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## BIOLOGICAL KINETICS PARAMETERS IN A PURE CULTURE BIOFILM DEVELOPED IN AN AIRLIFT REACTOR

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### ABSTRACT

A diffusion-reaction model was fitted to data obtained with *Pseudomonas fluorescens* biofilms developed in an airlift reactor under different limiting substrate conditions, in order to determine the biofilm kinetic constants and the substrate concentration profiles within the biological films. Model predicted concentration profiles within the biofilms demonstrate that all films were completely penetrated by the substrate and that the reaction rate inside the biofilms was of zero order. The estimated kinetic constants ( $\mu_{\max} = 0.24 \text{ h}^{-1}$ ;  $K_s = 0.73 \times 10^{-3} \text{ kg/m}^3$ ) differ from those obtained in a suspended culture ( $\mu_{\max} = 0.31 \text{ h}^{-1}$ ;  $K_s = 6.21 \text{ kg/m}^3$ ), as a result of the different metabolic state of microorganisms within biofilms.

### KEYWORDS

Airlift reactor; biofilm; diffusion-reaction model; kinetic parameters; *Pseudomonas fluorescens*

### INTRODUCTION

Biofilm reactors used in wastewater treatment processes are usually designed on the basis of an empirical approach that relies upon “common sense” values of the so-called eliminated load (mass of substrate consumed per unit volume and unit time), obtained from past experience with similar wastewaters (Henze *et al.*, 1995). This constitutes an obvious limitation for the optimisation of treatment processes, since the intrinsic parameters relating mass transfer and biological kinetics to the environmental conditions are not properly described and quantified. The difficulties in obtaining adequate correlations for those parameters reside not only on the complex physical structure of the biofilm matrix and its composition, but also on the poorly defined metabolic behaviour of microorganisms within the matrix. Although it is nowadays accepted that most biofilms are heterogeneous systems containing biomass clusters together with pores or channels between them, it is perfectly reasonable, from an engineering standpoint, to have a macroscopic quantification of the biofilm activity by using average values of properties and kinetic parameters within the biofilm. The diffusion-reaction models developed for heterogeneous catalysis can then be useful tools to describe overall substrate consumption rates, if diffusivities, specific biomass growth rate, saturation or affinity constants, etc are previously known. However, much work has still to be carried out to obtain reliable values of these variables in biofilms. The model used in the present work includes average mass transfer coefficients throughout the biofilm (instead of diffusivities) in order to encompass other mechanisms besides pure molecular diffusion. The paper focuses on the fitting of a diffusion-kinetic model to operating data from an airlift reactor containing biofilm of *Pseudomonas fluorescens* attached to basalt particles, under different limiting substrate concentrations. The outputs of the model are the concentration profiles inside the biofilms and the biological kinetic parameters.

### Model

A number of assumptions were made when applying the diffusion-reaction model to the experimental situation: the biofilm had a plane geometry, a homogeneous surface and only one substrate (glucose) was considered to be rate limiting. Mass transfer resistance in the bulk was considered negligible (see equation 2), as shown by the determination of the external mass transfer coefficient (Lopes, 1997). Assuming that substrate A is the rate limiting substrate inside the biofilm,  $C_{As}$  ( $\text{kg}_{\text{substrate}}/\text{m}^3$ ) is the substrate concentration at the surface of the biofilm,  $L$  (m) is the

thickness of the pellicle,  $C_A$  ( $\text{kg}_{\text{substrate}}/\text{m}^3$ ) is the substrate concentration in the biofilm at a distance  $z$  from the interface,  $D_A$  ( $\text{m}^2/\text{s}$ ) is the average effective diffusion coefficient inside the biofilm, and  $r_{fA}$  ( $\text{kg}_{\text{substrate}}/\text{m}^3_{\text{biofilm}}\cdot\text{s}$ ) is the substrate consumption rate, the mass balance for substrate A in a volume of thickness  $dz$  gives: (eq 2 and 3 are the boundary conditions).

$$D_A \frac{d^2 C_A}{dz^2} - r_{fA} = 0 \quad (1) \quad z=L \quad C_A = C_{AS} \quad (2) \quad z=0 \quad dC_A/dz = 0 \quad (3)$$

The development of the model is made taking into account the following equations:

$$r_{fA} = \frac{r_{f \max} C_A}{K_S + C_A} \quad (4) \quad r_{f \max} = \frac{\mu_{\max} X_a}{Y} \quad (5) \quad D_A \frac{d^2 C_A}{dz^2} - \frac{r_{f \max} C_A}{K_S + C_A} = 0 \quad (6) \quad r_f = \int_0^L \frac{r_{f \max} C_A}{K_S + C_A} dz \quad (7)$$

$r_{f \max}$  maximum substrate consumption rate ( $\text{kg}_{\text{substrate}}/\text{m}^3_{\text{biofilm}}\cdot\text{s}$ ),  $Y$  biomass yield ( $\text{kg}_{\text{biomass}}/\text{kg}_{\text{substrate}}$ ),  $X_a$  concentration of active bacteria in the biofilm ( $\text{kg}_{\text{intracellular protein}}/\text{m}^3_{\text{biofilm}}$ ),  $\mu_{\max}$  maximum specific growth rate of the microorganisms inside the biofilm ( $\text{h}^{-1}$ ),  $r_f$  consumption of the substrate by the entire biofilm ( $\text{kg}_{\text{substrate}}/\text{m}^2_{\text{biofilm}}\cdot\text{s}$ ). With dimensionless variables:  $x = z/L$  ( $0 < z < L$ ,  $0 < x < 1$ );  $C_A^* = C_A/C_{AS}$  and an average mass transfer coefficient within the biofilm  $K_b = D_A/L$  (8),

$$\frac{d^2 C_A^*(x)}{dx^2} = \frac{r_{f \max} L}{C_{AS} K_b} \frac{C_A^*(x)}{\frac{K_S}{C_{AS}} + C_A^*(x)} \quad (9) \quad C_A^*(x) = 1, x = 1 \quad d^2 C_A^*(x) / dx^2 = 0, x = 0$$

Suitable numerical methods (based on least squares techniques, implementation of the Gauss-Newton's method together with finite difference discretization) were used to determine the parameters  $r_{f \max}$  and  $K_S$  in the above non-linear second order differential equation. An iterative method was applied, until the difference between known experimental data ( $r_f$  values) and the theoretical model data was minimized. In order to apply this methodology, concentration profiles and values of the kinetic parameters were introduced as initial approximations. The model uses experimental values of substrate consumption rates, concentrations of the limiting substrate in the bulk, biofilm thicknesses and mass transfer coefficients in each test.

## MATERIALS AND METHODS

The microorganism used as biofilm producer was *Pseudomonas fluorescens* grown in an airlift reactor (5.9L) with basalt particles as the carrier. The basalt concentration in the reactor was 50 g/L (Lopes *et al.*, 1999). Glucose was the limiting substrate. The tested average glucose concentrations in the reactor were 13.5, 31, 42, 76.5 and 100 mg/L. The liquid velocity in the riser was 0.26 m/s. Glucose concentration was measured in the influent and effluent streams in order to determine the substrate consumption rate ( $r_f$ ,  $\text{kg}/\text{m}^2_{\text{Biofilm}}\cdot\text{s}$ ). When reaching steady-state, biofilm particles were removed from the reactor and an average film thickness was determined using an image analysis system (Lopes *et al.*, 1999).

## RESULTS AND DISCUSSION

The steady-state results used to evaluate the model are summarised in Table 1. This table also shows the model-predicted substrate consumption rates obtained for the different operational conditions.

TABLE 1 - Glucose concentration at the surface of the biofilm, corresponding thickness and substrate consumption rate in the different experiments

$C_{AS}$ (mg/L)	L (m)	Experimental $r_f$ ( $\text{kg}/\text{m}^2_{\text{Biofilm}}\cdot\text{s}$ )	Model $r_f$ ( $\text{kg}/\text{m}^2_{\text{Biofilm}}\cdot\text{s}$ )
13.5	$7 \times 10^{-6}$	$3.16 \times 10^{-8}$	$1.51 \times 10^{-8}$
31	$36 \times 10^{-6}$	$6.45 \times 10^{-8}$	$8.21 \times 10^{-8}$
42	$38 \times 10^{-6}$	$8.34 \times 10^{-8}$	$8.75 \times 10^{-8}$
76.5	$69 \times 10^{-6}$	$19.0 \times 10^{-8}$	$16.05 \times 10^{-8}$
100	$105 \times 10^{-6}$	$23.25 \times 10^{-8}$	$24.54 \times 10^{-8}$

As discussed in more detail elsewhere (Vieira and Melo, 1999), *Pseudomonas fluorescens* biofilms formed under different hydrodynamic conditions presented similar steady-state values of mass transfer coefficients. Taking that into account, an average value of  $K_b$  ( $1.70 \times 10^{-6}$  m/s) determined in a flow cell system (Vieira and Melo, 1999) was applied. Starting from initial values of  $r_{fmax}$  and  $K_s$ , as well as from the initial approximations of the concentration profiles, the numerical method was repeated until the convergence criteria were met. At the end of the iterative process,  $r_{fmax}$  ( $0.24 \times 10^{-2}$  kg/m<sup>3</sup><sub>Biofilm</sub>·s),  $K_s$  ( $0.73 \times 10^{-3}$  kg/m<sup>3</sup>) and the corresponding concentration profiles inside the biofilms were obtained (Figure 1). The model-predicted substrate consumption rates (Table 1) are calculated by taking into account the values of  $r_{fmax}$ ,  $K_s$  and the generated concentration profiles inside the biofilms. Taking into account the similarity between the experimental and model-predicted values of the substrate consumption rates, it may be concluded that the model fitting was reasonable.

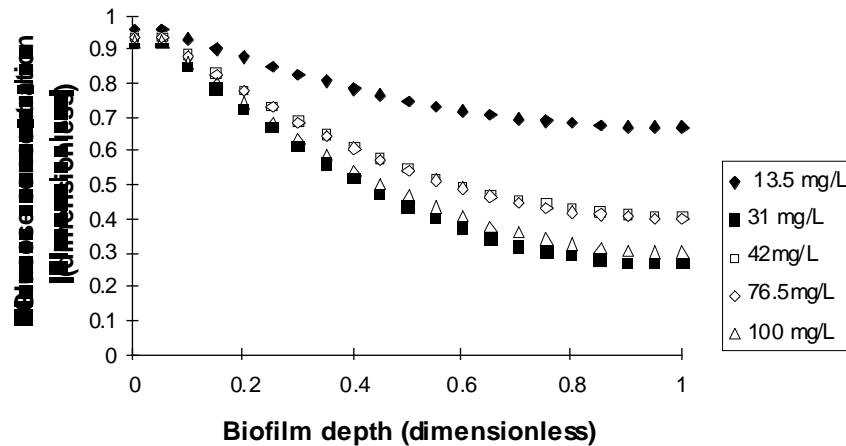


Figure 1 - Model-predicted substrate concentration profiles inside the biofilms

The model-predicted substrate concentration profiles (Figure 1) demonstrate that all biofilms are completely penetrated by the substrate. Despite the increase in the glucose concentration in the reactor and in the biofilm thickness (Table 1), no decrease in the biological activity was detected. The highly turbulent conditions in the reactor prevented the development of thick and partially active biofilms. Moreover, taking into account the concentration profiles (Figure 1) and the Monod kinetics, by applying the estimated parameters, a zero order reaction is expected within these biofilms. Equation 5 shows that the maximum substrate consumption rate ( $r_{fmax}$ ) is related to the biomass yield ( $Y$ ), the maximum specific growth rate of the microorganisms inside the biofilm ( $\mu_{max}$ ) and the concentration of active bacteria in the biological film ( $X_a$ ). A reliable relationship was verified between intracellular protein content and cell mass (Lopes, 1997; Lazarova and Manem, 1995).

The growth yield for *Pseudomonas fluorescens* inside the biofilm was assumed to be  $0.93 \text{ kg}_{\text{dry biomass}}/\text{kg}_{\text{glucose}}$  (Vieira and Melo, 1999). Steady-state values of the dry density of biofilm, its protein content and the ratio between volatile and dry biofilm were determined, being  $28 \text{ kg}_{\text{volatile biofilm}}/\text{m}^3_{\text{wet biofilm}}$ ,  $347 \text{ mg}_{\text{intracellular protein}}/\text{g}_{\text{volatile biofilm}}$  and  $0.832 \text{ kg}_{\text{volatile biomass}}/\text{kg}_{\text{dry biomass}}$ , respectively. From these data, a value of  $X_a = 9.7 \text{ kg}_{\text{intracellular protein}}/\text{m}^3_{\text{wet biofilm}}$  was obtained (Lopes, 1997). Considering all the values mentioned above, the maximum value of the “biofilm specific production rate” ( $\text{kg}_{\text{biofilm produced}}/\text{kg}_{\text{biofilm on the surface}} \cdot \text{s}$ ) is  $\mu_{max} = 0.24 \text{ h}^{-1}$ . The kinetic constants estimated by the model differed from those determined for a cellular suspension ( $\mu_{max} = 0.31 \text{ h}^{-1}$  and  $K_s = 6.21 \text{ kg}/\text{m}^3$ ) (Lopes, 1997), specially the Monod saturation constant ( $K_s$ ), which is much lower. Microorganisms in biofilms are subject to different micro-environmental conditions that may significantly alter their activity, in comparison with that encountered in a dispersed culture, conditioned by mass transfer phenomena, physicochemical characteristics of the solid-liquid interface and the presence of exopolymers, that may cause physiological changes in cells. There are several researches demonstrating that bacteria growth associated with surfaces may have a variety of effects, ranging from promotion to inhibition of physiological activities. However, it should be noticed that the type of results depends on the type of activity measured and the experimental conditions tested. In some cases, substrate uptake mechanisms may be affected by surface-induced modifications in cell membrane structure (Fletcher, 1984). These statements may give a possible explanation for such a large difference between the  $K_s$  values. Besides, it is known

that some bacteria have the ability to grow on high and low concentrations of substrate, due to the fact that they have both low and high affinity enzyme systems, depending on the type of substrates present to sustain growth (Fry, 1990). Data in literature show values of  $r_{\text{fmax}}$  ranging from  $0.28 \times 10^{-2}$  to  $1.7 \times 10^{-2} \text{ kg}_{\text{glucose}}/\text{m}^3_{\text{biofilm}} \cdot \text{s}$  for glucose oxidation in mixed population biofilms. The values vary considerably probably due to differences in bacterial population or density variations in the biofilm (Henze *et al.*, 1995). The  $r_{\text{fmax}}$  value obtained from the model is quite close to the ones reported in the literature. A large variety of  $K_s$  values can be found in literature, in a range of 0.23 to  $121 \text{ mg}_{\text{glucose}}/\text{L}$  (Henze *et al.*, 1995).

## CONCLUSIONS

The following main conclusions are: **1**-Biofilms developed under highly turbulent conditions in this airlift reactor were completely penetrated by the substrate and a zero order reaction is expected to occur within them; **2** - The biofilm kinetic parameters differed from the ones measured in a dispersed suspension, which shows that biofilm parameters should be preferably measured *in situ*; **3** - The theoretically predicted  $r_f$  appear to be in agreement with the experimental data which shows that model fitting was reasonable.

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