Growth, Death, and Oxygen Uptake
Kinetics of *Pichia stipitis* on Xylose

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Received July 17, 1990/Accepted November 19, 1990

*Pichia stipitis* NRRL Y-7124 has potential application in the fermentation of xylose-rich waste streams produced by wood hydrolysis. Kinetic models of cell growth, death, and oxygen uptake were investigated in batch and oxygen-limited continuous cultures fed a rich synthetic medium. Variables included rates of dilution (*D*) and oxygen transfer (*K*~*a*~) and concentrations of xylose (*X*), ethanol (*E*), and dissolved oxygen (*C*~*ox*~). Sustained cell growth required the presence of oxygen. Given excess xylose, specific growth rate (*μ*) was a Monod function of *C*~*ox*~. Specific oxygen uptake rate was proportional to *μ* by a yield coefficient relating biomass production to oxygen consumption; but oxygen uptake for maintenance was negligible. Thus steady-state *C*~*ox*~ depended only on *D*, while steady-state biomass concentration was controlled by both *D* and *K*~*a*~. Given excess oxygen, cells grew subject to Monod limitation by xylose, which became inhibitory above 40 g/L. Ethanol inhibition was consistent with Luong's model, and 64.3 g/L was the maximum ethanol concentration allowing growth. Actively growing cells died at a rate that was 20% of *μ*. The dying portion increased with *E* and *X*.

INTRODUCTION

A number of existing industries produce xylose-rich waste streams which are consistently available for fermentation to ethanol. Examples include sulfite liquor from the pulp and paper industry and hydrolyzates produced by steam explosion decompression of wood.† Successful utilization of these wastes by xylose-fermenting microbes, such as the yeast *Pichia stipitis*, requires an efficient bioreactor.‡ A complete model describing the kinetics and stoichiometry of the xylose fermentation process is not provided by past literature but would be a valuable tool for predicting optimum reactor configuration strategies. With this research, we began the development of such a model for *Pichia stipitis* NRRL Y-7124 by focusing on the kinetics and stoichiometry of oxygen uptake, growth, and death.

Past literature provided several important concepts which influenced both our experimental design and model development. First, total dry biomass concentration (*b*~*T*~) is the sum of viable (*b*) and dead biomass (*b*~*d*~), and in batch systems it increases at the rate

\[
\frac{db_T}{dt} = \mu b
\]

where *μ* is specific growth rate.13 Monod has expressed *μ* as the following function of limiting substrate concentration (*s*):11

\[
\mu = \mu_{max} s/(K_s + s)
\]

Here, *μ*~*max*~ is the maximum reaction velocity when the rate-limiting enzyme is saturated by its substrate and *K*~*s*~ is the saturation constant equal to *s* at *μ* = *μ*~*max*~/2. The identity of the limiting substrate may change with the extent of fermentation. At low conversions, xylose will be in excess, and oxygen limitation may prevail; but at high conversions either xylose, oxygen, or both may limit growth.

Second, accumulation rates of viable and dead biomass can be distinguished as

\[
\frac{db}{dt} = (\mu - k_d)b
\]

and

\[
\frac{db_d}{dt} = k_d b
\]

respectively. The specific death rate (*k*~*d*~) is likely to be a function of growth in light of reports that a fraction of newly produced cells are dead.13 In general, the kinetics of cell death are not as well documented as those of cell growth, but they are not to be ignored without experimental justification.

Third, the rate of oxygen accumulation in a batch culture is2,13

\[
\frac{dC_{ox}}{dt} = K_{1a}(C_{ox}^* - C_{ox}) - q_{ox} b
\]

The first term represents the oxygen solution rate, which depends on the difference between dissolved oxygen concentration (*C*~*ox*~) and oxygen solubility (*C*~*ox*~^*s*~), the overall gas/liquid mass transfer coefficient (*K*~*1a*~), and the gas/liquid interfacial area (*a*). The second term represents oxygen uptake by the viable biomass, where *q*~*ox*~ is the specific oxygen demand (or uptake rate). The de-
pendence of \( q_{ox} \) on growth has been observed for a variety of microorganisms and represented as

\[
q_{ox} = \left( \frac{\mu}{Y_{ox}} \right) + m_{ox}
\]

where \( Y_{ox} \) is the biomass yielded per oxygen consumed and \( m_{ox} \) is specific oxygen demand for maintenance.\(^{21}\) In the case of \( Pachysolen tannophilus \) and other pentose-fermenting yeasts, growth is an obligately aerobic process.\(^{15}\)

Finally, the parameters within expressions for \( \mu, k_d, \) and \( q_{ox} \) may vary subject to inhibition by xylose and/or ethanol.\(^{5,6,15}\) Such effects are important to our model because substrate consumption and product accumulation are responsible for dramatic compositional changes in the culture medium. Experimental data were collected from batch and continuous cultures to test the applicability of these concepts to modelling Y-7124. Variables included dilution rate, \( K_a \), and concentrations of ethanol, xylose, and dissolved oxygen. Their effects on \( \mu, k_d, \) and \( q_{ox} \) were measured and expressed in mathematical terms.

**MATERIALS AND METHODS**

**Organism and Media**

Lyophilized \( Pichia stipitis \) NRRL Y-7124 (CBS 5773) was acquired from the ARS Culture Collection (Northern Regional Research Center, Peoria, IL). Stock cultures maintained on agar slants were used to prepare fermentor inocula adapted to xylose broth.\(^6\) The complex media for slants (YM) and liquid cultures (CCY) have been described in detail.\(^18\) CCY medium was used in all liquid cultures except for anaerobic cultures which were on YM broth. CCY medium contained yeast extract, urea, potassium phosphate buffer, mineral salts, xylose, and 1 g/L Hodag FD-62 silicone anti-foam. Ethanol was also added in experiments designed to study product inhibition.

**Specific Death and Oxygen Uptake Rate Measurements**

**Continuous Cultures**

Cell death and oxygen uptake kinetics were studied in oxygen-limited continuous cultures carried out in B. Braun Biostat 2ER (2-L) fermentors. Each fermentor was controlled at 25°C and equipped with Ingold pH and dissolved oxygen electrodes for monitoring the process. The pH of the feed medium was 4.5, and automatic control was unnecessary because conversions were low, resulting in <0.2 unit pH shift. An FE211 diaphragm pump delivered the feed medium at a constant volumetric rate (\( Q \)) while a level sensor and peristaltic pump maintained the holding volume (\( V_R \)) at 850 mL. Cultures were operated at various dilution rates (\( D = Q/V_R \)) and feed compositions of xylose and ethanol. The feed reservoir (standardly CCY + 40 g/L xylose) was kept under a nitrogen atmosphere so that oxygen transfer to the culture was completely controlled by air flow and stirring rates in the fermentor. The air flow was routinely 113 mL/min, but the stirring (hence, \( K_a \)) was varied to examine the effect of oxygen transfer rate on steady-state concentrations. A 15-in. 18-gauge cannula provided access for daily sampling (ca. 5 mL). Steady state was identified by invariant viable biomass (\( b \)), dead biomass (\( b_d \)), xylose (\( X \)), xylitol (\( XOH \)), and ethanol (\( E \)) concentrations over at least three days. Fermentations were discontinued after one week to guard against complications of wall growth and culture adaptation.

**Evaluation of \( K_a \)**

The polarographic oxygen probe was calibrated at atmospheric pressure by setting zero and 100% saturation under nitrogen and air sparging, respectively. The stirring rate for the fermentation was set during probe calibration. In order to evaluate \( K_a \), each fermentor was configured as if for a continuous culture experiment, except that it was left uninoculated and without feed. Oxygen was stripped from the fermentor medium by sparging with nitrogen. The oxygen saturation time-course was then monitored as the air flow and stirring conditions chosen for use during fermentation were resumed. At \( b = 0 \), integration of eq. (5) gave the time course of dissolved oxygen in terms of the fraction of saturation (\( f = C_{ox}/C_{ox}^\ast \)), as follows:

\[
\ln[1 - f] = -K_ao t
\]

Linear regression of \( -\ln[1 - f] \) vs. time provided \( K_ao \) as the slope. The effect of cell density on \( K_ao \) was determined using heat-killed cells, and was found to be negligible in the range tested (ca. 0–12.5 g/L).

**Evaluation of \( C_{ox} \)**

The dissolved oxygen concentration during fermentation time courses was obtained from the electrode response as \( C_{ox} = fC_{ox}^\ast \). Given air (21% \( O_2 \)) at 25°C and 760 mm Hg, \( C_{ox}^\ast \) (mg/L) was determined as the following function of \( X \) (g/L), where the factor 1.08 adjusts the original relationship (for 30°C) to 25°C: \( C_{ox}^\ast = (0.21)(1.08)[34.6 - 0.0644X + 0.000156X^2] \).\(^{20}\)

**Calculation of Specific Rates at Steady State**

Equations (3)–(5) were modified to account for dilution, and the following differential mass balances on biomass and dissolved oxygen were obtained for continuous culture operation:

\[
\frac{db}{dt} = -Db - k_d b + \mu b
\]

\[
\frac{db_d}{dt} = -Db_d + k_d b
\]

\[
\frac{dc_{ox}}{dt} = k_o (C_{ox}^\ast - C_{ox}) - DC_{ox} - q_{ox} b
\]
Given that $\frac{db}{dt}$, $\frac{dD}{dt}$, and $\frac{dC_{ox}}{dt}$ equal zero at steady state, the following relationships apply:

$$\mu = D + k_d$$  \hspace{1cm} (11)  

$$k_d = \frac{D}{b_s}$$  \hspace{1cm} (12)  

$$q_{ox} = \frac{[K_\alpha(C_{ox} - C_{ox,s}) - D_{ox,s}]/b_s}{s}$$  \hspace{1cm} (13)  

The subscript "s" denotes steady-state concentrations. Measurements of $b_s$, $b_v$, $C_{ox,s}$, $K_\alpha$, $C_{ox}$, and $D$ allowed calculation of the specific rates—$k_d$, $\mu$, and $q_{ox}$.

**Specific Growth Rate Measurements in Batch**

Optical density time courses from batch cultures in early log phase were used to determine the dependence of specific growth rate ($\mu_{log}$) on ethanol, xylose, and oxygen concentrations. The procedure and calculations were the same as described earlier except that temperature was routinely controlled at 25°C, and pH at 4.5. If not chosen as experimental variables, oxygen concentration was controlled at 95% of saturation, and initial ethanol and xylose concentrations were set at 0 and 40 g/L, respectively. The dissolved oxygen probe was calibrated as in continuous cultures, except that 100% saturation was set under air at 902 mm Hg total pressure and corresponded to 7.9 mg/L oxygen.

**Comparison of Aerobic and Anaerobic Growth**

The ability of Y-7124 to grow anaerobically on xylose and glucose was assessed under strict anaerobic conditions on YM supplemented with 0.6 g/L l-cysteine (reducing agent) and 2 mg/L resazurin (oxygen indicator). Four flasks of oxygen-free medium were prepared in this manner—two with 10 g/L xylose and two with 10 g/L glucose. To provide controls, the rubber stoppers on one glucose and one xylose flask were replaced with air-permeable Bellco sponge stoppers. Inoculated flasks were incubated at 25°C and 150 rpm. The pH of both anaerobic (CO$_2$-saturated) and aerobic YM—4.5 and 6, respectively—were in the optimum range for growth.

**Sample Analyses**

**Biomass**

As previously reported, light absorbance ($A$) at 620 nm provided the total biomass concentration (g/L) as $b_r = 0.167 A_{620}$. The viable fraction was determined from light absorbance of methylene blue-stained cells at 663 nm. Our colorimetric procedure was a modification of an earlier method which assumed that dead cells absorbed dye while viable cells excluded it.

The total biomass in each viability assay was normalized based on 1 ml of culture broth with $A_{620}$ of 4.5. A volume of culture ($V = 4.5 \times 1$ mL/$A_{620}$) was delivered to a preweighed microfuge tube and spun ca. 5 min at $1.5 \times 10^4$ rpm in a Savant tabletop centrifuge. The supernate was removed, the cell pellet was resuspended to 1 g with 0.15 mM methylene blue in 0.1M sodium phosphate buffer (pH 7.4), and the suspension was allowed to stand 6 min at room temperature. After centrifuging again and removing the methylene blue supernate, the stained pellet was resuspended to 1 g in 0.1M phosphate buffer. The absorbance of the stained cell suspension was measured at 663 nm. This suspension was commonly diluted 20 times ($d = 20$) to obtain readings ($A_{663}$) between 0.05 and 0.5 units where absorbance was linearly dependent on stained cell concentration. The percentage ($P$) of viable cells was calculated from the calibration equation, $P = 138.773 - 11.574 A_{663}$. Calibration data were generated by staining log-phase and heat-killed cells mixed in various proportions. Both $A_{663}$ ($= A_{663} \times d$) and $P$ (the ratio of unstained to total cells counted in a hemacytometer) were evaluated for each mixture, and linear regression of the data provided equation parameters at 0.99 correlation. Viabilities of log-phase and heat-killed cells measured by the staining procedure were similar to those determined by plate counting on YM agar.

**Ethanol, Xylitol, and Xylose**

Sample treatment and chromatography (GC and HPLC) methods were as described previously.

**Furfural**

Since aqueous xylose was sterilized independently of other medium ingredients, furfural could be measured by UV absorption at 275 nm. Thus, the furfural concentration in the complete CCY medium was calculated as 88% of that in the sugar solution alone.

**RESULTS AND DISCUSSION**

**Cell Death Kinetics**

**Dependence of Cell Death on Growth**

The following relationship results when eqs. (11) and (12) are combined by eliminating $D$:

$$k_d = f_d \mu$$  \hspace{1cm} (14)  

where the dead biomass fraction is defined by $f_d \equiv b_{d,s}/(b_v + b_{d,s})$. Data from oxygen-limited continuous cultures indicated that $f_d$ remained near 0.20 (±0.03 standard deviation) regardless of the dilution or oxygen transfer rates used (Fig. 1). Thus 20% of the biomass produced was not viable, and growth-associated cell death needs to be accounted for in our model.

**Effect of Ethanol and Xylose on Cell Death**

Steady-state continuous cultures fed media with various compositions of xylose and ethanol were used to deter-
mine the influence of \( X \) and \( E \) on \( k_d \). The results are plotted in terms of \( f_d \) (Figs. 2 and 3). In experiments with \( X_c \) ca. 40 g/L, \( f_d \) increased with \( E \) according to the following second-order polynomial:

\[
f_d(X, E) = 0.22 - 0.000783E + 0.000122E^2 \tag{15}
\]

If \( f_d \) at \( E = 0 \) is termed \( f_d(X, 0) \), eq. (15) can be generalized by substituting \( f_d(X, 0) \) for 0.22 as follows:

\[
f_d(X, E) = f_d(X, 0)[1 - 0.00356E + 0.000555E^2] \tag{16}
\]

In experiments with \( E \), near zero, \( f_d \) approximated \( f_d(X, 0) \) and was a weak function of \( X \) which fit the following linear form:

\[
f_d(X, 0) = 0.194 + 0.000381X \tag{17}
\]

If this expression for \( f_d(X, 0) \) is substituted into eq. (16), then the following equation for the combined effects of \( E \) and \( X \) on \( f_d \) is obtained:

\[
f_d(X, E) = (0.194 + 0.000381X)(1 - 0.00356E + 0.000555E^2) \tag{18}
\]

which is consistent with the data of both Figures 2 and 3.

**Cell Growth Kinetics**

**Aerobic Versus Anaerobic Growth**

Figure 4 depicts our observation that oxygen was necessary to sustain vigorous growth regardless of whether the substrate was xylose or glucose. In aerobic batch cultures the population doubled 6.5 times and reached stationary phase within the first day. Under strict anaerobic conditions, however, the population doubled only once during six days and became stationary. These results are consistent with a previous report on *P. stipitis* strain CSIR Y-633 (CBS 7126). This obligatory dependence of growth on oxygen has been noted for *P. tannophilus* and other pentose-fermenting yeasts and has been linked to a necessity for electron transport function.\(^1^5\)
Oxygen-Limited Growth

A series of log-phase batch cultures were run with initial xylose and ethanol concentrations at 40 and 0 g/L, respectively. Figure 5(a) shows the dependence of the apparent specific growth rate ($\mu_{\log}$) on $C_{ox}$. This behavior was consistent with Monod kinetics as follows:

$$\mu_{\log} = \mu_{\max} C_{ox} / (K_{ox} + C_{ox})$$

(19)

when $\mu_{\max}$ and $K_{ox}$ were 0.53 h$^{-1}$ and 0.1 mg/L, respectively [Fig. 5(b)]. Since the slope for Figure 5(b) was biased by the point at $1/C_{ox} = 31.25$ L/mg, we repeated the calculation without this point and obtained $\mu_{\max} = 0.54$ h$^{-1}$ and $K_{ox} = 0.2$ mg/L at a lower correlation level (0.80). These results indicate that the cells have a very high affinity for oxygen. Oxygen levels as low as 0.1–0.2 mg/L allow a specific growth rate of half the maximum.

Effect of Xylose and Ethanol Concentrations on Growth

In air-saturated media, the expression $C_{ox} / (K_{ox} + C_{ox})$ is equal to 1 such that eq. (19) reduces to $\mu_{\log} = \mu_{\max}$. Thus, a series of air-saturated batch cultures in log phase allowed us to determine $\mu_{\max} = \mu_{\max}(X,0)$, a function of $X$ in the absence of ethanol. Figure 6 shows that $\mu_{\max}(X,0)$ was consistent with the following kinetics:

$$\mu_{\max}(X,0) = \mu_{\max}(X/(K_e + X))$$

$$- [K_i/(K_i + X - X_m)]$$

(20)

where $\mu_{\max}$ is the maximum specific growth rate which would be observed if no substrate inhibition ($K_i = 0$); $K_e$ is the Monod saturation constant; $K_i$ is an empirical inhibition constant; and $X_m$ is the xylose concentration above which growth cannot occur. The curve which best fit our experimental data (within an average error of 1.5%) had the following parameter values: $\mu_{\max} = 0.71$ h$^{-1}$; $K_e = 0.36$ g/L; $K_i = 60.2$ g/L; and $X_m = 253$ g/L. This model has several important features. It interprets growth and inhibition as independent positive and negative processes, respectively. Thus negative growth (or death) at a rate of $-0.136$ h$^{-1}$ is predicted when $X = 0$, and zero growth is predicted under two conditions—$X = 0.5X_m \pm (0.5)(X_m - 4K_iK_e)^{0.5}$—i.e., $X = 0.09$ or 253 g/L. Around 40 g/L, $\mu_{\max}(X,0)$ peaks broadly at 0.55 h$^{-1}$ which agrees within 3% of the value (0.53) determined for the oxygen-limited case [Fig. 5(b)].

Edwards reviewed common substrate inhibition models, but none of them fit our data. Inhibition of growth by furfural (in combination with xylose) was considered as a possible cause of this discrepancy. Furfural is a decomposition product of xylose which can be generated during autoclaving. Preparation of 1.5 L of CCY medium normally involved sterilizing the 1.32-L xylose solution in our fermentor for 0.5 h at 121°C (separate from other ingredients). This procedure was compared with one in which the xylose solution was sterilized via a 0.22-$\mu$m filter (Table I). Low xylose concentration and filter sterilization minimized the occurrence of furfural. However, the sugar sterilization method (and furfural production) did not affect $\mu_{\log}$ significantly until
Table I. Dependence of furfural accumulation and $\mu_{log}$ on xylose concentration and sterilization method.

<table>
<thead>
<tr>
<th>Xylose (g/L)$^b$</th>
<th>Furfural (g/L) ($\times 10^3$)</th>
<th>$\mu_{log}$ (h$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Steam</td>
<td>Filter</td>
</tr>
<tr>
<td>40</td>
<td>8.4</td>
<td>0.34</td>
</tr>
<tr>
<td>100</td>
<td>24.9</td>
<td>0.93</td>
</tr>
<tr>
<td>150</td>
<td>37.3</td>
<td>1.41</td>
</tr>
<tr>
<td>200</td>
<td>49.4</td>
<td>1.91</td>
</tr>
</tbody>
</table>

$^a$ The 1.32-L sugar solution used in preparing 1.5 L of CCY medium was either steam (121°C, 30 min) or filter (0.22 μm) sterilized.

$^b$ This value represents the concentration in the completed CCY medium and is 88% of the concentration in the sugar solution during sterilization.

xylose was >150 g/L. Even at 200 g/L xylose, $\mu_{log}$ (auto-claved) was within 83% of $\mu_{log}$ (filtered).

Growth inhibition by ethanol in the presence of 40 g/L xylose was assessed via an approach similar to that used to study xylose inhibition. We compared our $\mu_{log}$ vs. $E$ data with Luong’s model in the following form:

$$\mu_l/\mu_0 = 1 - (E/E_m)^4$$ (21)

The parameter $E$ is ethanol concentration; $\mu_l$ is $\mu_{log}$ in the presence of inhibitor ($E > 0$); $\mu_0$ is $\mu_{log}$ at $E = 0$; $E_m$ is the maximum $E$ allowing growth; and the parameter $A$ is a dimensionless constant.\(^{10}\) The linear plot of $\ln[1 - (\mu_l/\mu_0)]$ vs. $\ln(E)$ shows the behavior of $P. stipitis$ to be compatible with this model at $A = 1.324$ and $E_m = 64.3$ g/L (Fig. 7). This value of $E_m$ is 17% higher than ca. 55 g/L previously reported for $P. stipitis$ CBS 7126 at 25°C.\(^{5}\) Since $0 \leq (\mu_l/\mu_0) \leq 1$, we can make the interpretation that any given ethanol concentration will allow growth to occur at a particular fraction of the rate possible in the absence of ethanol. Therefore, recognizing that $\mu_l \equiv \mu_{max}(X, E)$ and $\mu_0 \equiv \mu_{max}(X, 0)$, we can combine eqs. (20) and (21) to obtain the final expression for $\mu_{max}$ in terms of $X$ and $E$:

$$\mu_{max}(X, E) = \mu_{MAX}[(X/(K_X + X))] - \{K_i/(K_i + X_m - X)}[1 - (E/E_m)^4]$$ (22)

**Oxygen Uptake Kinetics**

Specific oxygen uptake rate was examined in continuous cultures operated at various dilution and oxygen transfer rates, and steady state plots of $q_{ox}$ vs. $\mu$ indicated that $q_{ox}$ was growth associated (Fig. 8). Linear regression provided the biomass yield per oxygen consumed ($Y_{ox}$) as 2.70 g/g. Because the intercept was evaluated as $-0.00033$ g/g/h (i.e., $m_{ox} = 0$), eq. (6) simplifies to:

$$q_{ox} = (\mu/Y_{ox})$$ (23)

Figure 8 also shows that this equation was obeyed regardless of the rate of oxygen transfer to the culture. Data collected at various $E_s$ and $X_s$ (not shown) indicated $Y_{ox}$ to be independent of these variables.

Alexander et al. showed a similar relationship between $q_{ox}$ and $\mu$ for *Candida shehatae* ATCC 22984 under oxygen-limited conditions.\(^{1}\) In contrast to our findings, however, they obtained $Y_{ox} = 1.09$ g biomass per gram oxygen (their $Y_{ox} = 35$ g/mol). Apparently, *P. stipitis* uses oxygen more economically than *C. shehatae* does during growth. This phenomenon is consistent with earlier observations of ethanol production being growth-associated for *P. stipitis*\(^{10}\) but growth deterred for *C. shehatae*.\(^{1}\)

**Model Predictions of Steady-State Biomass and Oxygen Concentrations**

To further test our understanding of the kinetics, we incorporated our concepts of $O_2$-limited growth and growth-associated $O_2$ uptake into steady-state models describing biomass and dissolved oxygen concentrations as functions of $D$ and $K_ia$. When steady-state equations eqs. (11), (13), and (2) were combined

![Figure 7](image-url)  
**Figure 7.** Linearization of Luong’s model for the dependence of specific growth rate ($\mu$) on ethanol concentration ($E$). Regression indicated 0.98 correlation and provided model parameters [eq. (21)] via the slope ($A = 1.324$) and the intercept ($-A\ln[E_m] = -5.512$).

![Figure 8](image-url)  
**Figure 8.** Linear dependence of specific oxygen uptake rate ($q_{ox}$) on growth ($\mu$), given variable oxygen transfer ($K_ia$). Regression provided a slope ($1/Y_{ox}$) of 0.370 g/g at a correlation coefficient of 0.98.
with functions (14) and (23), the following expression was obtained:

\[ b_s = Y_{ox}(1 - f_d)\frac{[K_{ia}(C_{ox}^* - C_{ox,s}) - DC_{ox,s}]}{D} \]  

(24)

Note that at values of \( D < 0.3 \text{ h}^{-1} \), this equation simplified greatly because \( C_{ox,s} \) was ca. 0. Figure 9 shows the agreement between predicted and experimental behaviors of cultures operated at various \( D \) and \( K_{ia} \) settings. An important feature is that \( b_s \) was governed by both \( \mu \) (\( \equiv D/(1 - f_d) \)) and \( K_{ia} \). For a given \( K_{ia} \), \( b_s \) decreased as \( \mu \) (and \( q_{ox} \)) increased. As \( K_{ia} \) (and the oxygen transfer rate) increased, \( b_s \) shifted upward.

When steady-state equations eq. (11) and \( C_{ox} = C_{ox,s} \) were combined with our experimentally derived eqs. (14) and (19), the following model of \( C_{ox,s} \) was obtained:

\[ C_{ox,s} = K_{ox}D/(\mu_{\text{max}} - D') \]  

(25)

where \( D' \equiv D/(1 - f_d) \). It suggested that \( C_{ox,s} \) was governed by \( D \) and was independent of aeration conditions, as we observed (Fig. 10). Our data and model prediction indicated that \( C_{ox,s} \) was near zero for \( D' < 0.3 \text{ h}^{-1} \). As \( D' \) approached \( \mu_{\text{max}} \), washout of the population occurred as expected.

**CONCLUSIONS**

Classical batch culture models of cell death, growth, and oxygen uptake were applicable to xylose fermentation by *P. stipitis* NRRL Y-7124 [eqs. (1) and (3)-(5)]. Methylene blue staining of cell samples indicated that cell death occurred at a rate proportional to the specific growth rate [eq. (14)]. The fraction of dying cells increased with xylose and ethanol although the effect of substrate was weak, perhaps negligible [eq. (18)]. Specific growth rate was a Monod function of dissolved oxygen and xylose, but was inhibited by both the substrate and product [eqs. (19) and (22)]. Oxygen was key to sustaining growth, and the specific oxygen demand was proportional to growth rate [eq. (23)]. Negligible oxygen was consumed for cell maintenance in the absence of growth. In support of these findings and their implications on modelling, batch equations modified for dilution [eqs. (24) and (25)] accurately predicted steady-state biomass and oxygen concentrations in cultures operated at various combinations of oxygen supply and demand (i.e., \( K_{ia} \) and \( D \)).

**NOMENCLATURE**

- \( A \) exponent governing ethanol inhibition of growth (dimensionless)
- \( A_{620} \) light absorbance at 620 nm
- \( A_{663} \) light absorbance at 663 nm
- \( b \) viable biomass concentration (g/L)
- \( b_d \) dead biomass concentration (g/L)
- \( b_{d,s} \) steady-state dead biomass concentration (g/L)
- \( b_{s,v} \) steady-state viable biomass concentration (g/L)
- \( b_T \) total biomass concentration (g/L)
- \( b_{T,s} \) steady-state total biomass concentration (g/L)
- \( C_{ox} \) dissolved oxygen concentration (g/L)
- \( C_{ox,s} \) steady-state dissolved oxygen concentration (g/L)
- \( C_s^* \) oxygen solubility (g/L)
- \( D \) dilution rate (h\(^{-1}\))
- \( D' \equiv D/(1 - f_d) \), an expression equivalent to specific growth rate in terms of dilution rate (h\(^{-1}\))
- \( E \) ethanol concentration (g/L)
- \( E_m \) maximum ethanol concentration allowing growth (g/L)
- \( E_s \) steady-state ethanol concentration (g/L)
- \( f \) oxygen saturation fraction (\( C_{ox}/C_s^* \))
- \( f_d \) dead biomass fraction (\( b_d/b_T \))
- \( K_{ia} \) lumped oxygen mass transfer coefficient (h\(^{-1}\))
- \( k_d \) specific death rate (h\(^{-1}\))
- \( K_i \) parameter governing substrate inhibition of growth (g/L)
- \( K_{ox} \) saturation constant governing oxygen limited growth (g/L)
- \( K_s \) saturation constant governing xylose-limited growth (g/L)
- \( m_{ox} \) specific oxygen demand for maintenance (g/g/h)
- \( q_{ox} \) specific oxygen uptake rate (g/g/h)
- \( t \) time (h)
- \( X \) xylose concentration (g/L)
- \( X_m \) maximum xylose concentration allowing growth (g/L)
$X_c$ steady-state xylose concentration (g/L)

$Y_{ot}$ yield of biomass per oxygen consumed (g/g)

**Greek letters**

$\mu$ specific growth rate (h$^{-1}$)

$\mu_L$ specific growth rate during early log phase of a batch culture (h$^{-1}$)

$\mu_{\log}$ in the presence of inhibitor (h$^{-1}$)

$\mu_{\log}$ maximum specific growth rate function of $X$ and $E$ (h$^{-1}$)

$\mu_{\log-o}$ maximum specific growth rate which would be observed if no substrate inhibition (h$^{-1}$)

$\mu_{o}$ $\mu_{\log}$ in the absence of inhibitor (h$^{-1}$)

**References**