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# Quantifying selected growth parameters of *Leptothrix discophora* SP-6 in biofilms from oxygen concentration profiles

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**Abstract**

It is a dubious but common practice to use growth parameters measured in suspended cultures to predict substrate concentration profiles in biofilms. To obtain biofilm biokinetic parameters that apply to biofilms, a reliable method is needed that allows the computation of biokinetic parameters from substrate concentration profiles measured directly in biofilms. We have developed such a method and demonstrated its utility by evaluating biokinetic parameters from oxygen concentration profiles measured in biofilms of *Leptothrix discophora* SP-6 grown on a membrane, which was placed on top of an agar plate by fitting the data to Monod or Tessier growth kinetics, including maintenance substrate consumptions. We found that the Monod model represented the growth of *L. discophora* SP-6 biofilms marginally better than the Tessier model. The Monod half saturation coefficient was $0.333 \pm 0.077$ mg/l.


*Keywords:* Biofilms; Biokinetic parameters; Growth kinetics; Microelectrodes; Modeling

## 1. Introduction

Models of microbial growth kinetics quantify the relationship between the specific growth rate of microorganisms and substrate concentration. The constants in these models are termed "biokinetic parameters", and for some kinetics they may also have customary names, e.g. in the Monod equation they are termed half saturation constant and maximum specific growth rate. Biokinetic parameters that fit suspended culture growth models of various microorganisms, using various growth substrates, have been measured and reported in the literature for many microorganisms. Models of microbial growth in suspension are popular, and procedures of computing biokinetic parameters from such models are known. Because of lack of appropriate models and computational procedures for biofilms, to model microbial growth in biofilms biokinetic parameters measured in suspended cultures of the relevant microorganisms are commonly used (Kreft & Wimpenny, 2001; Eberl, Picioreanu, & Heijnen, 2000; Noguera, Okabe, & Picioreanu, 1999; Beyenal, Seker, Tanyolac, & Salih, 1997; Bakke, Characklis, Turakhia, & Yeh, 1989). However, using biokinetic parameters computed for microorganisms in suspension to predict the growth of these microorganisms in biofilms is questionable. It is obvious that these two modes of growth, suspended and attached, may be characterized by different biokinetic parameters, even if the growth models are the same, which is in itself an assumption worth verifying.

In principle, biokinetic parameters in biofilms can be estimated from diffusion–reaction models by fitting the model predictions to experimental data, e.g substrate consumption rate and bulk substrate concentrations (Cheton & Ellis, 2001; Samb, Deront, Adler, & Peringer, 1998; Riefler, Ahlfeld, & Smets, 1998; Nguyen & Shieh, 1995; Rittmann, Crawford, Tuck, & Namkung, 1986). Quantifying biokinetic parameters from substrate concentration profiles measured in biofilms is limited by two factors: (1) technical difficulties in measuring the substrate concentration profiles, and (2) lack of suitable computational procedures to quantify the biokinetic parameters from the substrate concentration profiles. While the technical difficulties of constructing and using microelectrodes have been overcome by several research groups, the lack of suitable computational procedures to quantify biokinetic parameters from substrate

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concentration profiles continues to be a problem. In our group we used the procedure developed by Lewandowski, Walser, and Characklis (1991) to quantify biokinetic parameters from dissolved oxygen concentration profiles measured across a biofilm. Their technique, however, was only applicable to the Monod kinetics and the computational procedures performed well only at low dissolved oxygen concentrations, near the half saturation concentration for oxygen (Lewandowski, 1994). Therefore, we have been working on constructing better computational procedures to calculate biokinetic parameters from substrate concentration profiles in biofilms (Visser, Hamilton, Lewandowski, Raquepas, & Tilt, 2003), and this paper gives such a procedure.

Attempts to compute biokinetic parameters characterizing microbial growth from substrate concentration profiles are not new, and are not limited to biofilms. In the stimulating paper Berg and Petersen (1998) described a method of predicting substrate concentration profiles from the zeroth order reaction rate in layers of the sediments as determined by microelectrode measurements. By increasing the number of the reacting layers, they successfully predicted the concentration profiles of $NO_3^-$ and $O_2$ using Nelder and Mead's (1965) down hill simplex method. Even though this method could not be used directly in biofilms because the authors assumed that the reaction rate in the biofilm was independent of substrate concentration (zeroth order), it was interesting to note that the authors successfully used the simplex method to predict substrate concentration profiles, which suggested that the simplex algorithm could be used in computational procedures searching for biokinetic parameters in biofilms. This work follows these principles in an attempt of computing biokinetic parameters characterizing microbial growth in biofilms.

The goal of this study was to develop an algorithm and a computational procedure to determine biokinetic parameters from substrate concentration profiles in biofilms. We decided to test the computational procedures by computing biokinetic parameters describing the growth of *L. discophora* SP-6 biofilms from dissolved oxygen concentration profiles measured in these biofilms. Our approach was based on an optimization technique that minimizes the squared differences between the oxygen concentrations measured at several locations in a biofilm and oxygen concentrations at the same locations predicted by the tested kinetic model. Since the procedure is based on improving the fit of experimental data to the model predicting concentration profiles in a biofilm, the computational procedures are not limited to any particular growth kinetics. To improve the stability and efficiency of the numerical procedures employed, we converted the model equations to dimensionless forms. We used the explicit Runge–Kutta (4,5) formula, the Dormand–Prince pairs, to solve the differential equations, and to calculate oxygen concentrations at the locations of microsensor measurements. To calculate the biokinetic parameters, we used a nonlinear optimization algorithm, including Nelder and Mead's (1965) simplex method developed by Lagarias, Reeds, Wright, and Wright (1998). Additionally, to determine the standard deviations of the predicted parameters, we used 21 experimentally measured oxygen concentration profiles and calculated the biokinetic parameters for each of them. All computations were performed using MATLAB®.

In our previous study, we found that *L. discophora* SP-6 growth in suspension was limited by the concentrations of pyruvate and oxygen in the solution, and the specific growth rate was adequately represented by combining Monod (or Tessier) growth kinetics with the maintenance substrate consumption (Yurt, Sears, & Lewandowski, 2002). Therefore, assuming that microbial growth in biofilms can be described by the same kinetics as microbial growth in suspension, we examined the Monod and the Tessier growth kinetics with maintenance substrate consumption to determine biokinetic parameters in *L. discophora* SP-6 biofilms.

The strategy of extracting biokinetic parameters that describe microbial growth in biofilms is composed of (1) growing relevant biofilms, (2) measuring nutrient concentration profiles, and (3) computing the biokinetic parameters characterizing microbial growth. In our case, we have quantified biokinetic parameters from the oxygen concentration profiles in a colony biofilm of *L. discophora* SP-6. Colony biofilms are somewhat different than the popular water-immersed biofilms; they are grown on solid media and are used to study antibiotic penetration (Anderl, Franklin, & Stewart, 2000). Even though their overall physical structure is similar to the biofilms grown immersed in water, i.e. microbial aggregates separated by interstitial voids, the colonies in colony biofilms are structurally uniform. The uniform structure of the biofilm was an important feature for our purposes, and needed to satisfy the assumptions of the biofilm model. We grew a colony biofilm of *L. discophora* SP-6 on membrane filters placed on the top of nutrient saturated agar layers. Dissolved oxygen concentration profiles were measured across these biofilms as shown in Fig. 1.

## 2. Materials and methods

### 2.1. Biofilms

The colony biofilms of *L. discophora* SP-6 (Fig. 1) were prepared according to the procedure described by Anderl et al. (2000). Forty-eight hour old cultures of *L. discophora* were diluted to an optical density of 0.02 at 600 nm (using 1-cm path length) in the growth medium specified below. Ten microliter of diluted culture was used to inoculate one sterile, polycarbonate membrane filter (25 mm ID, 0.2 μm pore size, Poretics Corporation, Livermore, CA, Cat. No. 11013) placed on an agar plate. Prior to inoculation, each side of the membrane filter was sterilized by UV light for 15 min. Inoculated Petri plates were kept at room temperature

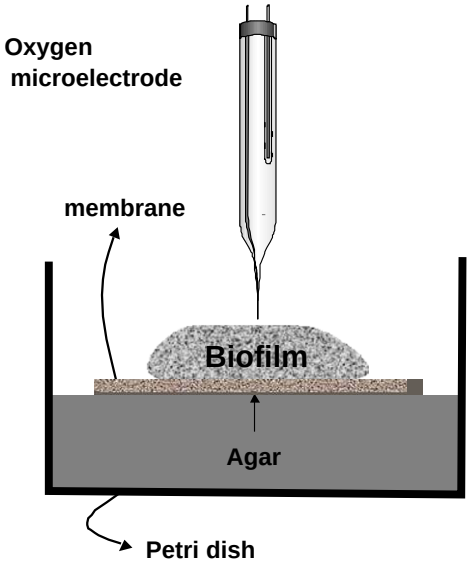


Fig. 1. A colony biofilm grown on the top of a membrane filter. The growth substrate is supplied by the agar, and is transferred through the polycarbonate membrane to the biofilm. The microorganisms receive oxygen from the air.

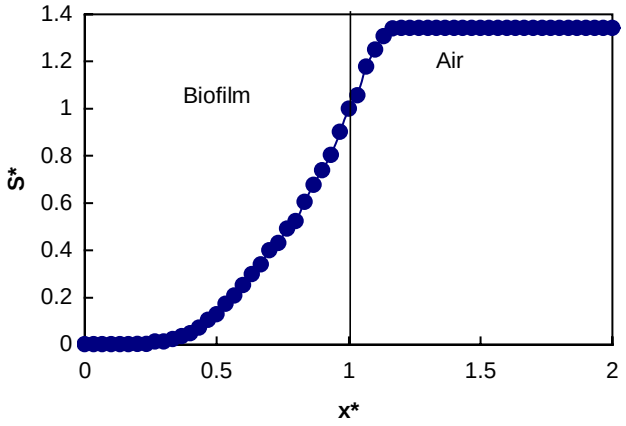


Fig. 2. Normalized dissolved oxygen concentration ($S^*$) is plotted versus normalized distance ($x^*$) from the bottom in a colony biofilm of *L. discophora* SP-6. The vertical line separates the data that were taken into account in calculating the biokinetic parameters (on the left side of the line) from those were not (on the right side of the line).

and the membrane filters with the colony biofilm were transferred to fresh agar plates every 24 h. The growth substrates were supplied by the agar and transferred through the membrane to the colony biofilm (Fig. 1), while the oxygen required for growth was delivered from the air.

To grow *L. discophora* SP-6, we used ATCC Culture 1917 MSVP (American Type Culture Collection Catalogue, ATCCC, 1992). It consisted of 0.24 g $(NH_4)_2SO_4$, 0.06 g $MgSO_4 \cdot 7H_2O$, 0.06 g $CaCl_2 \cdot 2H_2O$, 0.02 g $KH_2PO_4$, 0.06 g $Na_2HPO_4 \cdot 7H_2O$, and 2.383 g HEPES buffer in 950 ml distilled water. The pH of this medium was adjusted to 7.2 with NaOH or $H_2SO_4$, as needed. We added 15 g Agar Noble (Difco 214230) and autoclaved this solution at 121°C for 15 min per 1 l of the solution. Then, after cooling the solution to approximately 50°C, we added 1 ml of the vitamin solution (composition given below), 1 ml $FeSO_4$ (10 mM), and 48 ml of pyruvate solution (at various concentrations: 1; 5; 10; 20, and 30 g/l) aseptically by filter-sterilizing 0.2 μm sterile syringe filter (Corning, 431219). One liter of the vitamin solution consisted of 20 mg Biotin, 20 mg Folic acid, 50 mg Thiamine HCl, 50 mg D-(+) -calcium pantothenate, 1 mg Vitamin B12, 50 mg Riboflavin, 50 mg Nicotinic acid, 50 mg Pyridoxine HCl, and 50 mg *p*-Aminobenzoic acid. The agar solution was poured into sterilized petri dishes and kept in a cold place.

### 2.2. Microelectrode measurements

We measured dissolved oxygen concentration profiles using Clark type microelectrodes with guard cathodes, as described by Revsbech and Jorgensen (1986) (Fig. 1). The microelectrodes were made with tip diameters less than 10 μm to prevent damaging the biofilm structure during measurements. Local oxygen concentrations were measured in 5 μm intervals, and the response time of the microelectrodes was less then 1 s. The microelectrodes were calibrated before and after the measurements, purging water air and pure nitrogen gases.

To measure the oxygen concentration profiles, the microelectrode was mounted on a micromanipulator (Model M3301L, World Precision Instruments, New Haven, CT) equipped with a stepper motor (Model 18503, Oriel, Stratford, CT) controlled by the Oriel Model 20010 interface. Then, the tip of the microelectrode was positioned near the top of the biofilm and introduced into the biofilm perpendicularly to surface. The stepper motor was then interfaced with a computer and the microelectrode movement for the remaining time was handled by a controller (CTC-283-3, Micro Kinetics) with a positioning precision of 0.1 μm. Custom software was used to control and to coordinate microelectrode movement, the data acquisition, and to display the concentration profiles in real time. The position of the microelectrode was monitored during the measurement using a stereo microscope (Leica Stereo Zoom 7) with 40–70 times magnification.

### 2.3. An example of the measured profiles

Fig. 2 shows a typical dissolved oxygen concentration profile measured in a 120 h old colony biofilm of *L. discophora* SP-6. The continuous line shows the location of the biofilm surface, as determined using the microscope, we are sure that at this point the tip of the microelectrode was located in the biofilm. The biofilm thickness ($L_f$) at the location was 150 μm and the oxygen surface concentration ($S_s$) was 5.18 mg/l. The symbols $S^*$ ($=S/S_s$) and

$x^*$ ($=x/L_f$) designate the dimensionless concentration of oxygen and the dimensionless distance from the bottom. Since we could not see the exact location of the microelectrodes near the bottom, we measured the oxygen profile across the colony biofilm until at least two consecutive measurements showed the difference in normalized oxygen concentrations lower than 0.01.

## 3. Mathematical model

### 3.1. Microbial growth and substrate consumption

The Monod, Tessier, Moser, and Contois models are popular kinetic expressions describing microbial growth in suspension (Bailey & Ollis, 1986; Shuler & Kargi, 1992). In our previous study (Yurt et al., 2002), we demonstrated that Monod or Tessier models adequately describe the growth of *L. discophora* SP-6 in suspension. Therefore, we used these growth models to examine the growth of *L. discophora* SP-6 in biofilms.

$$\text{Monod} \qquad \mu = \mu_{\max}\frac{S}{K_{sM}+S} \quad \text{(Monod,1949)} \qquad (1)$$

$$\text{Tessier} \qquad \mu = \mu_{\max}(1-\mathrm{e}^{-S/K_{sT}}) \quad \text{(Tessier,1942)} \qquad (2)$$

Since it is not clear how important the maintenance substrate consumption is in biofilms, substrate consumption for maintenance (Powell, 1974; Pirt, 1965) was included in our computations, in agreement with our conclusions from the suspended culture study (Yurt et al., 2002);

$$-\frac{\mathrm{d}S}{\mathrm{d}t} = \frac{\mu}{Y_{x/o}}X + mX. \qquad (3)$$

Thus, the one-dimensional steady state diffusion–reaction equation is

*Monod*:

$$D_{\text{eff}}\frac{\mathrm{d}^2S}{\mathrm{d}x^2} = \frac{\mu_{\max}SX_f}{Y_{x/o}(K_{sM}+S)} + m_MX_f \qquad (4)$$

*Tessier*:

$$D_{\text{eff}}\frac{\mathrm{d}^2S}{\mathrm{d}x^2} = \frac{\mu_{\max}(1-\mathrm{e}^{-S/K_{sT}})}{Y_{x/o}} + m_TX_f. \qquad (5)$$

Searching for the best-fit model, we expected that the predicted Monod or Tessier biokinetics might produce different maintenance factors. To take this into account, we used two different symbols, $m_M$ and $m_T$, for the maintenance factors in Eqs. (4) and (5).

The following assumptions were made with respect to the experimental system, and to Eqs. (4) and (5). These assumptions are discussed and verified in Section 4.

1. Growth limiting substrate is oxygen.
2. Colony biofilms are uniform.
3. Substrates in the colony biofilm are transported only by diffusion.
4. Density and diffusivity in colony biofilms can be represented by their average values.
5. Transfer of oxygen is one-dimensional.
6. Colony biofilms are at pseudo steady state (with respect to the oxygen consumption rate).

Eqs. (4) and (5) cannot be solved analytically; numerical methods have to be applied. To improve the stability and efficiency of the numerical procedures employed, we converted Eqs. (4) and (5) to dimensionless forms:

*Monod kinetics* (*Eq.* (4))

$$\frac{\mathrm{d}^2S^*}{\mathrm{d}x^{*2}} = \Phi_M^2\frac{S^*}{(\beta_M+S^*)} + M_M, \qquad (6)$$

where

$$\Phi_M = \sqrt{\frac{\mu_{\max}L_f^2X_f}{Y_{x/o}D_{\text{eff}}S_s}}, \qquad (7)$$

$$\beta_M = \frac{K_{sM}}{S_s}, \qquad (8)$$

$$M_M = \frac{m_ML_f^2X_f}{D_{\text{eff}}S_s}. \qquad (9)$$

*Tessier kinetics* (*Eq.* (5))

$$\frac{\mathrm{d}^2S^*}{\mathrm{d}x^{*2}} = \Phi_T^2(1-\mathrm{e}^{-S^*/\beta_T}) + M_T, \qquad (10)$$

where

$$\Phi_T = \sqrt{\frac{\mu_{\max}L_f^2X_f}{Y_{x/o}D_{\text{eff}}S_s}}, \qquad (11)$$

$$\beta_T = \frac{K_{sT}}{S_s}, \qquad (12)$$

$$M_T = \frac{m_TL_f^2X_f}{D_{\text{eff}}S_s}. \qquad (13)$$

The boundary conditions for the solution of Eqs. (6) and (10) are

$$x^* = 0, \quad S^* = S^*_{\text{bottom}}, \qquad (14)$$

$$x^* = 0, \quad \mathrm{d}S^*/\mathrm{d}x^* = 0. \qquad (15)$$

We use the subscripts "$_M$" and "$_T$" to refer to the Monod kinetics (Eqs. (6)–(9)) and the Tessier kinetics (Eqs. (10)–(13)), respectively. Eqs. (7) and (11) refer to the same $\Phi$. Since, in search of the best-fit model we used two different growth kinetics, Monod and Tessier, to take into account that the predicted $\Phi$ values might be different for each of these kinetics, we used two different subscripts: $\Phi_M$ and $\Phi_T$. When referring to the conditions that apply to both $_M$ and $_T$, we use the subscript "$i$".

If the parameters $\Phi_i$, $\beta_i$, and $M_i$ are known, two boundary conditions are enough to solve Eq. (6) or Eq. (10). These

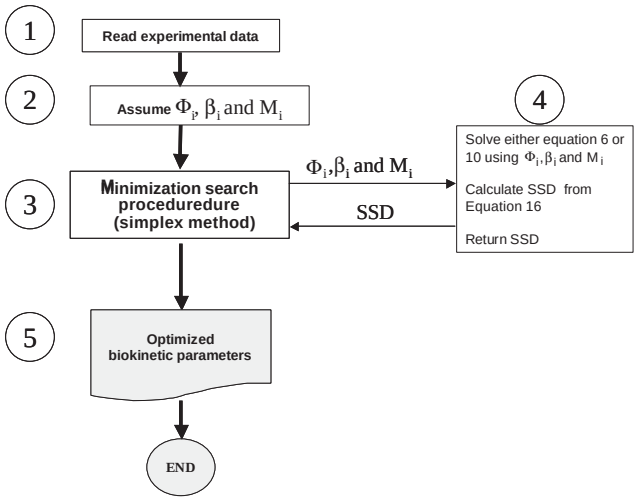


Fig. 3. The flowchart we used to compute biokinetic parameters in the colony biofilms.

boundary conditions are specified in Eqs. (14) and (15), and the meaning of these boundary conditions is examined by inspecting Fig. 2: Eq. (14) is the dimensionless oxygen concentration at the bottom of the colony biofilm, while Eq. (15) states that there is no flux of oxygen through the bottom.

The term "*biokinetic parameters*" refers to $\Phi_i$, $\beta_i$, and $M_i$. From $\beta_M$ we can calculate $K_{sM}$, and from $\beta_T$ we can calculate $K_{sT}$. However, we cannot calculate the maximum specific growth rate or the yield coefficient because these parameters are lumped into $\Phi_i$.

### 3.2. Solution algorithm

The algorithm we used to compute the biokinetic parameters is shown in Fig. 3, and we followed this algorithm to solve Eqs. (6) or (10). To facilitate computing the biokinetic parameters, we integrated the algorithm (Fig. 3) into a MATLAB® program, using MATLAB® version 12.1, and ran it on a PC with a Pentium® 3 processor using the Windows® operating system. This algorithm and MATLAB® program will be available on our web site, http://www.erc.montana.edu, prior to publication of this paper.

The procedures in the algorithm (Fig. 3) are best explained using a pseudo-code. The numbers in parenthesis refer to steps in the flowchart in Fig. 3.

- Read the sequence of pairs: dimensionless distances and dimensionless oxygen concentrations (1).
- Assume $\Phi_i$, $\beta_i$, and $M_i$ values (2), and run minimization search procedure (3).
- Minimization search procedure calls SSD calculator (3).
- SSD calculator solves Eq. (6) or (10) using boundary conditions (14) and (15) as an initial value problem, using Runge–Kutta Dormand pairs techniques (MATLAB® function *ode45*) (4).
- SSD calculator determines the predicted concentrations of oxygen, at the same locations where they were measured, and calculate the sum of squared differences (SSD) for the pairs of predicted and measured values as

$$\mathrm{SSD} = \sum_{n=1}^{n=n\_\mathrm{of}\_\mathrm{data}} (S^*_{\mathrm{predicted}} - S^*_{\mathrm{experimentally\_measured}})^2 \quad (16)$$

- Minimization search procedure (4) continues until it finds the $\Phi_i$, $\beta_i$, and $M_i$ values that minimize Eq. (16). Eq. (16) is considered the objective function in the search for optimum $\Phi_i$, $\beta_i$, and $M_i$ values.

To minimize SSD and calculate optimum $\Phi_i$, $\beta_i$, and $M_i$ values, we used the direct search method (Lagarias et al., 1998) integrated with the MATLAB®'s *fminsearh* function. The *fminsearh* is referred to as an unconstrained nonlinear optimization routine in MATLAB®'s help menu and in Lagarias et al. (1998). The *fminsearh* starts searching from the assumed $\Phi_i$, $\beta_i$, and $M_i$ values and calculates the respective SSD from the solution of Eq. (6) or (10). The SSD calculation is shown by step number 4 in Fig. 3, and called, "SSD calculator" in our procedure. We constrained the values of $\Phi_i$, $\beta_i$, and $M_i$ to be greater than or equal to zero in the SSD calculator because their values can not be smaller than zero. Then, the *fminsearh*, using Nelder and Mead's (1965) simplex algorithm, finds the optimum $\Phi_i$, $\beta_i$, and $M_i$ values for the next iteration. The iteration stops when the optimum $\Phi_i$, $\beta_i$, and $M_i$ values are found, i.e. when parameters from two consecutive iteration differ by less than 0.00001. We relied on the MATLAB's default criteria of convergence.

To test if the MATLAB® program predicted the biokinetic parameters correctly, we used a hypothetically produced concentration profile from a known set of $\Phi_i$, $\beta_i$, and $M_i$ values. The hypothetical concentration profile was generated using Eqs. (14) and (15) as boundary conditions and Runge–Kutta Dormand pairs technique to solve differential Eq. (10) or (16). The MATLAB® program predicted the $\Phi_i$, $\beta_i$, and $M_i$ values accurately for different known $\Phi_i$, $\beta_i$, and $M_i$ values and for the Monod or the Tessier models.

### 3.3. Calculation of the standard deviations

It is not expected that any single set of biokinetic parameters can accurately predict substrate concentration profiles at each location in the biofilm. It is expected that these biokinetic parameters can vary, within certain limits. However, the procedures we use cannot distinguish the measurement errors from the expected variability in the measured parameter. Therefore, we prescribed all the deviations to the variability in the measured parameter, and called it standard deviation, to refer to the fact that in real biofilms, the computed biokinetic parameters are expected to vary within these limits.

To calculate the standard deviations for $K_{sM}$ and $K_{sT}$, we performed 21 concentration profile measurements, similar to those in Fig. 2. Eight freshly prepared colony biofilms of

*L. discophora* SP-6 were used, and at least 2 to 3 oxygen profiles were measured in each colony. The concentration profiles were measured at the center of the biofilm colonies but also 100 μm away from the center. From each concentration profile, we calculated biokinetic parameters $\Phi_i$, $\beta_i$, and $M_i$. However, the standard deviations were only calculated for $K_{sM}$ and $K_{sT}$.

## 4. Results and discussion

The trustworthiness of the computed biokinetic parameters depends on how closely the experimental system, and the experimental procedures, adhere to the assumptions of the model we used. Therefore, we will discuss each of the assumptions separately.

*1. Growth limiting substrate is oxygen*: In our previous study (Yurt et al., 2002) we demonstrated that the growth of *L. discophora* in suspension was limited by pyruvate and by oxygen, but not by nitrogen or phosphorus. Depending on the experimental conditions, microbial growth in the colony biofilms can be limited by pyruvate or by oxygen. To make sure that this assumption applies, and to determine the condition for which microbial growth in the colony biofilms was limited by oxygen, we grew biofilms for 120 h on agar plates consisting of 1, 5, 10, 20, and 30 g/l pyruvate. Measuring oxygen concentration profiles in these biofilms, we concluded that when pyruvate concentrations exceeds 10 g/l, the oxygen profiles do not change much, indicating that oxygen limits the growth (Fig. 4). Therefore, to make sure that in our measurements oxygen limits microbial growth, we used 20 g/l pyruvate in agar.

*2. Colony biofilms are uniform*: We used a colony biofilm, and not a water-immersed biofilms, because colony biofilms are structurally uniform, and have large cell clusters without voids. A measurable, and expected, consequence

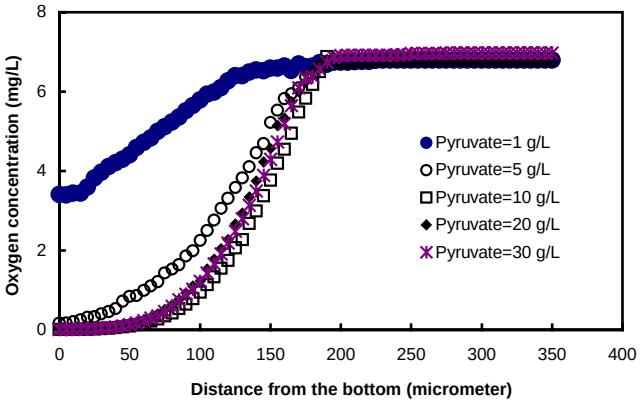


Fig. 4. Oxygen concentration profiles measured in a 120 h old colony biofilms grown on agar plates containing 1, 5, 10, 20 and 30 g/l pyruvate. When pyruvate concentrations exceeds 10 g/l, the oxygen profiles do not change much, indicating that pyruvate concentration is in excess and microbial growth is limited by oxygen.

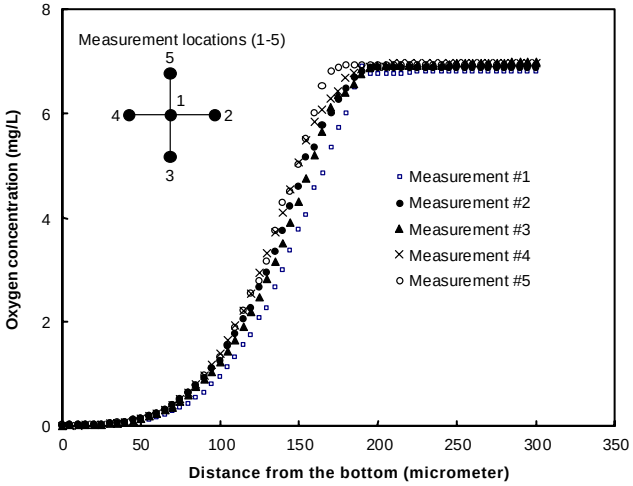


Fig. 5. Oxygen concentration profiles measured at different locations (1–5) in the colony biofilm. Location 1 was at the center of the microcolony and other locations were approximately 100 micrometers apart from the location #1.

of such structural uniformity is that substrate concentration profiles separated by reasonable close distances are very similar, which cannot be said about heterogeneous water immersed biofilms. To confirm uniformity of the biofilm, we measured vertical oxygen profiles at different locations in a microcolony, and found that the profiles measured approximately 100 micrometers apart were indeed almost identical (Fig. 5). Location 1 is the center of the colony and the other locations are at the vertexes of the cross sign at the location 1 (see the insert in Fig. 5). The results demonstrate that oxygen profiles are similar (the average $K_{sM}$ for all five profiles is $0.33 \pm 0.03$ mg/l), which is an expected consequence of uniform distribution of microorganisms.

*3. Nutrients in colony biofilms are transported only by diffusion*: This requirement can be satisfied by the careful design of the experiment, and by being aware at each stage of the measurement that precautions have to be made to satisfy this requirement. Convective flow from the agar layer to the biofilm was unlikely, and therefore all dissolved nutrients must have been transported by diffusion. However, avoiding convective transport of oxygen from air is more difficult. During the microelectrode measurements, when the reactor was opened to the atmosphere, we took all precautions to avoid air movement, and convectional flow of air. Therefore, we assumed that oxygen was transported to the biofilm by diffusion. In summary, we believe that in our reactor transport of dissolved substrates and oxygen could be described by Fick's law.

*4. Density and diffusivity in colony biofilms can be represented by their average values*: We are aware of the fact that biofilm density and effective diffusivity vary across biofilms (Beyenal & Lewandowski, 2000). However, to simplify the computations of biokinetic parameters we assumed that these variations could be neglected and that the average

biofilm density and diffusivity could be used in the computational procedures. In our models, density and effective diffusivity of the colony biofilm are lumped into the dimensionless module ($\Phi_i$), which represents their average value across the colony biofilm, which is consistent with the assumption that these values can be averaged. Verifying this assumption is work and time consuming, and we are currently developing experimental techniques to quantify the effect of effective diffusivity and density variations on the biokinetic parameters. As a result we will determine how much such variations would affect the biokinetic parameters.

*5. Transfer of oxygen is one-dimensional*: Results in Fig. 5 demonstrated that this assumption was acceptable, at least at the selected location, near the center of the colony. If the oxygen concentrations in profiles measured closer to the edge of the colony, locations 2; 3; 4, and 5, were significantly different than those measured at the center of the colony, the mass transfer could not be considered one-dimensional, as is often the case in heterogeneous biofilms (Beyenal & Lewandowski, 2001; de Beer, Stoodley, Roe, & Lewandowski, 1994). In our colony biofilm, the colony diameter, 3000 μm, was 20 times larger than the biofilm thickness, 150 μm. Therefore, we assumed that near the center of the colony, the substrate transport in radial and angular directions can be neglected, and therefore profiles measured at these locations can be used to compute the biokinetic parameters.

*6. The colony biofilms are at pseudo steady state* (*with respect to the oxygen consumption rate*): We define pseudo-steady state as a condition where oxygen concentration profiles do not change for reasonably long intervals, measured in hours. To test this assumption, we measured oxygen concentration profiles at the same location in a 120 and 144 h old colony biofilm. The profiles did not change much, as seen in Fig. 6, and therefore, the oxygen consumption rate was at the pseudo steady state.

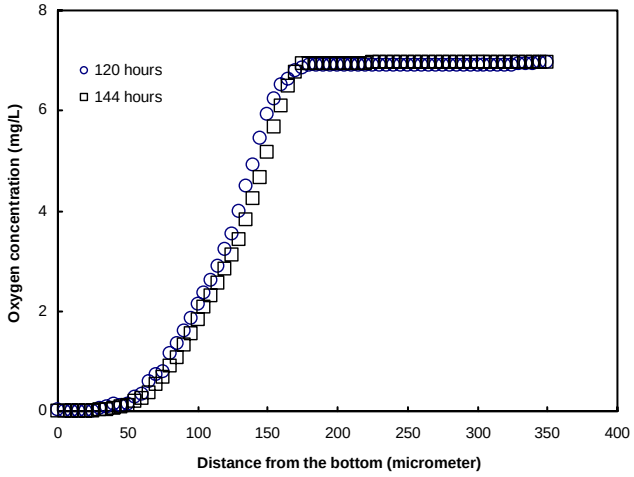


Fig. 6. Oxygen concentration profiles measured in a 120 and a 144 h old colony biofilms are similar, which supports the assumption that oxygen consumption is at pseudo steady state. The $K_{sM}$ values were 0.31 and 0.33 for the profiles measured at 120 and 144 h, respectively.

Table 1
Biokinetic parameters and their standard deviations evaluated for colony biofilm of *L. discophora* SP-6

| Parameters | Monod (Eq. (6)) | Tessier (Eq. (10)) |
|---|---|---|
| $K_{sM}$ (mg/l) | $0.333 \pm 0.077$ | na |
| $K_{sT}$ (mg/l) | na | $0.338 \pm 0.141$ |
| SSD | $0.099 \pm 0.095$ | $0.121 \pm 0.114$ |
| $M_M$ | $0.0006333 \pm 0.000616$ | na |
| $M_T$ | na | $0.000849 \pm 0.000584$ |

na = Not applicable.

### 4.1. Predictions of $\Phi_i$, $\beta_i$ and $M_i$

As an example, Eq. (6) was solved using the data in Fig. 2, and for this set of data we calculated $\Phi_M = 4.7401$, $\beta_M = 0.0645$, $M_M \approx 0$, and SSD = 0.0064 using the initial estimates of $\Phi_M = 1$; $\beta_M = 1$, and $M_M = 1$. To test how sensitive the computational procedure was to the initial estimates, we ran the program for randomly generated initial estimates; $\Phi_M$ between 0 and 1000, $\beta_M$ between 0 and 100, and $M_M$ between 0 and 10. Even though the initial estimates varied, the same optimal fit values of $\Phi_M$, $\beta_M$, and $M_M$ were calculated repeatedly. We also repeated this procedure using the Tessier model (Eq. (10)), and again found that the calculated biokinetic parameters were independent of the initial estimates. Therefore, our algorithm seems to be immune to selection of the initial estimates, within the tested limits. This could be caused by a variety of reasons, but we believe that the following two are important; (1) we used dimensionless equations which increase computational sensitivity and lower the round-off errors and (2) because we used the simplex method which is an efficient algorithm to find global minimum values.

### 4.2. Biokinetic parameters and their standard deviations

Table 1 shows the biokinetic parameters and their standard deviations for colony biofilms of *L. discophora* SP-6. The values in Table 1 were determined from 21 independent measurements in different colony biofilms, and at different locations. According to Table 1, the Monod model (Eq. (6)) represents the growth kinetics of *L. discophora* SP-6 colony biofilms better than the Tessier model; it has a slightly lower SSD value than those computed for the Tessier model.

The coefficient $K_{sT}$ in the Tessier equation should not be confused with the half saturation coefficient $K_{sM}$ in the Monod equation. Tessier (1942) developed his equation by hypothesizing that the dependence of specific growth rate on the substrate concentration was proportional to the difference

between $\mu$ and $\mu_{max}$:

$$\frac{d\mu}{dS} = \frac{1}{K_{sT}}(\mu_{max} - \mu). \quad (17)$$

Eq. (17), when integrated, gives the well-known form of the Tessier equation. To compare the Tessier coefficient $K_{sT}$ with the Monod's half saturation coefficient $K_{sM}$, the half saturation constant (substrate concentration for which $\mu = \mu_{max}/2$) can be estimated from Eq. (17), and is equal to $K_{sT} \log 2$ (Powell, 1974). In our model, the average $K_{sT} =$ 0.338 mg/l corresponds to the half saturation constant for oxygen in the Monod equation equal to $K_{sM} = 0.338 \times \log 2 =$ 0.102 mg/l.

Finally, once we verify the computational procedures, we fully expect to use the described procedures to estimate biokinetic parameters in water-immersed biofilms. However, to verify the computational procedures, we had to use colony biofilms, and not water immersed biofilms. The goal of this paper was to develop an algorithm of calculating biokinetic parameters in microbial biofilms from substrate (oxygen in this case) concentration profiles, and the choice of the attached microbial aggregates was arbitrary, as long as they satisfied the assumptions we specified when developing the model. We found water-immersed biofilms less suitable for verifying the computational procedures because of their inherently variable structure. In contrast to the colony biofilms, they form relatively uniform and large clusters and, as a result of that, some of the assumptions we have made, particularly those where we neglect the lateral mass transport, may have not been warranted in small colonies. Consequently, we were afraid that using water-immersed biofilms we could not distinguish between the effects of inherent variability of biofilm structure and the experimental errors, and we could not ignore the effects of biofilm structure variability. Whether the methods we developed using colony biofilms can be safely used in water immersed biofilms has to be tested in a separate set of experiments using appropriate tools of statistical analysis.

Computing biokinetic parameters in various biofilms should allow the comparison of biokinetic parameters of microorganisms growing in suspension (chemostats) and in biofilms, and test the reliability of the controversial procedures that use biokinetic parameters calculated in suspended microbial cultures to model biofilm activity.

In our previous study (Yurt et al., 2002) we determined several growth parameters for *L. discophora* SP-6 in suspension, and they can now be compared with the growth parameters we measured in the biofilm. Monod half saturation coefficient measured in the suspended culture was $K_{sM} = 0.237$ and in the biofilm $K_{sM} = 0.333$ mg/l. The difference between half saturation coefficients measured in the biofilm and in suspended culture is not dramatic, about 29%, and it is not clear whether this difference is significant. The calculated $K_{sM}$ values in suspension were close to the half saturation coefficient measured for other microorganisms grown in suspension (Beyenal, Chen, & Lewandowski, 2003; Seker, Beyenal, Salih, & Tanyolaç, 1997). Similarly, the calculated $K_{sM}$ values calculated for microbial growth in biofilms were similar to those reported for other organisms. Lewandowski et al. (1991) calculated $K_{sM}$ in biofilms as 0.25 mg/l, which is 25% less than the value we estimated in the colony biofilm of *L. discophora* SP-6. As expected, for both suspended and attached growth, Monod coefficient for oxygen has a small value, and there is little difference between its values estimated by various authors. We could not separate the maintenance factor from the other variables, and we had to lump it together with other variables in the Thiele modulus. So, we could not report any value for maintenance factor.

## 5. Conclusions

We have developed a computational procedure for calculating Monod half saturation coefficient, Tessier coefficient, and Thiele modulus for microbial growth in biofilms from substrate concentration profiles. To demonstrate the utility of this procedure we calculated these parameters from oxygen concentration profiles for *L. discophora* SP-6 biofilms. Testing the performance of the procedure for the Monod and the Tessier microbial growth kinetics, including maintenance substrate consumption, we concluded that

1. The algorithm presented in the paper successfully calculated the specified parameters.
2. Monod growth kinetics represented the growth of *L. discophora* SP-6 colony biofilms marginally better than the Tessier growth kinetics.
3. Monod half saturation coefficient for oxygen consumption in the colony biofilms of *L. discophora* SP-6 was $0.333 \pm 0.077$ mg/l.

## Notation

| | |
|---|---|
| $D_{eff}$ | effective diffusion coefficient of oxygen in colony biofilm, $m^2/s$ |
| $i$ | subscript refers to the growth model, $M$ for Monod and $T$ for Tessier |
| $K_{sM}$ | Monod half saturation coefficient, $g/m^3$ |
| $K_{sT}$ | Tessier coefficient, $g/m^3$ |
| $L_f$ | Colony biofilm thickness, m |
| $m$ | Maintenance coefficient for oxygen (mg microorganism/mg oxygen) |
| $M_M$ | $= \frac{m_M L_f^2 X_f}{D_{eff} S_s}$ |
| $m_M$ | maintenance coefficient for oxygen when growth is described according to Monod kinetics (mg microorganism/mg oxygen) |

| | |
|---|---|
| $M_T$ | $=\dfrac{m_T L_f^2 X_f}{D_{\text{eff}} S_s}$ |
| $m_T$ | maintenance coefficient for oxygen when growth is described according to Tessier kinetics (mg microorganism/mg oxygen) |
| $S$ | oxygen concentration in colony biofilm, g/m$^3$ |
| $S^*$ | dimensionless oxygen concentration in colony biofilm ($=S/S_s$) |
| $S^*_{\text{bottom}}$ | experimentally measured dimensionless oxygen concentration at the bottom of colony biofilm ($=S_{\text{bottom}}/S_s$) |
| $S^*_{\text{experimentally\_measured}}$ | experimentally measured dimensionless oxygen concentration in the colony biofilm |
| $S^*_{\text{predicted}}$ | calculated dimensionless oxygen concentration from the solution of the model in colony biofilm |
| $S_{\text{bottom}}$ | oxygen concentration at the bottom of colony biofilm ($=S/S_s$) |
| $S_s$ | oxygen concentration at colony biofilm–air interface, g/m$^3$ |
| $t$ | time, s |
| $x$ | distance from the bottom, m |
| $x^*$ | dimensionless distance from the bottom ($=x/L_f$) |
| $X$ | microorganism concentration, g/m$^3$ |
| $X_f$ | colony biofilm density, g/m$^3$ |
| $Y_{x/o}$ | yield coefficient (g microorganism produced/g oxygen consumed) |

*Greek letters*

| | |
|---|---|
| $\beta_M$ | $=\dfrac{K_{sM}}{S_s}$ |
| $\beta_T$ | $=\dfrac{K_{sT}}{S_s}$ |
| $\mu$ | specific growth rate, s$^{-1}$ |
| $\mu_{\max}$ | maximum growth rate, s$^{-1}$ |
| $\Phi$ | Thiele modulus ($\Phi_M$ or $\Phi_T$) |
| $\Phi_M$ | $=\sqrt{\dfrac{\mu_{\max} L_f^2 X_f}{Y_{x/o} D_{\text{eff}} S_s}}$ |
| $\Phi_T$ | $=\sqrt{\dfrac{\mu_{\max} L_f^2 X_f}{Y_{x/o} D_{\text{eff}} S_s}}$ |

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