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BIOTECHNOLOGY and BIOENGINEERING

Continuum Heterogeneous Biofilm Model—A Simple and Accurate Method for Effectiveness Factor Determination

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ABSTRACT: We present a novel analytical approach to describe biofilm processes considering continuum variation of both biofilm density and substrate effective diffusivity. A simple perturbation and matching technique was used to quantify biofilm activity using the steady-state diffusion–reaction equation with continuum variable substrate effective diffusivity and biofilm density, along the coordinate normal to the biofilm surface. The procedure allows prediction of an effectiveness factor, η, defined as the ratio between the observed rate of substrate utilization (reaction rate with diffusion resistance) and the rate of substrate utilization without diffusion limitation. Main assumptions are that (i) the biofilm is a continuum, (ii) substrate is transferred by diffusion only and is consumed only by microorganisms at a rate according to Monod kinetics, (iii) biofilm density and substrate effective diffusivity change in the x direction, (iv) the substrate concentration above the biofilm surface is known, and (v) the substratum is impermeable. With this approach one can evaluate, in a fast and efficient way, the effect of different parameters that characterize a heterogeneous biofilm and the kinetics of the rate of substrate consumption on the behavior of the biological system. Based on a comparison of η profiles the activity of a homogeneous biofilm could be as much as 47.8% higher than that of a heterogeneous biofilm, under the given conditions. A comparison of η values estimated for first order kinetics and η values obtained by numerical techniques showed a maximum deviation of 1.75% in a narrow range of modified Thiele modulus values. When external mass transfer resistance, is also considered, a global effectiveness factor, η_0, can be calculated. The main advantage of the approach lies in the analytical expression for the calculation of the intrinsic effectiveness factor η and its implementation in a computer program. For the test cases studied convergence was achieved quickly after four or five iterations. Therefore, the simulation and scale-up of heterogeneous biofilm reactors can be easily carried out.

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KEYWORDS: biofilm model; effectiveness factor; continuum heterogeneous biofilm

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Introduction

Biofilms are a common microbial ecosystem consisting of microbial cells, their extracellular polymeric substances and any sorbed substances or entrapped particles; they are associated with interfaces such as water–air, water–solid, and solid–air (Noguera et al., 1999; Saravanan and Sreekrishnan, 2006; Wuertz and Falkentoft, 2003). Biofilms are found in extremely varied environments, ranging from water distribution systems and wastewater treatment plants (Wuertz et al., 2003) to stream beds (Battin

et al., 2007), ship hulls, and teeth surfaces (O'Toole et al., 2000). They form different architectures in response to specific environmental conditions (Pereira et al., 2002; van Loosdrecht et al., 2002; Venugopalan et al., 2005) or the expression of certain genes as proposed for *Pseudomonas aeruginosa* (Battin et al., 2007). Modeling of biofilms has been the domain of engineering disciplines for some time and, generally, does not take into account biological factors other than those associated with growth and decay of cells. Early mathematical models of biofilms were one-dimensional (1-D) and derived from models used in chemical engineering to describe diffusion and reaction in a porous catalyst particle (Gonzo and Gottifredi, 2007; Harremoes, 1978; Kissel et al., 1984; Wanner and Gujer, 1986). Conceptually, they assume a homogeneous matrix containing uniformly distributed and biochemically reactive sites (Williamson and Mac Carty, 1976a,b). These models are numerically based, they can account for gradients of substrates normal to the biofilm substratum, and simulation software for biofilm calculations (Reichert, 1998a,b) is easy to implement. Similarly, analytical models make many simplifying assumptions and are 1-D, with the difference that they allow for the calculation of flux of dissolved substances into a biofilm by mathematical derivation instead of numerical techniques. They are suitable for biofilms where only one species or dominant process is analyzed, such as BOD removal (e.g., Perez et al., 2005, 2006).

Sáez and Rittmann (1988, 1992) developed an accurate pseudo-analytical solution for the flux of substrate into a steady-state homogeneous biofilm by calculating dimensionless parameters, which can be used to output substrate flux from the bulk liquid substrate concentration, C_0. Calculations provide algebraic solutions as output instead of numerical solutions. Multidimensional models have been developed as reviewed in Wanner et al. (2006), after experimental results obtained with powerful new experimental tools such as confocal laser scanning microscopy (CLSM; Neu and Lawrence, 1997), magnetic resonance imaging (Manz et al., 2003), and microsensors (De Beer et al., 1994) revealed the heterogeneous nature of many biofilms. These models were constructed to reflect the fact that microorganisms in biofilms are not uniformly distributed, which affects biofilm activity, resulting in variable biofilm density and substrate effective diffusivity with depth. For example, Beyenal and Lewandowski (2005) presented a stratified biofilm model. They subdivided the biofilm into a finite number of uniform layers modeling each of them as a homogeneous biofilm using a 1-D model. The effect of biofilm heterogeneity was imposed by the properties of the various layers. The authors solved the mass balance differential equation using numerical techniques. Others have employed three-dimensional approaches (Eberl et al., 2000; Picioreanu et al., 2004; von der Schulenburg et al., 2009; Xavier et al., 2005). For example, Alpkvist and Klapper (2007) have proposed a multidimensional continuum model for heterogeneous growth of a biofilm system with multiple species and multiple substrates. The model equations are solved using rigorous numerical simulation techniques.

Currently, there is no simple spreadsheet-based method available to perform biofilm substrate flux calculations and to quantify biofilm activity using the steady-state diffusion–reaction equation with continuum (rather than uniform) biofilm density. The objectives of the present study were, therefore, to (i) reliably predict the effectiveness factor, η, defined as the ratio between the observed rate of substrate utilization (reaction rate with diffusion resistance) and the rate of substrate utilization without diffusion limitation (Bischoff and Froment, 1980), (ii) to develop a simple analytical approach to modeling substrate flux in biofilms based on η, and (iii) to estimate the global effectiveness factor, η_0, that accounts for external mass transport resistance as a fundamental parameter for simulation and scale-up of biofilm reactors.

Materials and Methods

Computational Methods

For the purpose of comparison with the analytical solutions derived from equations a numerical technique was used to obtain the effectiveness factor values based on numerical integration of Equation (1). Results are referred to as "numerical" throughout this study; they were obtained with a high accuracy orthogonal collocation method (Villadsen and Michelsen, 1978), improved with spline collocations (Kubicek and Hlavacek, 1983). Calculations and simulations were performed using Fortran 4.0 programming language.

Model Formulation

The common practice in biofilm modeling is to divide components into dissolved (substrates) and particulate (microorganisms) phases experiencing different transport processes. Particulate materials do not diffuse, while soluble compounds do. The characteristic time scales are quite different: processes associated with the particulates (microorganisms) are much slower than those in the dissolved phase (Alpkvist et al., 2006). Therefore, simulation can be made more efficient by separating both processes. The substrate diffusion–reaction process can be considered in steady-state (or quasi steady-state), while microbial growth is time dependent.

The primary assumption in this model is that a heterogeneous biofilm is treated as a continuum (Wood and Whitaker, 1998) where both substrate effective diffusivity and biofilm density change in the direction normal to the biofilm surface. These changes in the perpendicular direction, x (Fig. 1) are more noticeable than those in the plane parallel to the surface. Therefore, the system can be solved by applying the substrate 1-D mass

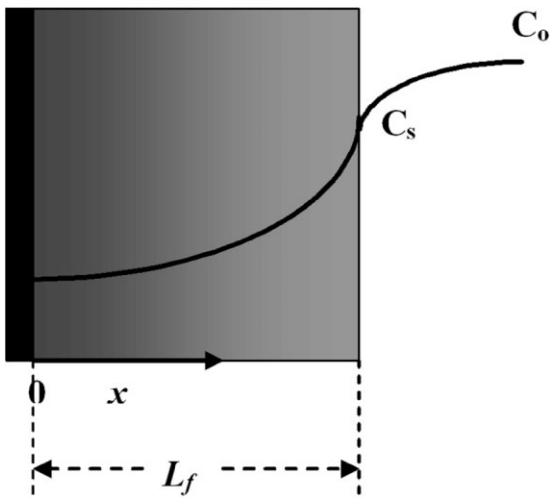

Figure 1. Structure of the continuum heterogeneous biofilm model. (–) substrate concentration profile. L_f biofilm thickness.

balance differential equation at steady-state (Lewandowski and Beyenal, 2003). Under these conditions the substrate continuity differential equation is

$$\frac{d}{dx}D_f(x)\frac{dC}{dx} = \frac{q_{max}X_f(x)C}{Y(K_s + C)} \tag{1}$$

For homogeneous films, Equation (1) is simplified to

$$\overline{D}_f\frac{d^2C}{dx^2} = \frac{q_{max}\overline{X}_fC}{Y(K_s + C)} \tag{2}$$

where $\overline{X}_f$ and $\overline{D}_f$ are the average values of X_f (density) and D_f (substrate effective diffusivity) along the x direction in the biofilm, C is the substrate concentration, K_s the Monod half-maximum rate constant, q_{max} the maximum specific growth rate, and Y the yield coefficient of substrates on biomass (mass of microorganisms/mass of substrates).

The following assumptions were accepted in Equations (1) and (2):

(a) The biofilm is a continuum.
(b) Substrate is transferred by diffusion only (Fick's law) (Lewandowski and Beyenal, 2003) and is consumed only by microorganisms at a rate according to Monod kinetics.
(c) Biofilm density and substrate effective diffusivity change in the x direction.
(d) The substrate concentration above the biofilm surface is known (C_s).
(e) The substratum (support where the biofilm grows) is impermeable.

Under these assumptions, the appropriate boundary conditions are

$$C = C_s \quad \text{at the bulk/boundary layer interface } (x = L_f) \tag{3}$$

$$\left(\frac{dC}{dx}\right)_{x=0} = 0 \quad \text{at the substratum } (x = 0) \tag{4}$$

As experimental data show, biofilm density increases toward the bottom, while the effective diffusivity decreases (Beyenal and Lewandowski, 2002). For example, when the surface averaged effective diffusivity of ferricyanide was experimentally measured for a biofilm consisting of *P. aeruginosa*, *Pseudomonas fluorescens*, and *Klebsiella pneumoniae* (Beyenal et al., 1998; Beyenal and Lewandowski, 2000, 2002; Lewandowski and Beyenal, 2003), the variation of D_f with the distance from the bottom, x, could be approximated by the linear relationship

$$D_f(x) = \alpha + \xi x \tag{5}$$

Parameters α and ξ depended on substrate (glucose) concentration and flow velocity at which biofilms were grown.

Lewandowski and Beyenal (2003) defined ξ as the effective diffusivity gradient in the biofilm. They found that for this biofilm the relative effective diffusivity, D_f^0, changed with the distance x from the substratum according to

$$D_f^0(x) = 0.001x + 0.2968 \tag{6}$$

with x in μm. Equation (6) was determined for a biofilm grown at a flow velocity of 3.2 cm/s and a glucose concentration of 40 mg/L (Beyenal and Lewandowski, 2000).
where

$$D_f^0(x) = \frac{D_f(x)}{D_w} \tag{7}$$

and D_w is the substrate diffusivity in the liquid medium.

Dimensionless Mass Balance Equations

To generalize the solution of this system, it is useful to work with dimensionless differential equations. In order to solve the mass balance differential equations it is fundamental to know $D_f(x)$ and $X_f(x)$. Fan et al. (1990), based on several experimental results, found the following empirical

correlation between relative diffusivity and biofilm density

$$D_f^0(x) = 1 - \frac{0.43X_f^{0.92}}{11.19 + 0.27X_f^{0.99}} \quad (8)$$

The higher the density of the biofilm, the less pore volume is available to the substrate to diffuse through the biofilm. After defining the following dimensionless variables and parameters:

$$x^* = \frac{x}{L_f} \quad C^* = \frac{C}{C_s} \quad \beta = \frac{K_s}{C_s} \quad (9)$$

$$\Psi = \frac{\alpha}{L_f \xi} \quad \kappa = \frac{D_w}{\alpha} \quad D_f^* = \frac{D_f}{\overline{D}_f} \quad X_f^* = \frac{X_f}{\overline{X}_f} \quad (10)$$

the mass balance Equations (1) yields

$$\frac{d}{dx^*} D_f^* \frac{dC^*}{dx^*} = \phi^2 X_f^* R(C^*) \quad (11)$$

$[X_f^* R(C^*)]$ and ϕ are the dimensionless reaction rate and Thiele modulus, respectively.

$$R(C^*) = \frac{(\beta + 1)C^*}{\beta + C^*} \quad (12)$$

$$\phi^2 = \frac{L_f^2}{\overline{D}_f C_s} \frac{q_{max} \overline{X}_f C_s}{Y(K_s + C_s)} = \frac{L_f^2}{\overline{D}_f C_s} r_s \quad (13)$$

where

$$r_s = \frac{q_{max} \overline{X}_f C_s}{Y(K_s + C_s)} \quad (14)$$

For the homogeneous model, the dimensionless differential equation is

$$\frac{d^2 C^*}{dx^{*2}} = \phi^2 R(C^*) \quad (15)$$

The dimensionless boundary conditions for both differential equations are

$$C^* = 1 \quad \text{at} \quad x^* = 1 \quad \text{and} \quad \frac{dC^*}{dx^*} = 0 \quad \text{at} \quad x^* = 0 \quad (16)$$

Next, we followed the procedure presented by Beyenal and Lewandowski (2005), who considered Equation (8) and that the average effective diffusivity in the biofilm, $\overline{D}_f$, is equal to

$$\overline{D}_f = \alpha + \frac{\xi L_f}{2} \quad (17)$$

They found the needed relation between the dimensionless effective diffusivity and biofilm density as a function of x^* (Beyenal and Lewandowski, 2005)

$$D_f^* = \frac{2(\Psi + x^*)}{2\Psi + 1} = c\left(1 + \frac{x^*}{\Psi}\right) \quad (18)$$

and

$$X_f^* = \frac{-38.856 + 38.976\kappa^{0.7782}\left(1 + \frac{x^*}{\Psi}\right)^{-0.7782}}{\overline{X}_f} = \frac{a + b\left(1 + \frac{x^*}{\Psi}\right)^{-0.7782}}{\overline{X}_f} \quad (19)$$

where $\overline{X}_f$ is the average biofilm density, given by

$$\overline{X}_f = -38.856 + \frac{38.976}{0.2218}\Psi\kappa^{0.7782}\left[\left(1 + \frac{1}{\Psi}\right)^{0.2218} - 1\right] \quad (20)$$

Effectiveness Factor Estimation

Continuum Heterogeneous Biofilm Model

The effectiveness factor η is the ratio between the observed substrate consumption rate, r_{ob} (reaction rate with diffusion resistance) and the substrate consumption rate without diffusion limitation (Bischoff and Froment, 1980), as given by Equation (21)

$$\eta = \frac{r_{ob}}{r_s} = \int_0^1 X_f^* R(C^*) dx^* \quad (21)$$

Thus, the actual observed reaction rate can be obtained from Equation (21).

In addition, taking into account the definition of η, the substrate steady-state mass balance at the biofilm–fluid interphase ($x^* = 1$) gives

$$D_{f(x=L_f)} \left.\frac{dC}{dx}\right|_{x=L} S_x = \eta \frac{q_{max} \overline{X}_f C_s}{Y(K_s + C_s)} S_x L_f \quad (22)$$

Considering the dimensionless variables and parameters previously defined, Equation (22) yields

$$\frac{D_f^*(1)}{\phi^2} \left.\frac{dC^*}{dx^*}\right|_{x^*=1} = \eta \quad (23)$$

Note that in the Thiele modulus definition (Equation 13), the reference reaction rate is the rate of reaction without

diffusion resistance (rate of reaction in a homogeneous biofilm, which is not diffusion limited).

Both Equations (21) and (23) can be used to obtain an expression for η. However, since $C^*(x^*)$ is not known, η cannot be estimated. Nevertheless, Equation (11) can be solved approximately with the perturbation procedure (Gottifredi and Gonzo, 1986), when $\phi \ll 1$ or when $\phi \gg 1$, and using a matching expression to find the analytical equation for η. This technique was successfully used in the simulation of hollow fiber and monolith reactors (Gonzo and Gottifredi, 2007, 2010; Gonzo, 2008). The application of the perturbation and matching technique to obtain the general solution for η versus ϕ for the system studied here is given in Supplemental Material A.

Perturbation Solutions

When $\phi^2 \ll 1$, the perturbation solution for η is a series with terms up to the order of ϕ^2, given by

$$\eta = 1 - \sigma\phi^2 \tag{24}$$

with

$$\sigma = \frac{\beta b \Psi^2}{\overline{X}_f^2(\beta+1)c(0.2218)}(F) \tag{25}$$

where F is the variable term in Equation (25) defined as

$$F = -\frac{a\Psi}{(0.2218)(1.2218)}\left[\left(1+\frac{1}{\Psi}\right)^{1.2218}-1\right]+\frac{a\left(1+\frac{1}{\Psi}\right)^{0.2218}}{(0.2218)}- -a\int_0^1 \ln\left[\frac{1+\frac{1}{\Psi}}{1+\frac{x^*}{\Psi}}\right]dx^* - \frac{b\Psi\left[\left(1+\frac{1}{\Psi}\right)^{0.4436}-1\right]}{(0.2218)(0.4436)}+ +\frac{b\Psi\left(1+\frac{1}{\Psi}\right)^{0.2218}}{(0.2218)^2}\left[\left(1+\frac{1}{\Psi}\right)^{0.2218}-1\right] - b\int_0^1\left(1+\frac{x^*}{\Psi}\right)^{-0.7782}\ln\left[\frac{1+\frac{1}{\Psi}}{1+\frac{x^*}{\Psi}}\right]dx^* \tag{26}$$

When $\phi^2 \gg 1$, the asymptotic expression for the effectiveness factor is

$$\eta = \frac{\left[2D_f^*(1)X_f^*(1)(\beta+1)\left(1+\beta\ln\frac{\beta}{(1+\beta)}\right)\right]^{1/2}}{\phi} = \frac{\rho}{\phi} \tag{27}$$

The matching expression proposed for the calculation of the effectiveness factor, valid for the entire range of ϕ values, is

$$\eta = \left[\phi^{*2} + \exp(-d\phi^{*2})\right]^{-(1/2)} \tag{28}$$

where

$$\phi^* = \frac{\phi}{\rho} \tag{29}$$

When parameter d becomes negative, it is simply set to equal zero. The procedure to obtain d is described in Supplemental Material A.

Homogeneous Biofilm Model

After applying the perturbation and matching technique to solve the differential Equation (15) and obtain the effectiveness factor, η_H, for a homogeneous biofilm model, the following solutions for small and large values of ϕ were found:

$$\eta_H = 1 - \sigma_H\phi^2 \tag{30}$$

with

$$\sigma_H = \frac{\beta}{3(\beta+1)} \tag{31}$$

and

$$\eta_H = \frac{\left[2(\beta+1)\left[1+\beta\ln\frac{\beta}{(1+\beta)}\right]\right]^{1/2}}{\phi} = \frac{\rho_H}{\phi} \tag{32}$$

To estimate η_H for the homogeneous biofilm model Equation (28) was used with

$$\phi^* = \frac{\phi}{\rho_H} \tag{33}$$

and

$$d = 1 - 2\sigma_H^* \tag{34}$$

where

$$\sigma_H^* = \sigma_H\rho_H^2 \tag{35}$$

Biofilm–Fluid Interphase Mass Transfer Effect

In various biotransformation reactions, the convective interphase biofilm–fluid mass transport can have a significant influence on the process rate. To account for

this mass transport limitation it is necessary to determine the concentration difference between the bulk liquid C_0, and the biofilm surface, C_s. The differential equation for a steady-state biofilm considering interphase mass transfer effect cannot be solved analytically, except in the case of a homogeneous biofilm where a first order substrate consumption reaction is considered. Therefore, for a heterogeneous biofilm a numerical procedure must be used.

We define

$$\eta_0 = \frac{r_{ob}}{r_0} = \eta \frac{r_s}{r_0} \tag{36}$$

as the effectiveness factor calculated in terms of the rate of substrate utilization evaluated at bulk fluid conditions, r_0. Hence η_0 is the global or overall effectiveness factor, which takes into account both external mass transport and internal diffusion limitations on the rate of substrate utilization.

We define r_0 as follows:

$$r_0 = \frac{q_{max}\overline{X}_f C_0}{Y(K_s + C_0)} \tag{37}$$

Considering Equations (13), (21), and (36), the following relationship for a heterogeneous biofilm is found:

$$\phi^2 \eta = \phi_0^2 \eta_0 \frac{C_0}{C_s} \tag{38}$$

where

$$\phi_0^2 = \frac{L_f^2}{\overline{D}_f C_0} \frac{q_{max}\overline{X}_f C_0}{Y(K_s + C_0)} \tag{39}$$

After the value of the mass transport coefficient k_c (Bischoff and Froment, 1980; De Beer et al., 1994; Gonzalez-Brambilla and Lopez-Isunza, 2008) is estimated, a steady-state mass balance at the biofilm surface gives

$$k_c(C_0 - C_s)S_x = \eta S_x L_f r_s \tag{40}$$

$$\frac{k_c L_f}{\overline{D}_f} \frac{(C_0 - C_s)}{C_s} = \phi^2 \eta \tag{41}$$

and, finally

$$\frac{C_s}{C_0} = \frac{1}{1 + \frac{\phi^2 \eta}{Bi}} \tag{42}$$

where Bi represents the Biot number for mass transport

$$Bi = \frac{k_c L_f}{\overline{D}_f} \tag{43}$$

The mass Biot number is a dimensionless parameter that combines interphase transport and internal effective diffusion coefficients; it is a measure of the relative importance of the external and internal resistance to mass transport.

An iterative procedure was used to estimate η_0 and C_s. Assuming negligible interphase resistance ($\eta = \eta_0$; $\phi = \phi_0$; $C_s = C_0$), an approximate correction was made using Equation (42). With the resulting value of C_s, new values of η, η_0, and ϕ were obtained. The procedure was iterated until two successive calculations of C_s indicated that the desired convergence was achieved. Therefore, with this simple procedure the values of η, η_0, and C_s for each value of C_0 were found.

Results

Relationship of Effectiveness Factor With Thiele Modulus

According to our definition in Equation (13), the Thiele modulus, ϕ, is the ratio between the reference reaction rate and the diffusion rate. The reference reaction rate is the reaction rate in a homogeneous biofilm with a density equal to the average values in the biofilm and which is not diffusion limited. Then, when $\phi \to 0$, both continuum heterogeneous and homogeneous biofilm should have the same value for the effectiveness factor ($\eta = \eta_H \approx 1$).

Figure 2 shows the profiles of the effectiveness factor as a function of the Thiele modulus considering a continuum heterogeneous biofilm, a homogeneous biofilm, and η_N values obtained from the simulation with a rigorous and highly time intensive numerical method for the heterogeneous biofilm for $\Psi = 0.5$, $\kappa = 4$, and $\beta = 0.125$. From the comparison of effectiveness factor profiles it can be said that

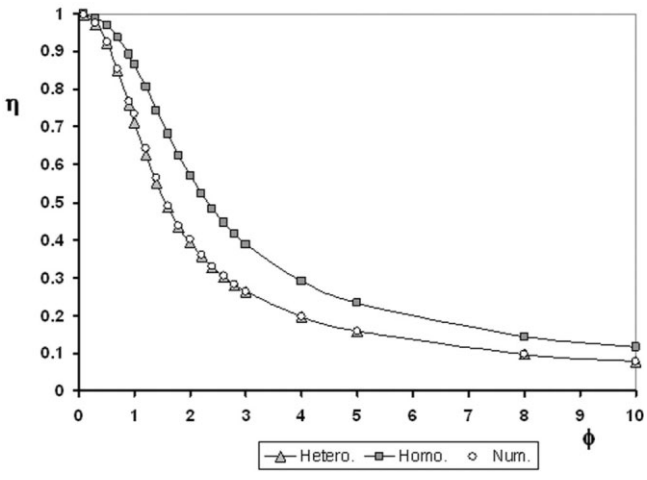

Figure 2. Effectiveness factor as a function of Thiele modulus for $\kappa = 4$, $\Psi = 0.5$, and $\beta = 0.5$ [$\rho = 0.7866$, $\rho_H = 1.1628$, $\sigma^* = 0.1767$, $\sigma_H^* = 0.1502$, $d = 0.6466$, $d_H = 0.6995$]. All plots refer to analytical solutions.

the activity of a homogeneous biofilm could be as much as 47.8% higher than that of a heterogeneous biofilm, under the given conditions. Numerical results of η_N show that our estimation procedure predicts η values in very close agreement with the corresponding numerical finding, for the entire range of Thiele modulus. Also, for $\phi \to 0$, the effectiveness factor for both types of biofilm models, goes to one, as expected (Fig. 2). Relative percent deviation $[(\eta - \eta_N) \times 100/\eta_N]$ between estimated and numerical η values increases from 0.01% at $\phi = 0.1$ to 1.6% at $\phi = 1$ and decreases to 0.02% for $\phi = 8$.

Relationship Between Effectiveness Factor, Thiele Modulus, and Parameters κ, Ψ, and β

Parameter κ is equal to the ratio between the substrate diffusivity in water and the effective diffusivity at the bottom of the biofilm. This means that the higher the κ value the lower the effective diffusivity value at the bottom of the biofilm. However, the effect of parameter κ is stronger on biofilm density (activity) than on effective diffusivity. Increasing values of κ also resulted in a substantial increase in biofilm activity (Fig. 3). For a better insight into the effect of parameter κ on the effectiveness factor, we can start from the definition of Ψ and κ. By considering Equation (10), the effective diffusivity gradient ξ (a parameter directly related to the biofilm heterogeneity, where $\xi = 0$ represents a homogeneous biofilm) can be obtained

$$\Psi = \frac{\alpha}{L_f \xi} = \frac{D_w}{L_f \Psi} \frac{1}{\kappa} \quad \text{then} \quad \xi = \frac{D_w}{L_f \Psi} \frac{1}{\kappa} \tag{44}$$

Figure 3 shows the effect of κ on η at constant values of parameters β and Ψ. Therefore, considering Equation (44), as κ increases, the value of ξ decreases, which represents a more homogeneous biofilm. This is in agreement with results presented here, where η tends to values closer to that corresponding to a homogeneous biofilm.

For $\kappa > 12$, the heterogeneous biofilm showed a microbial activity (as revealed by the effectiveness factor) differing by no more than 6% from that of a homogeneous biofilm.

As stated in Equation (10), the parameter Ψ is the ratio between the effective diffusivity at the bottom of the biofilm and the effective diffusivity difference between the top and the bottom of the biofilm. Therefore, as the value of Ψ increases the more homogeneous the biofilm becomes. Similar to Figure 3, when Ψ is greater than near 20, the effectiveness factor estimated for a heterogeneous biofilm approaches that of a homogeneous biofilm (Fig. 4). These results are as expected because with increasing Ψ the difference in effective diffusivity between the surface and the bottom of the biofilm goes to zero (as in a homogeneous biofilm).

Parameter β is directly related to the kinetics of the substrate consumption rate and plays an important role in the system behavior. By definition, β is the ratio between the Monod half -maximum rate constant and the substrate biofilm surface concentration. When $\beta \to 0$ (very high substrate surface concentration, $C_s \gg K_s$), the kinetics tend towards a zero order reaction (Fig. 5) while, when $\beta \to \infty$ (very low substrate surface concentration, $C_s \ll K_s$), the kinetics approximate a first order reaction. However, very low values of β are quite difficult to find in practice since the maximum value of C_s is the substrate saturation concentration in the fluid.

Comparison of Estimated and Numerically Determined Effectiveness Factor

A comparison of effectiveness factor values estimated with Equation (41) for first order kinetics and η_N values obtained

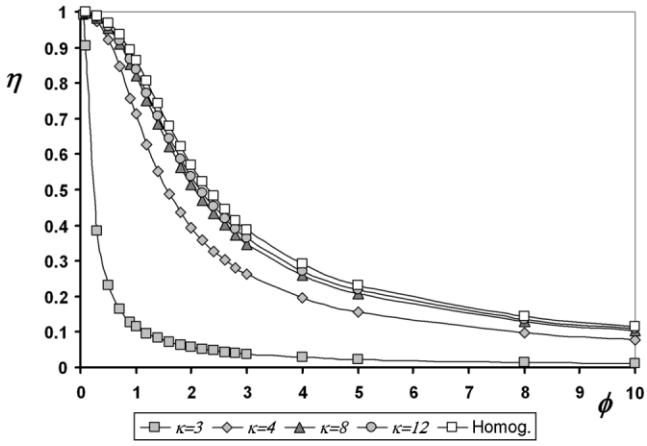

Figure 3. Effectiveness factor as a function of Thiele modulus and parameter κ, for $\Psi = 0.5$ and $\beta = 0.5$. All plots refer to analytical solutions.

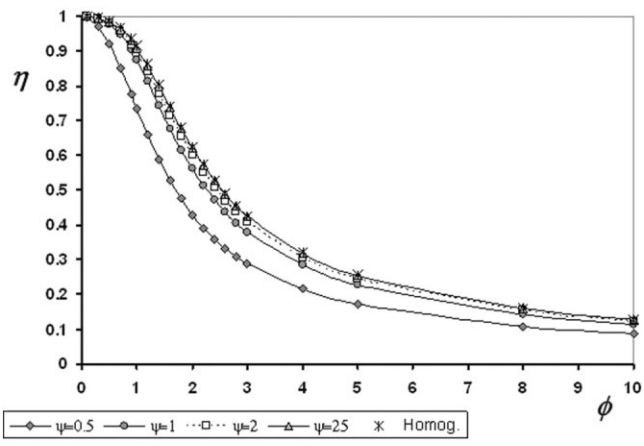

Figure 4. Effectiveness factor as function of Thiele modulus and parameter Ψ, for $\kappa = 4$ and $\beta = 0.125$. All plots refer to analytical solutions.

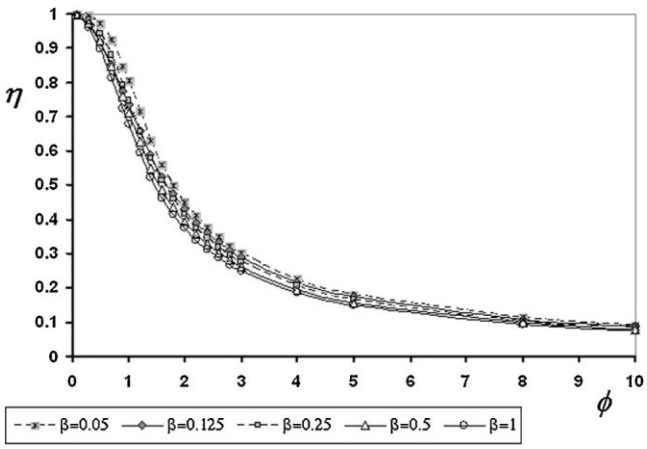

Figure 5. Effectiveness factor as function of Thiele modulus and parameter β, for $\kappa = 4$ and $\Psi = 0.5$. All plots refer to analytical solutions.

by numerical techniques (Table I) reveals that the maximum deviation is 1.75% in a narrow range of modified Thiele modulus values ($0.7 < \phi^* < 1.2$). Also listed are the estimated effectiveness factor and the analytical (exact) values of η_T for a zero order reaction for $\kappa = 4$ and $\Psi = 0.5$. The maximum deviation between the predicted and the exact values is 12.5% for the most unfavorable situation since the analytical solution presents a discontinuity at $\phi^* = 1$. Nevertheless, this maximum deviation is found at $\phi^* = 1$. For ϕ^* values lower than 0.5 and higher of 1.6, the deviations are less than 1%.

Table I. Comparison between estimated and numerical or exact values of effectiveness factor for first order ($\beta \to \infty$) and zero order ($\beta = 0$) reaction rate.

	First order kinetics $\rho = 0.6765$, $\sigma^* = 0.3922$, $d = 0.2156$			Zero order kinetics $\rho = 0.9567$, $\sigma^* = 0$, $d = 1$		
ϕ	ϕ^*	η	η_N	ϕ^*	η	η_T
0.1	0.1478	0.9915	0.9925	0.1045	1	1
0.3	0.4435	0.9304	0.9370	0.3136	0.9977	1
0.5	0.7391	0.8347	0.8466	0.5226	0.9834	1
0.7	1.0347	0.7323	0.7454	0.7317	0.9446	1
0.9	1.3304	0.6385	0.6497	0.9407	0.8778	1
1	1.4782	0.5966	0.6064	1.0453	0.8369	0.9567
1.2	1.7738	0.5231	0.5299	1.2543	0.7494	0.7973
1.4	2.0695	0.4623	0.4666	1.4634	0.6653	0.6834
1.6	2.3651	0.4119	0.4145	1.6724	0.5915	0.5979
1.8	2.6608	0.3702	0.3717	1.8815	0.5293	0.5315
2	2.9564	0.3353	0.3362	2.0905	0.4777	0.4784
2.4	3.5477	0.2811	0.2813	2.5086	0.3986	0.3986
3	4.4346	0.2254	0.2254	3.1358	0.3189	0.3189
4	5.9128	0.1691	0.1691	4.1810	0.2392	0.2392
8	11.8256	0.0846	0.0846	8.3621	0.1196	0.1196

For: $\kappa = 4$ and $\Psi = 0.5$.

Table II. Kinetics and transport parameter values for the test case.

Symbol	Parameter	Value
Bi	Biot number	1.68 and 16.79
C_0	Bulk fluid nutrient concentration (kg/m^3)	0.1–0.7
D_w	Diffusivity of nutrient in the liquid (m^2/s)	4×10^{-10}
K_s	Monod half rate constant (kg/m^3)	0.07
k_c	Mass transfer coefficient (m/s)	1×10^{-6} and 1×10^{-5}
L_f	Average biofilm thickness (μm)	300
q_{max}	Maximum specific growth rate (s^{-1})	1.7×10^{-5}
Y	Yield coefficient of substrate on biomass	0.36
κ	Parameter defined by Equation (10)	3.369
Ψ	Parameter defined by Equation (10)	0.989

Test Case Results

To consider the effect of biofilm–fluid interphase mass transport, a test case was studied, with kinetics and transport parameters values given in Table II. The parameter values are within the range of those currently found in the literature (Beyenal and Lewandowski, 2005; Gonzalez-Brambilla and Lopez-Isunza, 2008; Rama Rao et al., 2010).

With the iterative procedure presented here to estimate η_0 and C_s, no more than four or five iteration cycles are needed to achieve the desired convergence (in the worst situation).

Figure 6 shows the profiles of relative percent difference between the bulk fluid and the biofilm surface substrate concentrations $[(C_0 - C_s) \times 100/C_0]$ as a function of substrate bulk concentration, for a low and a normal value of the interphase mass transport coefficient. The profiles are significantly different; for a low flux ($Bi = 1.68$), the substrate concentration drops by as much as 60% and the effect of the external mass transport plays an important role in the whole process. For a Biot number one order of

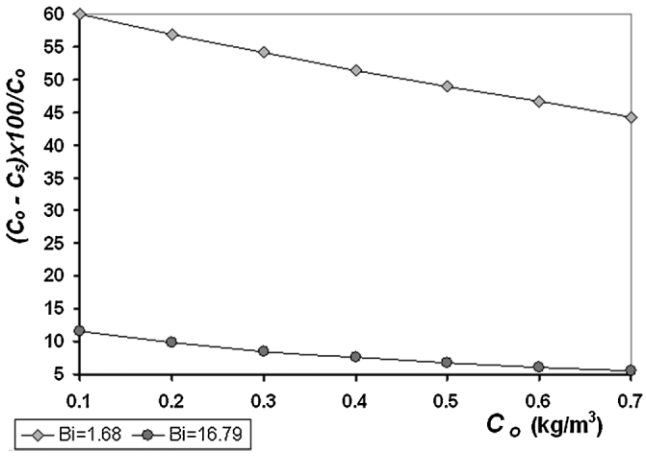

Figure 6. Relative percent difference between substrate bulk and biofilm surface concentration for two values of the Biot number. The iterative procedure takes into account interphase mass transport resistance.

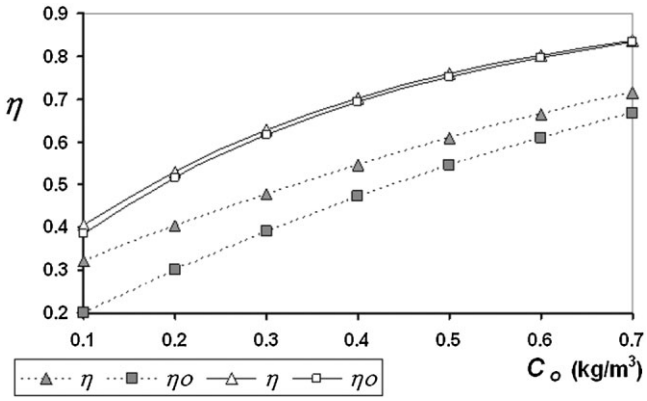

Figure 7. The variation of the intrinsic and bulk effectiveness factors as a function of substrate bulk fluid concentration. $\kappa = 3.369$, $\Psi = 0.989$. Dashed lines, $Bi = 1.68$; continuous lines, $Bi = 16.79$. All plots refer to analytical solutions with the matching expression taking into account interphase mass transport resistance.

magnitude higher than the previous one, the influence of the interphase mass transport decreases substantially, but can still be important (12%) when the substrate concentration is low.

The influence of the external mass transport limitation on intrinsic (η) and bulk (η_0) effectiveness factors for a heterogeneous biofilm as a function of the substrate bulk fluid concentration, for two values of the Biot number, is depicted in Figure 7. As the mass transfer coefficient decreases the difference between the intrinsic and bulk effectiveness factors increases. This trend of η as a function of substrate bulk concentration for the two values of the Biot number is as expected.

Discussion

We have presented the first analytical approach to describe biofilm development considering continuum variation of both biofilm density and substrate effective diffusivity. Effectiveness factor estimation carried out using an algebraic expression gives accurate values of η when compared with numerical findings. The procedure builds on work by Beyenal and Lewandowski (2005) who, using a stratified biofilm model, investigated the effectiveness factor as a function of the Thiele modulus for heterogeneous biofilm with different values of the dimensionless parameters: κ, Ψ, and β. The main difference is that the new approach does not require numerical simulation.

Our method includes a procedure to account for the interphase mass transport (external) effect on the whole process. For large values of ϕ, the results obtained by Beyenal and Lewandowski (2005) for the relationship of η versus ϕ agree very well with those reported in this study. In contrast, for low values of ϕ, we found important differences, which are due to inherently different definitions in the Thiele modulus in the two studies. The effectiveness factor is defined as the ratio between the observed substrate utilization rate, r_{ob}, (diffusion limited reaction rate in the heterogeneous biofilm) and the diffusion free rate, r_s (equal to the rate in a homogeneous biofilm, with $C = C_s$ throughout the biofilm). Therefore, for r_s, the biofilm density and the effective diffusivity are $\overline{X}_f$ and $\overline{D}_f$. We know from Equation (21) that $r_{ob} = \eta r_s$, and if r_s is used to define η and to obtain the value of r_{ob}, the Thiele modulus should be given by

$$\phi^2 = \frac{L_f^2}{\overline{D}_f C_s} r_s = \frac{L_f^2}{\overline{D}_f C_s} \frac{q_{max} \overline{X}_f C_s}{Y(K_s + C_s)} = \frac{L_f^2}{\overline{D}_f C_s} \frac{q_{max} \overline{X}_f}{Y(\beta + 1)} \quad (45)$$

leading to the dimensionless mass balance differential equation

$$\frac{d}{dx^*} D_f^* \frac{dC^*}{dx^*} = \frac{L_f^2}{\overline{D}_f C_s} \frac{q_{max} \overline{X}_f}{Y(\beta + 1)} X_f^* \frac{(\beta + 1) C^*}{\beta + C^*} = \phi^2 X_f^* \frac{(\beta + 1) C^*}{\beta + C^*} \quad (46)$$

However, in Beyenal and Lewandowski (2005) the term $(\beta + 1)$ is simplified in such a way that Thiele modulus and reaction rate are defined differently than derived here in Supplemental Material B. As a result, the solution for small values of ϕ_{BL} is

$$\eta_{BL} = \frac{1}{(\beta + 1)} - \frac{\sigma}{(\beta + 1)} \phi_{BL}^2 \quad (47)$$

with subscript BL indicating the parameter used as defined by the authors in their study and σ being the parameter found in our case. Hence the difference in outcome between the two studies is due to the fact that η_{BL} and ϕ_{BL} no longer represent the effectiveness factor and Thiele modulus corresponding to a Monod kinetic expression. One consequence is that $\eta_{BL} \rightarrow [1/(\beta + 1)]$ for small ϕ_{BL} values.

Others have used analytical approaches to estimate the flux of substrate through the biofilm by the weighted average of the flux corresponding to the zero and first order reaction rate (Perez et al., 2005, 2006). However, the authors considered neither heterogeneity in the biofilm nor the external mass transfer effect. The method presented here is most comparable to several approaches (Atkinson and Davies, 1974; Atkinson and How, 1974; Rittman and McCarthy, 1981) that resulted in an accurate pseudo-analytical solution for a steady-state biofilm (Saez and Rittman, 1992). The solution coupled external mass transport resistance to Monod kinetics (Rittman and McCarty, 1981) and included a biofilm mass balance (Rittman and McCarthy, 1980). The model can be linked with a reactor mass balance to calculate substrate concentration and substrate flux in a given biofilm system (Rittman and McCarthy, 2001). It has also been applied to describe multi-species biofilms (Wanner et al., 2006). These

pseudo-analytical approaches differ from the analytical method in the present study in that they all assume uniform biofilm density, a major simplification. Biofilm density, however, can vary considerably, for example, with mechanical stress, characteristics of organisms, and several other environmental factors. Observations in biofilm research are frequently made at the microscale with powerful experimental tools such as CLSM, magnetic resonance imaging, or microsensors (Battin et al., 2007). Our analytical approach can interpret such results, which are based on nonuniform biofilm density. By comparison, current 3-D models were developed for a particular biofilm (geometry) configuration, which cannot be generalized, and do not lend themselves to incorporating measurements from CLSM and microsensor measurements. In contrast, our analytical solution is generalized resulting in a continuum heterogeneous model.

Conclusions

The procedure introduced here is a general framework that can be used to describe the behavior of a heterogeneous biofilm and to calculate the rate of substrate utilization through estimation of the effectiveness factor. This approach allowed us to evaluate, in a fast and effective way, the effect of different parameters that characterize a heterogeneous biofilm and the kinetics of the rate of substrate utilization on the behavior of the biological system. The procedure can take into account external mass transfer resistance based on the analytical expression for the intrinsic effectiveness factor calculation, Equation (41), which allows a rapid and accurate calculation of η for each trial. Convergence is reached very quickly. A value of $[(C_s(i+1) - C_s(i))/C_s(i)] < 0.001$, was achieved in four or five cycles (i) for the test cases studied. Therefore, the simulation and scale-up of heterogeneous biofilm reactors can be easily carried out. The method is applicable to any biofilm growing on a substratum with different geometry including membrane, wall, pipe, or pellet. In the latter two cases, it is applicable if the ratio of biofilm thickness to diameter of the pipe or pellet is small, that is, planar geometry can be assumed for the biofilm.

Nomenclature

a parameter defined by Equation (19) (kg/m^3)
Bi Biot number, defined by Equation (43)
b parameter defined by Equation (19) (kg/m^3)
C^* dimensionless nutrient concentration
C nutrient concentration (kg/m^3)
C_s nutrient concentration at the surface of the biofilm (kg/m^3)
C_0 bulk fluid nutrient concentration (kg/m^3)
c dimensionless parameter defined by Equation (18)
D_f surface average effective diffusivity of nutrient (m^2/s)
D_f^* dimensionless relative effective diffusivity defined by Equation (10)
$\overline{D}_f$ average effective diffusivity along the (x) direction in the biofilm (m^2/s)
D_f° relative effective diffusivity defined by Equation (7)
D_w diffusivity of nutrient in the liquid medium (m^2/s)
d dimensionless parameter defined by Equation (28)
K_s Monod half maximum rate constant (kg/m^3)
k_c mass transfer coefficient (m/s)
L_f average biofilm thickness (m)
q_{max} maximum specific growth rate (s^{-1})
$R(C^*)$ dimensionless kinetic expression defined by Equation (12)
r_s nutrient consumption rate evaluate with the biofilm surface concentration (kg/s m^3)
r_0 nutrient consumption rate evaluate with the bulk fluid concentration (kg/s m^3)
r_{ob} average rate of nutrient consumption of the whole biofilm (kg/s m^3)
S_x biofilm-fluid interphase surface area (m^2)
X_f biofilm density (kg/m^3)
$\overline{X}_f$ average biofilm density along the (x) direction (kg/m^3)
X_f^* dimensionless relative effective diffusivity defined by Equation (10)
x distance from the bottom of the biofilm (m)
x^* dimensionless distance defined by Equation (9)
Y yield coefficient (kg microorganism/kg nutrient)

Greek Letters

α effective diffusivity at the bottom of the biofilm (m^2/s)
β dimensionless parameter defined by Equation (9); β is the ratio between the Monod half maximum rate constant and the substrate biofilm surface concentration
ξ effective diffusivity gradient (m/s)
η effectiveness factor for a continuum heterogeneous biofilm
η_0 bulk effectiveness factor
η_H effectiveness factor for a homogeneous biofilm
η_N effectiveness factor calculated with numerical technique
η_T analytical (exact) value of the effectiveness factor
κ parameter defined by Equation (10); κ is equal to the ratio between the nutrient diffusivity in water and the effective diffusivity at the bottom of the biofilm
ϕ Thiele modulus defined by Equation (13)
ϕ^* normalized Thiele modulus, defined by Equation (29)
Ψ parameter defined by Equation (10); Ψ is the ratio between the effective diffusivity at the bottom of the biofilm and the effective diffusivity difference between the top and the bottom of the biofilm

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