ELSEVIER

Enzyme and Microbial Technology 32 (2003) 92–98

ENZYME and MICROBIAL TECHNOLOGY

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# The double substrate growth kinetics of *Pseudomonas aeruginosa*

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Received 14 June 2001; received in revised form 19 September 2002; accepted 23 September 2002

**Abstract**

Growth parameters of *Pseudomonas aeruginosa* were quantified based on steady-state concentrations, utilization rates of glucose and dissolved oxygen, and microorganism concentration in a chemostat that was operated at 25 °C, pH 7.2, and an agitation rate 350 rpm. The results showed that the microbial growth was limited by the concentration of glucose and the concentration of oxygen. A dual-substrate, Tessier growth kinetics for oxygen and glucose, was in good agreement with the experimental data using the following biokinetic parameters: $\mu_{max} = 0.29\,h^{-1}$, $K_g = 26.9$ mg/l, $K_o = 1.18$ mg/l, $Y_{x/g} = 0.628$ g microorganism/g glucose and, $Y_{x/o} = 0.635$ g microorganism/g oxygen. Maintenance factors for glucose and oxygen were: $m_g = 0.0078$ g glucose consumed/g microorganism h, and $m_o = 0.014$ g oxygen consumed/g microorganism h.


*Keywords:* *Pseudomonas aeruginosa*; Growth kinetics; Multiple-substrate; Biokinetics

## 1. Introduction

To our best knowledge, there are not any available multiple-substrate growth kinetic model developed for *Pseudomonas aeruginosa*. The goal of this study is to develop such a model and to calculate biokinetic parameters associated with the model. *P. aeruginosa* is often used in biofilm studies and modeling biofilm accumulation, probably because microbial geneticists have been studying this organism intensively and its physiology and genetics are well known [1–3]. Biokinetic parameters for microbial growth of *P. aeruginosa* have been determined in biofilms by Bakke et al. [4], and in planktonic cultures by Robinson et al. [5]. However, in both papers the growth parameters of *P. aeruginosa* have been determined at relatively low glucose concentrations, less than 7.5 mg/l in the chemostat [4], and less than 1.4 mg/l in the biofilm reactor [5]. We can only guess that the reason for using such low glucose concentrations was to assure that glucose—not oxygen—was the limiting substrate. In biofilms, however, there is little control over the substrates that may act as growth-limiting factors [6,7]. Concentrations of electron donors and electron acceptors in biofilms decrease toward the bottom because of mass transfer limitations and microbial consumption, and it may be difficult to assess which of them is exhausted first in the deeper biofilm layers. In the excess of glucose in solution, it is reasonable to assume that oxygen rather than glucose will be the growth-limiting factor. However, to quantify biofilm accumulation rate, models accounting for multiple-substrate utilization by the microorganisms should be used to judge whether the electron donor or the electron acceptor is the growth-limiting factor [8].

The inherent difficulty associated with developing relevant multiple-substrate growth models stems from the necessity of providing relevant experimental data and solving non-linear equations. Appropriate techniques to build such models are available [5,9]. We have generated experimental data and constructed a multiple-substrate growth model for *P. aeruginosa*. To acquire experimental data we used a chemostat, measured concentrations, consumption rates of glucose and dissolved oxygen, and concentration of the microorganism. All measurements were made at steady states.

## 2. Materials and methods

### 2.1. Microorganism and growth conditions

A pure culture of *P. aeruginosa* (ATCC 700829) was used throughout the study. To grow the microorganism we used an artificial growth medium containing $Na_2HPO_4$ (1.83 g/l); $K_2HPO_4$ (0.35 g/l); $MgSO_4{\cdot}7H_2O$ (0.01 g/l); yeast extract (0.001 g/l); $(NH_4)_2SO_4$ (0.1 g/l) and glucose

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PII: S0141-0229(02)00246-6

**Nomenclature**

| | |
|---|---|
| $B_g$ | constant in Contois model for glucose |
| $B_i$ | constant in Contois model for substrate $i$ |
| $B_o$ | constant in Contois model for oxygen |
| $D$ | dilution rate ($h^{-1}$) |
| $K_g$ | Monod saturation constant for glucose (g/l) |
| $K_o$ | Monod saturation constant for oxygen (g/l) |
| $K_{si}$ | half-saturation constant for substrate $i$ (g/l) |
| $m_g$ | maintenance factor for glucose ($h^{-1}$) |
| $m_i$ | maintenance factor for limiting substrate $i$ ($h^{-1}$) |
| $m_o$ | maintenance factor for oxygen ($h^{-1}$) |
| mo | microorganism |
| $N$ | number of experimental data |
| OUR | oxygen uptake rate (mg oxygen/h) |
| $Q$ | flow rate (l/h) |
| $S_{ei}$ | substrate concentration in effluent stream (g/l) |
| $S_{fg}$ | concentration of glucose in fresh feed (g/l) |
| $S_{fi}$ | substrate concentration in influent stream (g/l) |
| $S_{fn}$ | concentration of ammonium sulfate in fresh feed (g/l) |
| $S_g$ | concentration of glucose in chemostat (g/l) |
| $S_i$ | concentration of substrate $i$ (g/l) |
| $S_o$ | concentration of dissolved oxygen (g/l) |
| SOUR | specific oxygen uptake rate (g oxygen/g microorganism/h) |
| SSD | sum of squares of the differences (see Eq. (10)) |
| $T$ | temperature (°C) |
| $V$ | reactor volume (l) |
| $X$ | microorganism concentration in chemostat (g/l) |
| $Y_{x/g}$ | yield coefficient for glucose (g microorganism/g glucose) |
| $Y_{x/o}$ | yield coefficient for oxygen (g microorganism/g oxygen) |
| $Y_{x/si}$ | yield coefficient for limiting substrate $i$ (g microorganism/g limiting substrate) |

*Greek letters*

| | |
|---|---|
| $\mu$ | specific growth rate ($h^{-1}$) |
| $\mu_{experimental}$ | experimental specific growth rate ($h^{-1}$) |
| $\mu_i$ | specific growth rate for limiting substrate $i$ ($h^{-1}$) |
| $\mu_{max}$ | maximum specific growth rate ($h^{-1}$) |
| $\mu_{predicted}$ | theoretical specific growth rate ($h^{-1}$) |
| $\lambda_g$ | Moser's constant for glucose (g/l) |
| $\lambda_i$ | Moser's constant for substrate $i$ (g/l) |
| $\lambda_o$ | Moser's constant for oxygen (g/l) |

(1 g/l). One milliliter of trace elements was added to the growth medium for every liter of growth medium. The solution of trace elements had the following composition; $MnCl_2{\cdot}4H_2O$ (527 mg/l); $CuCl_2{\cdot}2H_2O$ (228 mg/l); $CoCl_2{\cdot}2H_2O$ (317 mg/l); $(NH_4)Mo_7O_4{\cdot}H_2O$ (231 mg/l); $Na_2B_4O_7{\cdot}10H_2O$ (127 mg/l); $ZnCl_2$ (363 mg/l); $FeCl_3$ (2160 mg/l); and $CaCl_2$ (3700 mg/l). The growth medium was prepared using distilled water sterilized in an autoclave at 121 °C and 1 atm absolute pressure for 3 h. Glucose, yeast extract, and $(NH_4)_2SO_4$ were autoclaved separately. Trace elements were added to the sterile growth medium using a disposable sterile syringe filter (0.2 μm, Corning).

### 2.2. Experimental setup

We used a New Brunswick (BioFlo 2000) chemostat with a working volume of 2 l, equipped with pH, agitation, temperature, and dissolved oxygen controllers. The sensitivities of the control units for dissolved oxygen, pH, and agitation rate were 0.05%, 0.1 unit, and ±1 rpm, respectively. Prior to use, the chemostat was autoclaved for 30 min at 121 °C. The pH was controlled using solutions of 0.1N NaOH and 0.1N $H_2SO_4$. The agitation rate was 350 rpm (optimized) to maintain a homogeneous culture. Dissolved oxygen concentration was controlled in the range of 0.5–7.3 mg/l by sparging

filtered air or mixtures: air + oxygen or air + nitrogen, depending on the needs. The air flow rate was fixed between 1 and 5 l/h. The reactor was inoculated with microorganisms using 10% (v/v) solution at exponential growth phase.

Microorganisms used to inoculate the chemostat were prepared as follows: 1 ml frozen stock sample (stored at −70 °C) of *P. aeruginosa* (ATCC 700829) was introduced into separate flasks containing growth medium composed of glucose (5 g/l), yeast extract (0.2 g/l), $(NH_4)_2SO_4$ (1 g/l), and the remaining components of the growth media (as described before). The volume of the solution was 100 ml, and the microorganisms were grown for 20–30 h. The flasks were placed on a shaker, set at 150 rpm, at room temperature, approximately 25 °C. The typical cell concentration, when plated on R2A agar and grown for 24 h in the incubator at 30 °C, was $9.4 \times 10^6$ CFU/ml of solution.

After inoculation, the chemostat was initially run in a batch mode. Continuous pumping of fresh feed started after the culture had entered the exponential growth phase (ca. after 20–30 h). To establish a steady state, the reactor was run for six to seven retention times, and steady state was assumed if the absolute differences in consecutive measurements of effluent substrate concentration differed by less than 3%. Several dilution rates up to the washout point were used, and the corresponding steady-state data were recorded. To find a new steady state, the dilution rate was increased by a gradual increase in the feed-flow rate. The chemostat was operated using different combinations of influent substrate concentration, pH, agitation rate, and temperature.

### 2.3. Analytical methods

The microorganism concentration was determined using a standard dry-weight method [10]. The glucose concentration was measured using Sigma® procedure 510 (Sigma® Diagnostics, St. Louis, MO). The ammonium sulfate concentration was measured using a Hatch® ion-selective electrode (Loveland, CO). Dissolved oxygen and pH were monitored by Ingold® dissolved oxygen and pH electrodes integrated with the chemostat. The ammonium electrode was calibrated using a standard ammonia solution from Hatch® (catalog #24065–49). The dissolved oxygen electrode was calibrated in the autoclaved growth medium purging the solution with pure nitrogen and air. The electrode that measured pH was calibrated using pH buffers at pH 4, 7 and 10 from Fisher (catalog numbers SB98-1, SB108-1, and SB116-1, respectively).

The oxygen uptake rate was estimated using a method suggested by Bandyopdhyay et al. [11]. In this method, the gas space of the chemostat is flushed with nitrogen gas to remove the oxygen, and the decrease of dissolved oxygen concentration in the reactor is recorded against time, the value of $(-dS_o/dt)$ at the linear region yields the oxygen uptake rate per unit volume. The term $(-dS_o/dt)/(X)$ is the specific oxygen uptake rate (SOUR) [12].

### 2.4. Estimation of biokinetic constants

Three types of multiple-substrate growth models can be considered when growth is limited by more than one substrate [6]:

Interactive or multiplicative form:

$$\frac{\mu}{\mu_{max}} = [\mu(S_1)][\mu(S_2)] \cdots [\mu(S_i)] \quad (1)$$

Additive form:

$$\frac{\mu}{\mu_{max}} = \frac{\mu(S_1) + \mu(S_2) + \cdots + \mu(S_i)}{i} \quad (2)$$

Non-interactive form:

$$\frac{\mu}{\mu_{max}} = \mu(S_1) \text{ or } \mu(S_2) \text{ or } \ldots \text{ or } \mu(S_i) \quad (3)$$

Many mathematical models are available to correlate the single substrate concentration with microbial growth rate, $\mu$ versus $S_i$ (presented in the literature [6,13–16]). To develop multiple-substrate growth kinetics, these individual models can be combined in a manner described by Eqs. (1)–(3), to obtain equations consistent with the experimental data [16]. In our tests, we used the following growth kinetics [13,17–19] for a single substrate, $S_i$:

$$\text{Monod} \quad \mu = \mu_{max}\frac{S_i}{K_{si} + S_i} \quad (4)$$

$$\text{Tessier} \quad \mu = \mu_{max}(1 - e^{-S_i/K_{si}}) \quad (5)$$

$$\text{Moser} \quad \mu = \mu_{max}(1 + K_{si}S^{-\lambda_i})^{-1} \quad (6)$$

$$\text{Contois} \quad \mu = \mu_{max}\frac{S_i}{B_i X + S_i} \quad (7)$$

The mass balance for the microorganism in an ideal chemostat yields Eq. (8):

$$\mu = D = \frac{Q}{V} \quad (8)$$

To develop growth models for multiple-substrates, the specific growth rate, $\mu$, is calculated from Eq. (8) for each steady state. Different growth models based on single substrate (Eqs. (4)–(7)) are then inserted into Eq. (1) or (2), or (3) to find the best multiple-substrate model. From the same data, the maintenance factor, $m_i$, and yield factor, $Y_{x/si}$ are calculated from the mass balance for the substrate as given by Eq. (9):

$$D(S_{fi} - S_{ei}) = \frac{\mu_i X}{Y_{x/si}} + m_i X \quad (9)$$

### 2.5. Non-linear regression

To estimate the biokinetic parameters from the experimental data, we used Microsoft Excel 2000® Solver®, which solves non-linear regression problems using Newton's method [20]. The equations were solved to find values of

Table 1
The search range for biokinetic constants of *P. aeruginosa* in the non-linear regression analysis

| Constants | Allowable range |
|---|---|
| $\mu_{max}$ ($h^{-1}$) | 0–1 |
| $K_g$ (mg/l) | 0–1000 |
| $K_o$ (mg/l) | 0–7.8 |

the biokinetic parameters that minimize the objective function, the sum of squares of the differences (SSD) between experimental and theoretical data for specific growth rates, as given by Eq. (10):

$$\text{SSD} = \sum_{i=1}^{N} (\mu_{\text{experimental}} - \mu_{\text{predicted}})^2 \quad (10)$$

To calculate the maintenance and yield factors (Eq. (9)), we have described the objective function as the SSD between the substrate consumption rates, which were experimentally measured and theoretically estimated, separately for oxygen and glucose. Because the solution was sensitive to initial guesses, the search was constrained to a predetermined range, determined as the range of biokinetic constants for bacteria reported in literature and shown in Table 1 [12,15].

### 2.6. Best kinetic model

Using non-linear regression and the selected multiple-substrate growth kinetics, we calculated the biokinetic parameters as described before. The best multiple-substrate growth model was selected from among different combinations of Eqs. (4)–(7) to give the minimum sum of squares of differences (SSD) between the experimental data and model solutions.

## 3. Results and discussion

### 3.1. Optimum operating conditions

In aerobic systems, dissolved oxygen acts as the electron acceptor during substrate oxidation [21]. Research on suspended cell cultures has revealed that the SOUR (g oxygen/g dry biomass/h) is proportional to microbial activity, which makes it suitable as an indicator of microbial activity [13,22–25]. Therefore, we selected SOUR to determine the optimum operating conditions in the chemostat.

Fig. 1 shows SOUR for different agitation rates in the chemostat. It was expected that at low agitation rates the growth of microorganisms was limited by external mass transport. Therefore, when the agitation rates increased, the mass transfer rate to the microorganisms increased, along with the SOUR, which reached a maximum value. Increasing the agitation rate beyond this maximum actually decreased the SOUR, probably because the agitation was injuring the microorganisms. Based on the results in Fig. 1, we selected 350 rpm as the working agitation rate. Microscopic examinations showed that the microorganisms were distributed uniformly in the chemostat, and neither flocculation nor agglomeration were observed.

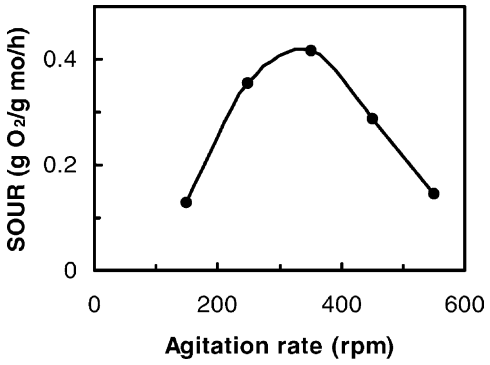


Fig. 1. The SOUR for different agitation rates in the chemostat ($D = 0.124\,h^{-1}$, pH 7.2, $T = 25\,^{\circ}C$, $S_{fg} = 5\,g/l$, and $S_{fn} = 0.1\,g/l$, air flow rate = 3 l/h).

We have measured the effect of pH on SOUR (Fig. 2). The SOUR increases, reaches a maximum value, and then decreases. The optimum pH 7.2 was used in all runs.

The data (Table 2) collected at steady states were used for the modeling of growth kinetics.

### 3.2. Modeling microbial growth kinetics

In addition to the data in Table 2, we also tested the growth-limiting substrates and their interactions. According to these results, the growth rate of *P. aeruginosa* in the absence of oxygen or glucose was negligible. Furthermore, changing $NH_4^+$ concentration in the feed did not affect the effluent concentrations of glucose, oxygen, and microorganisms (results not shown). These observations, combined with the results in Table 2, demonstrate that glucose and oxygen influenced the growth kinetics of *P. aeruginosa*. Hence, the growth of *P. aeruginosa* should be represented by a kinetic

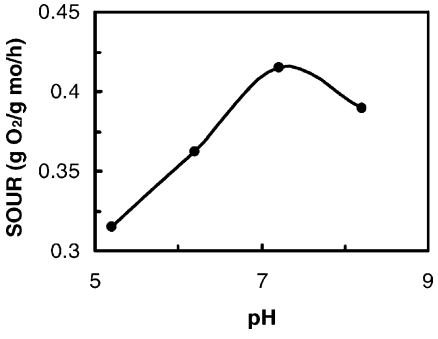


Fig. 2. The effect of pH on SOUR in the chemostat ($D = 0.124\,h^{-1}$, agitation rate = 350 rpm, $T = 25\,^{\circ}C$, $S_{fg} = 5\,g/l$, and $S_{fn} = 0.1\,g/l$, air flow rate = 3 l/h).

Table 2
The results of steady-state experiments ($T = 25\,^{\circ}C$, pH 7.2, agitation rate = 350 rpm, $S_{fg} = 5$ g/l, $S_{fn} = 0.1$ g/l)

| $D$ ($h^{-1}$) | $S_o$ (mg/l) | $S_g$ (mg/l) | $X$ (mg/l) | OUR (mg oxygen/h) |
|---|---|---|---|---|
| 0.03 | 1 | 5 | 1725 | 323 |
| 0.04 | 7.2 | 3.8 | 3000 | 424 |
| 0.0556 | 0.5 | 19.4 | 2381 | 581 |
| 0.069 | 7.2 | 7.1 | 2820 | 719 |
| 0.118 | 0.8 | 45.7 | 3150 | 1212 |
| 0.124 | 7.2 | 15.1 | 3070 | 1273 |
| 0.162 | 7.2 | 22.7 | 3225 | 1658 |
| 0.187 | 1.5 | 69.4 | 3285 | 1912 |
| 0.24 | 6.6 | 255 | 2760 | 2565 |
| 0.24 | 2.5 | 87.4 | 3105 | 2440 |
| 0.275 | 3.3 | 112.65 | 3090 | 2799 |
| 0.299 | 5.4 | 217 | 2850 | 2599 |
| 0.325 | 5.6 | 154.9 | 3045 | 3306 |

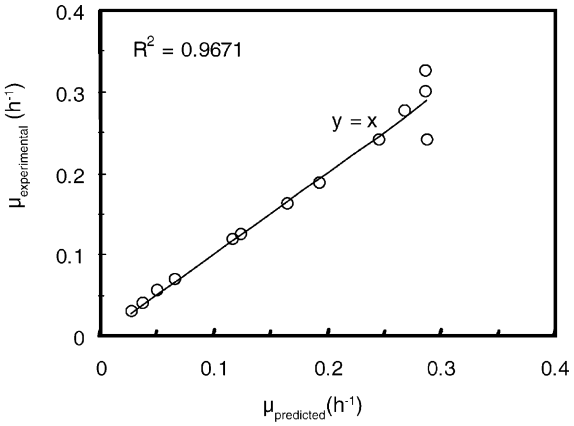


Fig. 3. The specific growth rates predicted from Eq. (12), Double Tessier kinetics vs. measured specific growth rates.

expression taking into account the dual-substrate limitations of oxygen and glucose combined.

Table 3 shows possible growth models using combinations of Eqs. (4)–(7), calculated biokinetic parameters, SSD, and regression coefficients ($R^2$). The minimum SSD were found for models #12 and #17, with $R^2$ between 0.96 and 0.97. Model 17 combined the Moser and Tessier kinetics. However, according to the Moser kinetic (model #17), *P. aeruginosa* should grow in the absence of the oxygen, which contradicts experimental results. Consequently, as the best growth model we have selected the double Tessier kinetics:

$$\mu = \mu_{max}(1 - e^{-S_o/K_o})\,(1 - e^{-S_g/K_g}) \quad (11)$$

For the selected model, the biokinetic parameters are $\mu_{max} = 0.29\,h^{-1}$, $K_g = 26.9$ mg/l, $K_o = 1.18$ mg/l, $Y_{x/g} = 0.628$ g biomass/g glucose, and $Y_{x/o} = 0.635$ g biomass/g oxygen. Maintenance factors are $m_g = 0.0078$ g glucose consumed/g microorganism h, and $m_o = 0.014$ g oxygen consumed/g microorganism h. Fig. 3 shows the growth rates both measured and predicted from Eq. (11). The high

Table 3
Growth models, biokinetic parameters, SSD, and regression coefficients ($R^2$)

| Model number | Equation number for oxygen | Equation number for glucose | $\lambda_o$ | $\lambda_g$ | $B_o$ | $B_g$ | $K_o$ | $K_g$ | $\mu_{max}$ | SSD | $R^2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | – | 4 | – | – | – | – | – | 31.97 | 0.30 | 0.0272074 | 0.78 |
| 2 | – | 5 | – | – | – | – | – | 39.76 | 0.26 | 0.0256887 | 0.80 |
| 3 | – | 6 | – | 1.06 | – | – | – | 37.42 | 0.29 | 0.027141 | 0.79 |
| 4 | – | 7 | – | – | – | 1.13E−02 | – | – | 0.30 | 0.0308807 | 0.76 |
| 5 | 4 | – | – | – | – | – | 0.65 | – | 0.21 | 0.1055993 | 0.17 |
| 6 | 4 | 4 | – | – | – | – | 0.50 | 20.32 | 0.32 | 0.0192399 | 0.85 |
| 7 | 4 | 5 | – | – | – | – | 0.51 | 20.00 | 0.21 | 0.1012759 | na |
| 8 | 4 | 6 | – | 1.49 | – | – | 0.62 | 66.67 | 0.30 | 0.0174575 | 0.86 |
| 9 | 4 | 7 | – | – | – | 6.81E−03 | 0.55 | – | 0.32 | 0.021275 | 0.83 |
| 10 | 5 | – | – | – | – | – | 0.98 | – | 0.19 | 0.0980918 | 0.22 |
| 11 | 5 | 4 | – | – | – | – | 0.85 | 19.13 | 0.29 | 0.016236 | 0.87 |
| 12 | 5 | 5 | – | – | – | – | 1.18 | 26.89 | 0.29 | 0.0041595 | 0.97 |
| 13 | 5 | 6 | – | 1.49 | – | – | 0.96 | 60.30 | 0.27 | 0.0146219 | 0.88 |
| 14 | 5 | 7 | – | – | – | 1.13E−02 | 9.9E−06 | – | 0.30 | 0.0308807 | 0.76 |
| 15 | 6 | – | 3.32 | – | – | – | 0.76 | – | 0.20 | 0.0956579 | 0.24 |
| 16 | 6 | 4 | 2.5 | – | – | – | 0.40 | 18.78 | 0.29 | 0.0163252 | 0.87 |
| 17 | 6 | 5 | – | 1.51 | – | – | 0.78 | 27.30 | 0.30 | 0.0045242 | 0.96 |
| 18 | 6 | 6 | 2.29 | 1.47 | – | – | 0.59 | 105.56 | 0.27 | 0.0154927 | 0.88 |
| 19 | 6 | 7 | 2.56 | – | – | 6.36E−03 | 0.44 | – | 0.29 | 0.0176969 | 0.86 |
| 20 | 7 | – | – | – | 1.97E−04 | – | – | – | 0.20 | 0.1126745 | 0.11 |
| 21 | 7 | 4 | – | – | 1.53E−04 | – | – | 21.14 | 0.32 | 0.0202808 | 0.84 |
| 22 | 7 | 5 | – | – | 2.05E−04 | – | – | 25.96 | 0.29 | 0.0164883 | 0.87 |
| 23 | 7 | 6 | – | 1.53 | 2.00E−04 | – | – | 76.13 | 0.30 | 0.0181868 | 0.86 |
| 24 | 7 | 7 | – | – | 1.64E−04 | 7.27E−03 | – | – | 0.32 | 0.0232337 | 0.82 |

The second and third columns show numbers of the equations we combined to assemble the double substrate kinetics. na, not available.

Table 4
Comparison of biokinetic parameters calculated in this study for dual-substrate growth models with literature studies

| Study | $\mu_{max}$ ($h^{-1}$) | $K_g$ (mg/l) | $K_o$ (mg/l) | $Y_{x/g}$ (g mo/g glucose) | $Y_{x/o}$ (g mo/g oxygen) | $m_g$ (g glucose/g mo h) | $m_o$ (g oxygen/g mo h) |
|---|---|---|---|---|---|---|---|
| Our work | 0.29 | 26.9 | 1.18 | 0.628 | 0.635 | 0.0078 | 0.014 |
| Bakke et al. [4] chemostat | 0.4 | 2 | na | 0.34 | na | na | na |
| Robinson et al. [5] biofilms | 0.45 | 2 | na | 0.3 | na | na | na |

na: not available.

correlation coefficient (0.97) demonstrates that the growth model accurately represents the growth of *P. aeruginosa*.

Though the literature shows double Monod kinetics (#6) as more commonly used for this procedure, the double Tessier (model #12) proved to better fit our needs with a correlation of $R^2 = 0.97$. To select the kinetic expressions, we accepted the $R^2$ higher than 0.85 as a cutoff value. Models indicating $R^2 > 0.85$ were considered options. Double Monod kinetics showed $R^2 = 0.85$, which according to our definition was a borderline for the kinetic expressions we decided to consider.

The comparison of the biokinetic parameters evaluated in this study (Table 4) with those reported by Bakke et al. [4] and Robinson et al. [5] shows that our $\mu_{max}$ was slightly lower but on the same order of magnitude as those reported. However, our $K_g$ value was nearly an order of magnitude higher than those reported. This difference was most likely caused by our chemostat being operated at much higher glucose concentration than the systems used by Bakke et al. [4] and Robinson et al. [5]. As mentioned earlier, Bakke et al. [4] and Robinson et al. [5] used relatively low glucose concentration to assure that glucose—not oxygen—was the limiting substrate. This is to say they did not consider oxygen as a limiting substrate. Also, Bakke et al. [4] and Robinson et al. [5] considered only Monod kinetics to describe microbial growth, a model that was not found below the acceptance level ($R^2 = 0.85$) in our study.

Our half-saturation constant for oxygen, $K_o = 1.18$ mg/l, was fairly high compared to those reported in the literature: 0.022 mg/l by Beyenal and Tanyolac [12], 0.048 mg/l by Seker et al. [26], 0.075 mg/l by Tado and Sato [27]. We do not offer any explanation for these differences.

## 4. Conclusions

We have found that a dual-substrate microbial growth kinetics, dual Tessier kinetics for oxygen and glucose, was in agreement with the chemostat data, yielding the following biokinetic parameters describing growth of *P. aeruginosa*: $\mu_{max} = 0.29\,h^{-1}$, $K_g = 26.9$ mg/l, $K_o = 1.18$ mg/l, $Y_{x/g} = 0.628$ mg microorganism/mg glucose and, $Y_{x/o} = 0.635$ mg microorganism/mg oxygen. Maintenance factors for glucose and oxygen were $m_g = 0.0078$ g glucose consumed/g microorganism h, and $m_o = 0.014$ g oxygen consumed/g microorganism h.

## Acknowledgments

The research was supported by the cooperative agreement EED-8907039 between the National Science Foundation and Montana State University.

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