, continuous pumping of fresh feed was started. In order to establish a steady state, the reactor was left to equilibrate over four or five retention times and steady state was assured if the absolute differences in consecutive measurements of microorganism and dissolved oxygen concentrations were less than 3%. Several dilution rates up to washout point were applied and corresponding steady-state data were recorded. For a new steady state, dilution rate was increased carefully by a gradual increase in feed rate. A large perturbation was given to feed rate to enter the inhibition region of substrate phenol, and this region was completely scanned by slowly decreasing the dilution rate.

Differential fluidized-bed biofilm reactor

For the evaluation of effective diffusion coefficients by the suggested diffusion-reaction model, a well-defined biofilm reactor was necessary. The kinetic predictions of the biofilm model, together with practical considerations, led to the design of a glass column made of Pyrex glass that was 0.30 m in height and 0.025 m in diameter, having a tapered expansion section at the top. Around 2,500–3,000 carbon particles with 5.075×10^{-4} ($\pm 5 \times 10^{-6}$) m diameter were placed in the reactor before the startup of the system. The differential fluidized-bed biofilm reactor (DFBBR) system was designed with the experience of literature (Shieh and Keenan, 1986) and is presented in Figure 1.

During the runs, the flow rates in both columns were controlled by Watson-Marlow peristaltic pumps (Watson-Marlow

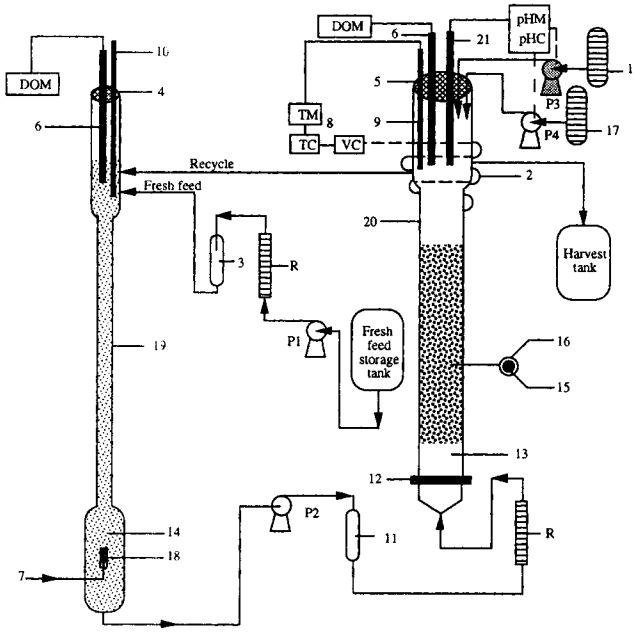

Figure 1. Differential fluidized bed biofilm reactor setup.

P1: Pump for fresh feed; P2: fluidized-bed combined feed pump; P3: pump for base; P4: pump for acid; R: rotameter; DOM: dissolved oxygen meter; pHM: pH meter; pHC: pH controller; TC: temperature controller; TM: temperature measurement; VC: voltage control; 1: base storage; 2: heating strip 3: trap; 4,5: sponge plugs; 6: dissolved-oxygen electrode; 7: air feed; 8: temperature control unit; 9: thermocouple; 10: thermometer; 11: pulse-fader; 12: steel screen; 13: nonfluidized medium (river sand); 14: air bubbles; 15: support particle (carbon); 16: biofilm; 17: acid storage; 18: air sparger; 19: oxygenerator; 20: differential fluidized-bed biofilm reactor (DFBBR); 21: pH electrode.

Limited, England). To prevent pulsing effect of the pumps, a pulse-fader was placed at the entrance of the DFBBR.

Differential fluidized-bed biofilm reactor runs

For the first propagation of the microorganism on carbon particles, the fluidized-bed system was recirculated with the fresh culture grown in the fermenter. The system was operated in batch mode until a thin layer of biofilm was developed on support particles. Then the whole system together with bioparticles was rinsed with sterile distilled water to remove any microorganism other than in the biofilm. Subsequently, a flow of feed was started at an average dilution rate of 6.94×10^{-4} s^{-1} and minimum fluidization rate was used so as not to strip the biomass due to high shear rate in the DFBBR. Using high dilution rate (> four times of the maximum specific growth rate of the culture) provided growth only on the support material and enabled washout of any free suspended biomass. In all the runs, high recycle ratio (> 100) was employed for DFBBR so as to establish continuous stirred tank reactor behavior in the differential bed, which was assessed theoretically and checked experimentally. Neither concentration gradient of any substrate nor nonhomogeneous biofilm properties were detected within the reactor. The recycle and fresh feed flow rates were maintained around 9.0×10^{-4} m^3/s and 7.2×10^{-6} m^3/s, respectively. The biofilm thickness was the main parameter to terminate a run. When a definite thickness of the biofilm was obtained, the experiment was terminated and all the bioparticles were used to measure the biofilm density. For every run, medium temperature and pH were controlled at 25°C and 6.8 units, respectively.

Analytical methods

Phenol concentration in the liquid phase was measured by means of an HPLC (Cecil 1100) column (Chromosorb) constructed with a liquid chromatography pump (CE 1100), a variable wavelength monitor (CE 11220), and an integrator (HP 4495). Dissolved-oxygen concentration was monitored with a Gallencamp DO meter (Gallencamp, England) for DFBBR. Oxygen consumption rate was measured using a dynamic method suggested by Bandyopdhyay et al. (1967).

The biofilm thickness was measured by an optical microscope (Zeiss, D-73446, Germany) equipped with a micrometer. The average biofilm thickness was determined by measuring the biofilm thickness of 80–100 bioparticles taken from various heights of the bed (Fan et al., 1990).

The density of the biofilm as well as microorganism concentration in continuous fermenter experiments were determined in accord with standard dry-weight measurements (APHA, 1962). After a run, all the bioparticles were discharged and rinsed several times with distilled water, then filtered and dried at 105°C until constant biofilm weight was obtained. This weight is the dry weight of the biomass in the biofilm (W_d), and total biofilm volume (V_b) was calculated from the biofilm thickness and support particle radius. The biofilm density (X_f) was calculated from W_d/V_b.

Mathematical Model

Biokinetic-parameter estimation of the culture

A mass balance for the microorganism over a continuously fed stirred-tank reactor (CFSTR) yields $\mu = D = Q/V$, if nutrient feed does not include any microorganism (Bailey and Ollis, 1986).

During the experimental study, steady-state concentrations of microorganism, phenol, and oxygen at several dilution rates were measured, and phenol and oxygen consumption rates were calculated. These results were evaluated by a nonlinear regression program, namely Systat (1986), to obtain maintenance and yield factors in Eqs. 1 and 2.

A mass balance for phenol in a CFSTR, at steady state, gives

$$Q(S_{Pf} - S_P) = \left(\frac{\mu}{Y_{X/P}} + m_P\right) XV. \tag{1}$$

Experimentally determined oxygen consumption rate, OCR, can be related to oxygen balance over a CFSTR at steady state;

$$\mathrm{OCR} = \frac{\mu}{Y_{X/O}} + m_O. \tag{2}$$

According to the literature, the growth rate of *Pseudomonas putida* in a phenolic medium is limited by both phe-

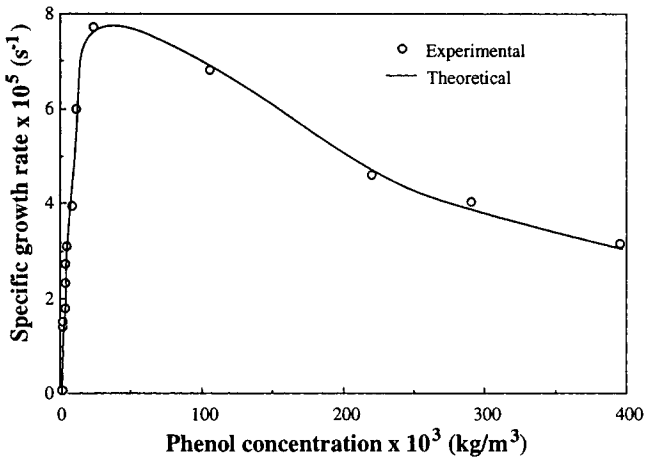

Figure 2. Change of experimental- and theoretical-specific growth rates with phenol concentration at corresponding experimental conditions.

nol and oxygen interactively, and the Monod equation for oxygen and Haldane inhibition kinetics for phenol were found to be the most appropriate models for dual-substrate expression (Livingston and Chase, 1989; Tang and Fan, 1987):

$$\mu = \mu_{\max} \frac{S_P}{K_P + S_P + \dfrac{S_P^2}{K_I}} \frac{S_O}{K_O + S_O}. \qquad (3)$$

Biokinetic parameters of the dual-substrate growth kinetics expression in Eq. 3 were evaluated by Systat as, $\mu_{\max} = 1.58 \times 10^{-4}$ s^{-1}, $K_P = 18.539 \times 10^{-3}$ kg/m^3, $K_I = 99.374 \times 10^{-3}$ kg/m^3, and $K_O = 0.048 \times 10^{-3}$ kg/m^3 ($R = 0.998$, least squared error (LSE) $= 2.75 \times 10^{-10}$).

Figure 2 shows the variation of experimental- and theoretical-specific growth rates calculated from Eq. 3 by phenol concentration at corresponding experimental conditions. A good agreement between the results were observed in Figure 2 with a regression coefficient value of $R = 0.998$.

Equations 1 and 2 were solved for phenol and oxygen—again with Systat—yield, and maintenance factors related to phenol and oxygen were found as $Y_{X/P} = 0.521$-kg micoorganism/kg phenol ($R = 0.979$, LSE $= 3.72 \times 10^{-4}$); $m_P \approx 0$-kg phenol/kg microorganism/s; and $Y_{X/O} = 0.338$-kg microorganism/kg oxygen ($R = 1$, LSE $= 8.18 \times 10^{-4}$), $m_O \approx 0$-kg oxygen/kg microorganism/s, respectively.

The value of Monod saturation constant for oxygen becomes important when phenol degradation rate is controlled by oxygen concentration. Atkinson and Mavituna (1983) listed values of K_O in the range of $0.4\text{–}0.0001 \times 10^{-6}$ kg/m^3 for different cultures. Wagner and Hempel (1983) experimentally determined K_O for *Pseudomonas sp.* A3 growing on naphthalene sulfonate as 2.6×10^{-6} kg/m^3 at 30°C. Livingston and Chase (1989) used Wagner and Hempel's (1988) results in their study considering both phenol and naphthalene sulfonate were aromatic compounds. However, our K_O for phenol degradation was 18 times greater than that of naphthalene sulfonate and was in the range of literature values listed by Atkinson and Mavituna (1983).

The value of $Y_{X/P} = 0.521$-kg microorganism/kg phenol in this study agreed well with the literature values for microorganisms growing on phenol [Hill and Robinson (1975), 0.52; Pawlowsky and Howell (1973), 0.545–0.616]. The value of $Y_{X/O} = 0.338$-kg microorganism/kg oxygen was found smaller than in the literature [Tang and Fan (1987), 0.496; Livingston and Chase (1989), 0.465]. Livingston and Chase (1989) also stated that the maintenance term was negligible for the culture according to dilution rate-yield coefficient correlation.

Calculation of effective diffusion coefficients

The fluidized-bed system can be divided into two major phases: the bulk phase and the microorganism phase (biofilm). In our experimental system, the fluidized bed was operated at high recycle ratio (> 100) and the difference between reactor and outlet phenol and dissolved oxygen concentrations in the fluidized bed were observed lower than 7×10^{-3} kg/m^3 and 2.5×10^{-4} kg/m^3, respectively. Thus fixed-bulk phenoland dissolved oxygen concentrations were assumed through the fluidized bed in the model.

For the substrate to be consumed, first it has to diffuse through a thin stagnant liquid film surrounding the bioparticle and then into the microorganism layer. Even though external mass-transfer effects may be important, a number of workers have concluded that they were not significant in their systems and therefore excluded from the model (Grady, 1983). For our system, external mass-transfer coefficients were calculated according to the given correlation for fluidized beds (Shieh and Keenan, 1986):

$$k_c = \frac{0.81}{\epsilon} \left(\frac{D_w^{4/3} U \rho_l^{1/3}}{\mu_l^{1/3} d_p} \right)^{1/2}. \qquad (4)$$

Using k_c, the difference between bulk and surface concentrations of the substrate were found smaller than 2.5×10^{-5} kg/m^3 and 4×10^{-5} kg/m^3 for phenol and oxygen, respectively. Moreover, Biot numbers (Bailey and Ollis, 1986) for the biofilm were calculated to be greater than 200. Thus, external mass-transfer resistance was neglected in the development of the model and surface concentrations of the limiting substrates were taken equal to bulk concentrations.

The biofilm grew on support particles in a reasonable spherical form. The average values of biofilm thickness and corresponding statistical data are presented in Table 1. In any run, the average biofilm thickness variation in Table 1 was $\pm 2.9 \times 10^{-6}$ m, so all bioparticles were assumed to have uniform diameter and spherical geometry. For a unique biofilm thickness in DFBBR, biofilm density can be assumed to be uniform in the system (Grady, 1983; Hoehn and Ray, 1973). The multisubstrate growth model developed for suspended culture was assumed valid for biofilm type of growth under pseudo-steady-state conditions (Andrews, 1982).

Buffer capacity of carbon particles was found to be lower than 1×10^{-6} kg phenol per carbon particle at highest-bulk phenol concentration during the operations, and no buffer capacity was observed for dissolved oxygen within the limits of instrumental sensitivity. Thus, adsorbed substrates on the carbon particles were neglected in the model. With these assumptions, the equations of continuity were written for phe-

Table 1. Measured Biofilm Thickness, Number of Measurements, and Statistical Outputs

Biofilm Thickness	Run No. 1 $\times 10^6$ m	Run No. 2 $\times 10^6$ m	Run No. 3 $\times 10^6$ m	Run No. 4 $\times 10^6$ m	Run No. 5 $\times 10^6$ m
Model	35	51	76	104	143
Arithmetic mean	35.30	51.40	75.55	104.48	143.20
Avg. deviation	2.68	2.02	2.72	3.94	3.35
Std. deviation	3.99	2.82	5.35	6.54	5.07
Geometric mean	35.13	51.34	75.35	104.27	143.12
Std. dev. for population	3.96	2.80	5.31	6.50	5.02
Variance	15.92	7.93	28.57	42.74	25.75
Total data no.	79	90	83	91	105

nol and oxygen on a spherical bioparticle at pseudo-steady state (Bird et al., 1960), respectively:

$$\frac{D_{fP}}{r^2}\frac{d}{dr}\left(r^2\frac{dS_P}{dr}\right)=\frac{\mu}{Y_{X/P}}X_f+m_P X_f \tag{5}$$

$$\frac{D_{fO}}{r^2}\frac{d}{dr}\left(r^2\frac{dS_O}{dr}\right)=\frac{\mu}{Y_{X/O}}X_f+m_O X_f. \tag{6}$$

Boundary conditions for these equations are

$$r=r_b \qquad S_P=S_{bP} \tag{7}$$

$$r=r_b \qquad S_O=S_{bO} \tag{8}$$

$$r=r_b \qquad \frac{T_{\text{biofilm}-P}}{4\pi r_b^2}=J_{rp}=D_{fP}\frac{dS_P}{dr}\Big|_{r=r_b} \tag{9}$$

$$r=r_b \qquad \frac{T_{\text{biofilm}-O}}{4\pi r_b^2}=J_{ro}=D_{fO}\frac{dS_O}{dr}\Big|_{r=r_b} \tag{10}$$

$$r_p\le r<r_b \qquad S_P\to 0 \qquad dS_P/dr=0 \quad \text{or}$$

$$r=r_p \qquad dS_P/dr=0 \tag{11}$$

$$r_p\le r<r_b \qquad S_O\to 0 \qquad dS_O/dr=0 \quad \text{or}$$

$$r=r_p \qquad dS_O/dr=0. \tag{12}$$

Boundary conditions in Eqs. 7–10 refer to experimental conditions that exist in the reactor. Substrate bulk concentrations in Eqs. 7 and 8 were measured, and substrate fluxes in Eqs. 9 and 10 were calculated from total surface area of the bioparticles and experimental overall substrate uptake rates. The boundary conditions of Eqs. 11 and 12 point out that there is no substrate flux at the biofilm–carbon interface owing to the rigid surface of support material. This boundary condition also prevails anywhere in the biofilm if the concentration of any substrate goes to zero (Andrews, 1982).

There is no analytical solution available for Eqs. 5 and 6, so they must be solved numerically. Moreover, effective diffusion coefficients are required *a priori* for the solution so, an iterative algorithm is developed. First, appropriate startup values for effective diffusion coefficients of phenol and oxygen (smaller than those in water) were assigned and the boundary conditions of Eqs. 9 and 10 were calculated from experimental data for each substrate. Then using the boundary conditions of Eqs. 7 to 10, Eqs. 5 and 6 turned into an initial-value problem of simultaneous differential equations for phenol and oxygen, respectively. The set of equations were simultaneously solved with fourth-order Runge-Kutta-Gill algorithm (Hewlett-Packard Contributed Program Series). During the progress of iterations, Eqs. 11 and 12 were repeatedly controlled either at $r=r_p$ or at any point of the biofilm. If substrate concentration goes to zero anywhere in the biofilm, the substrate derivative value also had to be zero at this point (Hoehn and Ray, 1973), and if flux goes to zero anywhere in the biofilm except at the biofilm–carbon interface, substrate concentration also had to be zero at this point. If these conditions for any substrate were not satisfied, a new set of effective diffusion coefficients were proposed automatically by the algorithm. This control also gives the active biofilm thickness for the biofilm since it is defined as the thickness in which any limiting substrate concentration and corresponding flux are greater than zero.

Equations 5 and 6 were solved simultaneously and predicted substrate consumption rates for a single bioparticle were calculated according to Eqs. 13 and 14:

$$T'_{\text{biofilm}-P}=\int_{r_p}^{r_b}\left(\frac{\mu}{Y_{X/P}}+m_P\right)X_f 4\pi r^2\,dr \tag{13}$$

$$T'_{\text{biofilm}-O}=\int_{r_p}^{r_b}\left(\frac{\mu}{Y_{X/O}}+m_O\right)X_f 4\pi r^2\,dr. \tag{14}$$

In the model solution, the shooting method (Vos et al., 1990) was used, and the relative error of predicted consumption rate of any substrate was found to be less than 3%.

Results and Discussion

The measured biofilm thickness values were presented in Table 1 along with statistical calculations. Table 2 represents the measured and calculated results of experimental runs in DFBBR.

Table 2. Experimental Results of the Study

X_f kg/m^3	L_f $\times 10^6$ m	S_{bp} $\times 10^3$ kg/m^3	S_{bo} $\times 10^3$ kg/m^3	$T_{\text{biofilm}-P}$ kg Phenol/ Bioparticle·s	$T_{\text{biofilm}-O}$ kg Oxygen/ Bioparticle·s
107	35	52.7	2.959	0.536×10^{-14}	0.825×10^{-14}
89	51	57.3	4.023	0.697×10^{-14}	1.074×10^{-14}
73	76	60.7	5.306	0.934×10^{-14}	1.443×10^{-14}
61	104	61.0	6.332	1.182×10^{-14}	1.822×10^{-14}
47	143	61.1	6.744	1.782×10^{-14}	2.746×10^{-14}

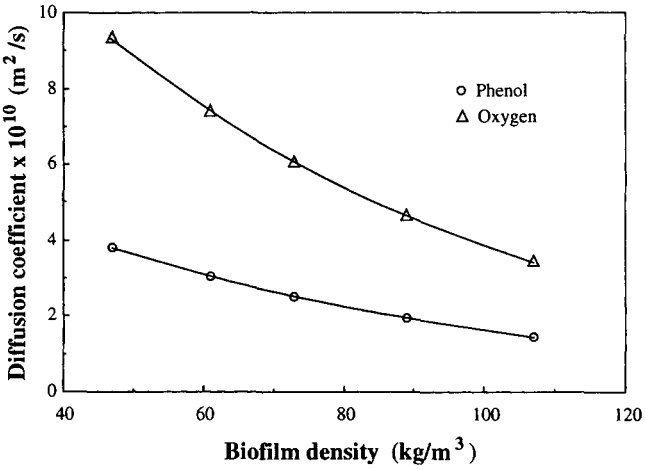

Figure 3. Variation of effective diffusion coefficients of phenol and oxygen by biofilm density.

The calculated effective diffusion coefficients of the substrates are sketched in Figure 3 as a function of biofilm density. The general trend observed in Figure 3 is that effective diffusion coefficients decrease with increasing biofilm density for both limiting substrates. For any biofilm density, diffusion coefficient of phenol was found to be smaller than oxygen because it has greater molecular weight than oxygen. The ratio of effective diffusion coefficient in active biofilm to that of water varied between 17–44% and 9–24% for phenol and oxygen, respectively, for different biofilm densities.

The calculated effective diffusion coefficients were processed with Systat to yield the model of best fit as a function of biofilm density. An exponential form for effective diffusion coefficients was found as the most appropriate equation with LSE for each substrate.

$$D_{fP} = 8.154\times10^{-10}\times10^{-0.0070053\,X_f} \qquad (\text{LSE} = 0.58\times10^{-21}) \tag{15}$$

$$D_{fO} = 2.017\times10^{-9}\times10^{-0.0072367\,X_f} \qquad (\text{LSE} = 0.67\times10^{-18}). \tag{16}$$

If the biofilm density was taken equal to zero, the values of the diffusion coefficients in Eqs. 15 and 16 were found to be 8.154×10^{-10} m^2/s and 2.107×10^{-9} m^2/s, respectively. These values had to be the corresponding diffusion coefficients of the substrates in the fermentation medium, which were smaller than in water owing to the presence of many chemicals and metabolites. A similar approach was carried out for oxygen by Onuma et al. (1985). The values of diffusion coefficients for phenol and oxygen in pure water and in the fermentation medium (at zero biofilm density) for the literature and for this study are given in Table 3. The substrate diffusion coefficients of this work were found to be smaller than those of Onuma et al. (1985) and the Wilke and Chang correlation (Wilke and Chang, 1955). The Wilke and Chang correlation estimates the coefficients in pure water; thus, these coefficients are anticipated to be greater than those in the fermentation medium. The difference in coefficient values of this study and Onuma's work can be attributed to different

Table 3. Diffusion Coefficients in Pure Water and Fermentation Medium (at Zero Biofilm Density) at 25°C

	This Study	At Zero Biofilm Density	
		Onuma et al., 1985	Wilke and Chang, 1955
	m^2/s	m^2/s	m^2/s
Phenol	8.154×10^{-10}	—	8.47×10^{-10}
Oxygen	2.107×10^{-9}	$1.97\text{–}2.09\times10^{-9}$	2.11×10^{-9}

experimental techniques employed as well as to different cultures and fermentation media.

In Figure 4, the effective diffusion coefficients of phenol and oxygen along with biofilm density were plotted against active biofilm thickness. In this figure, both diffusion coefficients increased with increasing active biofilm thickness, while biofilm density decreased. For all experimental runs, phenol and oxygen concentrations within the biofilm were found to be greater than zero; thus, reported biofilm thickness was always active. Hoehn and Ray (1973) experimentally showed that biofilm density increased by the biofilm thickness until a maximum value, and then started to decrease, finally remaining constant for larger biofilm thickness. The same trend was also observed by Şeker et al. (1995). Those results were in agreement with the literature results for small biofilm thickness that was completely active.

Equations 15 and 16 were used to estimate the effective diffusion coefficients of the substrates in the biomass for various studies in the literature (Fan et al., 1990), and the results are presented in Table 4. This table reveals biofilm density and (D_f/D_W) ratio for biofilms formed/grown in a diffusion cell, fixed, and fluidized beds. Fair agreement was observed between this model prediction and the literature results for low biofilm densities. The deviations observed may be caused by different types of cultures, biofilm preparation techniques, and experimental systems. In the works of Fan et al. (1990) and Fujie et al. (1987, imported from Fan et al., 1990) the gap widened at high biofilm densities; however, they used dead microorganism in the biofilm, so their results did not include the effects of metabolites and live interactions within the biofilm. In many studies in the literature active biofilm thickness was not checked for the whole biofilm; instead, the total biofilm thickness including the inactive mi-

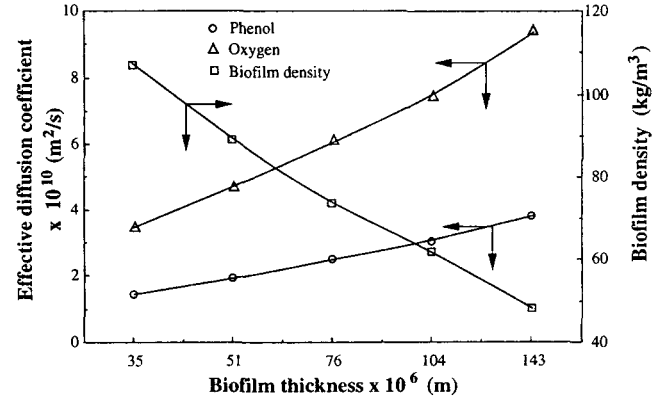

Figure 4. Variation of effective diffusion coefficients of phenol and oxygen and biofilm density by active biofilm thickness.

Table 4. D_f/D_w Ratios for Substrates Predicted by this Study and Model Results of Other Studies

Authors	Substrate	Biomass Type	Exp. Method	X_f kg/m^3	D_f/D_w (Literature)	D_f/D_w (This Study)
Smith and Coackley (1984)	Oxygen	Mixed-culture floc	Diffusion cell	84.0	0.36	0.25
				65.1	0.33	0.34
				42.0	0.34	0.54
				29.4	0.31	0.61
Onuma and Omura (1982)	Oxygen	Mixed-culture floc	Diffusion cell	< 10.0	0.97	0.85
Fujie et al. (1979)	Oxygen	Biofilm	Fixed-bed column	23.5	0.5	0.68
Tang and Fan (1987)	Phenol	Mixed-culture biofilm	Fluidized-bed reactor	151	0.10	0.084
				152	0.12	0.082
				78	0.18	0.273
				72	0.28	0.301
Fan et al. (1990)	Phenol	Mixed-culture biofilm	Fluidized-bed reactor	182	0.26	0.051
				178	0.39	0.054
				170	0.13	0.062
				130	0.38	0.118
Fujie et al. (1987)*	Phenol	Mixed-culture biofilm	Fluidized-bed reactor	199	0.073	0.039
				181	0.143	0.052
				144	0.185	0.094
				135	0.194	0.109
				96	0.256	0.204
				14	0.736	0.763

*Adapted from the study of Fan et al. (1990).

total biofilm thickness including the inactive microorganism layer was utilized in the model calculations. Therefore, controversial results, such as the increase of effective diffusion coefficients with enhanced biofilm density, were seen.

Fan et al. (1990) gave a correlation for the variation of effective diffusion coefficient by dead biofilm density for all types of biofilm and substrates. However, their correlation did not predict diffusivity in the fermentation media and would not be useful in developing a correlation for all types of biofilm. Beer et al. (1994) recently showed that a biofilm was composed of microorganism clusters and that this cluster structure varied by microorganism type. So in biofilm modeling, the designer must choose a model equation for an effective diffusion coefficient for a specific microorganism. Our work presented a sound model, with an appropriated experimental setup, to simultaneously evaluate effective diffusion coefficients of phenol and oxygen in the live biofilm of *Pseudomonas putita* and in the fermentation medium.

Conclusion

A pure culture of *Pseudomonas putida* was grown as a film on carbon particles in a differential fluidized bed biofilm reactor (DFBBR) for this study. In the biofilm, effective diffusion coefficients of the essential substrates, phenol, and dissolved oxygen were simultaneously calculated. The results of the model solution revealed that the effective diffusion coefficients for substrates through the biofilm decreased by biofilm density. An experimental expression was derived for the estimation of effective substrate diffusion coefficients in biofilm as well in fermentation media. The ratio of effective diffusion coefficients through active biofilm to that of water varied between 17–44% and 9–24% for phenol and oxygen, respectively, at different biofilm densities, which showed a fair agreement with the literature at low biofilm density values.

In conclusion, during the operation of a biofilm reactor the biofilm density varies and due to increasing biofilm density more than a single substrate may limit the growth. Thus, modeling strategies of the biofilm reactors may be better modified to include variable simultaneous effective diffusion coefficients as a function of biofilm density.

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Notation

D = dilution rate, s^{-1}
D_f = effective diffusion coefficient of limiting substrate, m^2/s
D_{fO} = effective diffusion coefficient of oxygen, m^2/s
D_{fP} = effective diffusion coefficient of phenol, m^2/s
d_p = particle diameter, m
D_w = diffusion coefficient of substrate in bulk fluid or in water, m^2/s
J_{ro} = oxygen flux at biofilm surface, kg/m^2·s
J_{rp} = phenol flux at biofilm surface, kg/m^2·s
k_c = external mass-transfer coefficient, s^{-1}
K_I = inhibition constant for phenol, kg/m^3
K_o = saturation constant for oxygen, kg/m^3
K_p = saturation constant for phenol, kg/m^3
L_f = observed biofilm thickness, m
OCR = oxygen consumption rate (kg oxygen consumed/kg microorganism produced·s)
Q = volumetric nutrient feed rate, m^3/s
r = radial distance, m
r_b = radius of biofilm-covered bioparticle, m
r_p = radius of clean particle, m

S_{bO} = bulk dissolved-oxygen concentration in the bed, kg/m^3
S_{bP} = bulk phenol concentration in the bed, kg/m^3
S_O = oxygen concentration in the reactor, kg/m^3
S_P = phenol concentration in the reactor, kg/m^3
S_{Pf} = phenol concentration in fresh feed solution, kg/m^3
$T_{\text{biofilm}-O}$ = experimental oxygen consumption rate for a bioparticle in the bed, kg oxygen/bioparticle·s
$T_{\text{biofilm}-P}$ = experimental phenol consumption rate for a bioparticle in the bed, kg phenol/bioparticle·s
U = superficial up-flow velocity, m/s
V = reactor volume, m^3
X = microorganism concentration in reactor, kg dry microorganism/m^3
ϵ = bed porosity
μ = specific growth rate, s^{-1}
μ_l = dynamic viscosity of the liquid, kg·s/m^2
$\mu_{\max}$ = maximum specific growth rate, s^{-1}
ρ_l = liquid density, kg/m^3

Literature Cited

Andrews, G. F., and C. Tien, "Bacterial Film Growth in Adsorbent Surfaces," *AIChE J.*, **27**, 396 (1981).

Andrews, J. F., "Fluidized Bed Fermenters: A Steady State Analysis," *Biotechnol. Bioeng.*, **24**, 2013 (1982).

APHA, *Standard Methods for the Examination of Water and Wastewater*, Amer. Public Health Assoc., Washington, DC (1962).

Atkinson, B., and F. Mavituna, *Handbook of Biochemical Engineering*, Macmillan, London (1983).

Bailey, J. E., and D. F. Ollis, *Biochemical Engineering Fundamentals*, 2nd ed., McGraw-Hill, New York (1986).

Bandyopdhyay, B., E. Humhrey, and N. Taguchi, "Dynamic Measurement of the Volumetric Oxygen Transfer Coefficient in Fermentation Systems," *Biotechnol. Bioeng.*, **9**, 533 (1967).

Beer, D., P. Stoodley, F. Roe, and Z. Lewandowski, "Effects of Biofilm Structures on Oxygen Distribution and Mass Transport," *Biotechnol. Bioeng.*, **43**, 1131 (1994).

Beyenal, H., and A. Tanyolaç, "The Calculation of Simultaneous Effective Diffusion Coefficients of the Substrates in a Fluidized Bed Biofilm Reactor," *Water Sci. Technol.*, **29**(10–11), 463 (1994).

Bird, R. B., W. E. Steward, and E. N. Lightfoot, *Transport Phenomena*, Wiley, Singapore (1960).

Bungay, H. R., and D. M. Harold, "Simulation of Oxygen Transfer in Microbial Slimes," *Biotechnol. Bioeng.*, **13**, 569 (1971).

Denac, M., I. Miguel, and J. Dunn, "Modeling Dynamic Experiments on The Anaerobic Degradation of Molasses Wastewater," *Biotechnol. Bioeng.*, **31**, 1 (1988).

Fan, L.-S., R. L. Ramos, K. D. Wisecarver, and B. J. Zeher, "Diffusion of Phenol Through a Biofilm Grown on Activated Carbon Particles in a Draft-Tube Fluidized Bed Bioreactor," *Biotechnol. Bioeng.*, **35**, 279 (1990).

Fujie, K., T. Sekizawa, and T. Kubota, "Mass Transfer in Liquid Phase with Tubular Wastewater Treatment Contraction," *J. Ferment. Technol.*, **55**(5), 532 (1979).

Grady, C. P. L., "Modeling of Biological Fixed Films—A State of The Art Review," *Fixed Film Biological Process for Wastewater Treatment*, Y. C. Wu and E. D. Smith, eds., Noyes Data Corporation, Park Ridge, NJ, p. 75 (1983).

Hewlett-Packard Contributed Program Series: Math and Numerical Analysis (300). H-P Software Department, Stevens Creek Blvd., Santa Clara, CA 95050.

Hill, C. A., and C. V. Robinson, "Substrate Inhibition Kinetics: Phenol Degradation by *Pseudomonas putida*," *Biotechnol. Bioeng.*, **17**, 1599 (1975).

Hoehn, R. C., and A. D. Ray, "Effects of Thickness on Bacterial Film," *J. Water Pollut. Control Fed.*, **45**, 2302 (1973).

LaMotta, E. J., "Internal Diffusion and Reaction in Biological Films," *Environ. Sci. Technol.*, **10** (8), 765 (1976).

Livingston, A. G., and H. A. Chase, "Modeling of Phenol Degradation in a Fluidized Bed-Bioreactor," *AIChE J.*, **35**, 1980 (1989).

Matson, J. V., and W. G. Characklis, "Diffusion Into Microbial Aggregates," *Water Res.*, **10**, 877 (1976).

Mulcahy, L. T., W. K. Shieh, and E. J. LaMotta, "Simplified Mathematical Models for a Fluidized Bed Biofilm Reactor," *AIChE Symp. Ser.*, **77**, 273 (1981).

Ngian, K. F., and S. H. Lin, "Diffusion Coefficient of Oxygen in Microbial Aggregates," *Biotechnol. Bioeng.*, **29**, 1623 (1976).

Onuma, M., and T. Omura, "Mass Transfer Characteristics Within Microbial Systems," *Water Sci. Technol.*, **14**, 553 (1982).

Onuma, M., T. Omura, T. Umita, and J. Aizava, "Diffusion Coefficient and Its Dependency on Some Biochemical Factors," *Biotechnol. Bioeng.*, **27**, 1533 (1985).

Pawlowsky, U., and J. A. Howell, "Mixed Culture Bioxidation of Phenol: I. Determination of Kinetic Parameters," *Biotechnol. Bioeng.*, **15**, 889 (1973).

Şeker, Ş., H. Beyenal, and A. Tanyolaç, "The Effects of Biofilm Thickness on Biofilm Density and Substrate Consumption Rate in a Differential Fluidized Bed Biofilm Reactor (DFBBR)," *J. Biotechnol.*, **41**, 39 (1995).

Shieh, W. K., and J. D. Keenan, "Fluidized Bed Biofilm Reactor for Wastewater Treatment," *Advances in Biochemical Engineering*, Vol. 33, A. Fiechter, ed., Springer-Verlag, Berlin, p. 141 (1986).

Smith, P. G., and P. Coackley, "Diffusivity, Tortuosity and Pore Structure of Activated Sludge," *Water Res.*, **18** (1), 117 (1984).

Systat Version 3 by Systat Incorporated, 2902 Central St., Evanston, IL 60201 (1986).

Tang, W. T., and L. S. Fan, "Steady State Phenol Degradation in a Draft Tube, Gas-Liquid-Solid Fluidized Bed-Biofilm Reactor," *AIChE J.*, **33**, 239 (1987).

Vos, H. J. P., J. Heedrik, J. J. Potters, and K. C. A. M. Luyben, "Effectiveness Factor for Spherical Biofilm Catalysts," *Bioprocess Eng.*, **28**, 314 (1990).

Wagner, K., and D. C. Hempel, "Biodegradation of Immobilized Bacteria in an Airlift-Loop Reactor-Influence of Biofilm Diffusion Limitation," *Biotechnol. Bioeng.*, **31**, 559 (1988).

Wang, S. C. P., "The Interaction Between Adsorption and Microbial Growth in Biological Activated Carbon (BAC) Process," PhD thesis, Syracuse Univ., Syracuse, NY (1981).

Wilke, C. R., and P. Chang, "Correlation of Diffusion Coefficients in Dilute Solutions," *AIChE J.*, **1**, 264 (1955).

Yang, R. D., and A. E. Humphrey, "Dynamics and Steady State Response Studies of Phenol Biodegradation in Pure and Mixed Cultures," *Biotechnol. Bioeng.*, **17**, 1211 (1975).

Zbigniew, L., G. Walser, and W. G. Characklis, "Reaction Kinetics in Biofilms," *Biotechnol. Bioeng.*, **38**, 877 (1991).

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