Analysis of the logistic function model: derivation and applications specific to batch cultured microorganisms

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Abstract

Mathematical models are useful for describing microbial growth, both in natural ecosystems and under research conditions. To this end, a rate expression that accounted for depletion of nutrients was used to derive the logistic function model for batch cultures. Statistical analysis was used to demonstrate the suitability of this model for growth curve data. Two linear forms of the model and two procedures for calculating growth rate constants were derived to facilitate statistical evaluation of growth curves. The procedures for calculating growth rate constants were found to be useful for calculation of growth rate constants at each time point, or for estimating growth rate constants from early growth curve data. The utility of the logistic function model and its alternative forms is discussed with respect to planning experiments, analyzing growth curves for the effects of factors other than nutrient limitation, and developing more complete descriptions of cell proliferation.

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1. Introduction

The logistic function model is increasingly being used to describe microbial growth (Annadurai et al., 2000; Classen et al., 2000; Dalgaard and Koutsoumanis, 2001; Tobajas and Garcia-Calvo, 2000), as well as for predicting inhibition or inactivation of microbial growth (Bozkurt and Erkmen, 2001; Zhao et al., 2001). The logistic function model describes the growth of microbial populations as a function of initial population density, time, growth rate, and final population density. The original logistic function model was developed by Pearl and Reed (1920), based on earlier insights by Verhulst (1838). These authors recognized that, for various organisms, plots of population density vs. time had a sigmoidal shape. The rate of increase in population growth decreased as the population neared the saturation point of the environment. The saturation point could consist of many interacting factors, including nutrient availability, crowding, behavioral changes, predation, and pestilence. Use of the logistic function model to describe growth in these cases has been empirical, however, the model also may be derived so that the coefficients have biological meaning. We re-examined this model as a specific equation for characterizing growth of microorganisms, in order to better define where the model may be appropriately applied as a tool for planning and analyzing growth curve experiments. A unique aspect of this work is the use of the early parts of the logistic function model to predict time to stationary phase, or maximum population density. Conditions are discussed under which application of the logistic function model is appropriate, as well as approaches for identifying conditions that inhibit microbial growth. A linear form of the model which gives an excellent fit to growth curves for both aerobic and anaerobic batch culture is presented.

In batch cultures of microorganisms, the maximum population size that can be achieved is determined by fewer factors than is the case for metazoan populations. The most significant determinants include nutrient depletion, accumulation of inhibitory fermentation
products, and changes in physical properties, such as viscosity and osmolarity. Nutrient availability can be represented by the probability of individual cells receiving individual molecules of substrate. Fermentation products can inhibit bacterial growth by a variety of mechanisms, including the effects of pH on various enzyme activities, feedback inhibition of various enzymes, and changes in the mechanisms of transport of nutrients into the cells. Different fermentation products may be produced at different rates, and the combined effects of fermentation products may be synergistic. Therefore, models that describe the effects of fermentation products must be considered on a case by case basis.

A general empirical approach for inhibitory effects on Monod kinetics has been proposed (Han and Levenspiel, 1988), and similar modifications of the logistic function model may be useful. Similarly, the effects of changes in the growth medium must be considered with respect to each organism involved. The present derivation of the logistic function model is specific for the effects of competition for nutrients. By examining the degree to which growth is described by the logistic function model, the relative influences of these parameters on the growth rate can be examined.

The growth rate of a microorganism is a function of the concentration of a limiting substrate (Monod, 1949). In a batch culture, the maximum cell density (X_max) is a function of the yield of the organisms due to the fermentation of substrate, throughout the growth period. In a differential increment of time, only some of the cells (X_{grow}) out of the total (X_{tot}) will be in a given state of division. The ratio of X_{grow} to X_{tot} is the probability that an individual cell will be in the given state of division at that time, and is determined by the availability of nutrients that support cell replication. Nutrient availability can be represented by the difference between X_max and X_{tot}, at a given state in the growth curve, as suggested by Carlson (1913). The ratio X_{grow} to X_{tot} is then:

\[
\frac{X_{grow}}{X_{tot}} = K \frac{X_{max} - X_{tot}}{X_{max}}
\]

where K is a proportionality constant and X_{max} > X_{tot} > 0. Then

\[
X_{grow} = K X_{tot} (1 - X_{tot}/X_{max})
\]

Therefore, X_{grow} reflects the probability that a given cell will obtain the nutrients that are required for replication in a differential increment of time. This probability depends both on the concentration of nutrients that remain after earlier depletion by growing cells, and on the cell density. The overall growth rate for X_{tot} is the sum of the growth rates for X_{grow} and for cells that are viable and not replicating. By definition, the growth rate for cells which do not replicate is zero. Therefore, the rate of change in the total number of cells, X_{tot}, is given by:

\[
\frac{dX_{tot}}{dt} = k' X_{grow}
\]

where, k' is the constant which reflects growth rate for cells which have obtained nutrients. Combination of Eqs. (2) and (3), and defining k = Kk' followed by integration over the entire time (0 to t) of growth (4), gives

\[
X_{tot,t} = \frac{X_{tot,0} \exp(kt)}{1 - (X_{tot,0}/X_{max}) (1 - \exp(kt))}
\]

where, X_{tot,0} is the cell density at time 0, and X_{tot,t}, the cell density at time t. Eq. (4) can also be represented by Eq. (5):

\[
X_{tot,t} = \frac{X_{t}}{X_{e} + (X_{t}/X_{max})}
\]

where

\[
X_{t} = X_{tot,0} \exp(kt)
\]

\[
X_{e} = (X_{max} - X_{tot,0})/X_{max}
\]

Although X_t is commonly used to describe exponential growth of microorganisms, in the present context X_t serves only as a part of the overall logistic function equation. The variable X_e represents the proportion of cell density that results from cell proliferation during the growth of the population from the initial time, to the time at which X_{max} is effectively achieved. Two linear forms of Eq. (5) are:

\[
\frac{1}{X_{tot,t}} = \frac{1}{X_{max}} + \frac{1}{X_{e}} (X_{t})
\]

and

\[
\frac{X_{t}}{X_{tot,t}} = X_{e} + \frac{X_{t}}{X_{max}}
\]

Eq. (8) is analogous to the Lineweaver-Burke transformation of Michaelis-Menten enzyme kinetics, while Eq. (9) is analogous to the Hanes-Wolf transformation of Michaelis-Menten enzyme kinetics (Wong, 1975). Eqs. (8) and (9) differ from these enzyme kinetic equations since the resultant product is cell density, rather than reaction rate. When X_{max} is not already known, k can be estimated from the limiting case of Eq. (4). If substrate concentrations are high, the value for X_{max} will also be high, compared to X_{tot,0}. Therefore, early in the growth curve, Eq. (4) approaches Eq. (6), and X_{tot,t} = X_t, so k can be estimated from early data points using the equation:

\[
k_{F} = \left(\ln(X_{tot,t}/X_{tot,0})\right)/t
\]

where, k_{F} \approx k, as determined from early growth data. When there is a lag phase, k_{F}, as calculated from each pair of consecutive data points will increase until the effects of nutrient depletion become greater than the effects of acclimation to new growth conditions. At the inflection point where k_{F} ceases to increase and begins to
decrease, \( k_L \) will therefore be at its highest value, and will serve as an estimate for \( k \).

If \( \dot{X}_{\text{max}} \) is already known, \( k \) can be calculated for each time point in the growth curve by directly solving Eq. (4) for \( k \):

\[
k_L = (1/t) \ln \left[ \frac{(X_{\text{tot},t})(X_{\text{max}} - X_{\text{tot},0})}{(X_{\text{tot},0})(X_{\text{max}} - X_{\text{tot},0})} \right]
\]

(11)

where, \( k_L = k \), as calculated using Eq. (11).

Therefore, after a growth curve is completed, Eq. (11) can be used for determining whether the model is appropriate for the growth curve in question. The shape of a plot of \( k \) vs. cell density may provide insight into what types of factors need to be examined. If \( k_L \) changes systematically during the growth curve, or if there is an abrupt change in \( k_L \) at some point in the growth curve, then factors other than depletion of nutrients have influenced the growth rate. For example, if \( k_L \) decreases with cell density, the effects of fermentation product accumulation may be significant. An abrupt and persistent change in \( k_L \) may indicate a shift in fermentation pathways, or a diauxic effect. If \( k_L \) increases with cell density, then the presence of toxic substances in the original growth medium that are metabolized to less toxic ones may be a factor influencing early growth. Such substances may be present in some complex media and would have less effect on the growth rate as the cell density increases. Alkaloids in plant extracts (Nishikawa et al., 1988), low levels of antibiotics in gastrointestinal tract fluids, and heat degradation products of sugars are possible sources of such toxic substances. In effect, then Eq. (11) allows one to calculate values of \( k_L \) which aid identification of whether factors other than nutrient depletion are influencing growth rate.

This derivation of the logistic function model describes growth which is limited by the availability of nutrients, rather than growth, which is limited by the effects of inhibitory fermentation products. By separating the effects of nutrient depletion from alternative factors that influence the growth rate, the model facilitates a more detailed examination of growth curve data than would otherwise be possible. The model describes proliferation of cells from a low density after the lag phase (if any) is completed, through the stationary phase.

The decline in cell density that occurs in the death phase is not described by the logistic function model. While the logistic model will also not describe the overall growth curve when diauxic growth occurs, it is useful in describing specific regions of the growth curve, exclusive of shifts from one fermentation pattern to another. Poor fit of this derivation of the logistic model may also be used to suggest that factors other than nutrient limitation may be affecting growth.

2. Methods

2.1. Microorganisms

*Escherichia coli* W3110, and *Serratia marcescens* ATCC 8195, were maintained on plates of tryptic soy agar (DIFCO Laboratories, Ann Arbor, MI). *Streptococcus lactis* W2, *Selenomonas ruminantium* HD4, and *Eubacterium limosum* ATCC 10825 were maintained anaerobically in the general growth medium, frozen with glycerol as a cryoprotectant, in the authors’ culture collection.

2.2. Growth medium

All cultures were grown in the same basal medium, with some modifications. The basal medium contained (g/l): trypicase, 1.5; yeast extract, 1.0; ammonium acetate, 0.5; ammonium citrate, 0.5; KH₂PO₄, 2.5; KC₁, 0.5; CaCl₂·2H₂O, 0.05; MgSO₄, 0.05; and FeSO₄·7H₂O, 0.01. Anaerobic growth medium was prepared from the basal medium by addition of (g/l): resazurin, 0.001; hemin, 0.0005; Na₂CO₃, 4.0; and L-cysteine hydrochloride, 0.25. When prepared for anaerobic cultures, the medium was boiled for 1 min under a stream of anaerobic CO₂ prior to sealing and sterilization (Bryant, 1972). Carbonate and cysteine were added to the cooled, anaerobic growth medium as a separate sterile, anaerobic (CO₂) solution. For all cultures, glucose was also added as a separately autoclaved, anaerobic solution. The final concentration of glucose was 0.4 g/l for *E. limosum*, and 0.5 g/l for all of the other cultures. The final pH was 6.8 for the anaerobic medium, and 6.9 for the aerobic medium.

2.3. Growth curves

Inocula were prepared by growing each of the cultures for three successive passages in the medium that was used for the growth curve experiment. The growth curves were initiated with 1% (v/v) inocula of approximately mid-exponential phase cultures, in pre-warmed growth medium. *S. ruminantium*, *E. limosum*, and *S. lactis*, were grown in sealed, anaerobic tubes, under a CO₂ atmosphere, and strict anaerobic procedures were used (Bryant, 1972). *E. coli* and *S. marcescens* were grown aerobically. Cell densities were measured using a Spectronic 70 spectrophotometer (Bausch and Lomb, Rochester, NY), as optical density (OD) at 660 nm, 16 mm path length. The initial OD was too low to be accurately measured until about 30 min had elapsed. Therefore, cell densities were measured at 30 min intervals for the first 1.5 h, and at 15 min intervals thereafter. Measurements were taken until the cultures reached a stable OD, and remained at that value for at
least four consecutive readings. All cultures were incubated in triplicate at 37 °C.

Bacterial cultures used for growth experiments were in the author's culture collection and represented both anaerobic and aerobic bacterial species. Experimental growth data for Bacillus polymyxa ATCC 12321, grown at two rates of mixing, was obtained from de Mas et al. (1988) and growth data from an unnamed species of yeast (probably Saccharomyces cerevisiae) was obtained from Carlson (1913). The yeast data were analyzed in the present investigation because they were used for Carlson's early model and as an example of logistic growth by Pearl (1925).

2.4. Statistical analysis

For the experimental growth curves, \( k \) was estimated as \( k_F \) using Eq. (10). At the earliest point at which measurements were reliable, cell densities were still low (approximately 2–5% of the final cell densities), and the growth rates, when calculated for each pair of consecutive readings, were at their highest point in the growth curves. Therefore, \( k_F \) was assumed to be a reasonable estimate of \( k \). For the growth curves that were taken from the published work of other investigators, \( k \) was estimated as the inflection point \( k_I \), as described earlier. The experimental \( X_{\text{max}} \) was calculated for each culture as the mean value for the final stable maximum OD. The lowest cell densities that could be reliably measured (at approximately OD = 0.02) were used for \( X_{\text{tot,0}} \). The time at this point was considered to be \( t = 0 \).

For evaluation of whether the logistic function model applied to the growth curves, Eq. (4) was employed to generate models for each growth curve, using the experimental values for \( X_{\text{max}}, X_{\text{tot,0}}, t, \) and \( k_I \) as described above. Least squares regression analysis (SAS, 1990) was used to compare the curves generated from the models with the actual data (Neter et al., 1985). If the correlation coefficient (\( r^2 \)) and the slope were near unity, the models were considered to be satisfactory descriptions of the experimental data.

Eqs. (8) and (9) were evaluated as general linear descriptions of the data, using least squares regression analysis. Values for \( X_I \) were calculated using Eqs. (6) and (10), and the experimental values for \( X_{\text{tot,0}} \), for each time point, \( t \). The resultant values for \( X_{\text{max}} \) from the intercept for Eq. (8) or the slope for Eq. (9) were compared with the actual \( X_{\text{max}} \) values. As a more stringent test of the equations, and to evaluate their utility for predicting cell densities (including estimates for final cell density), the same regression procedures were also used for early portions of the growth curves. Two cases were examined. For the first case, cell densities less than \( (1/2)X_{\text{max}} \) were used. For the second case, cell densities up to \( (3/4)X_{\text{max}} \) were used. In these analyses, regression analysis was used for estimation of \( X_{\text{max}} \) only if they were significant using the \( F \) test (\( H_0: \) intercept = 0, or \( H_0: \) slope = 0, for Eqs. (8) or (9), respectively), with a cutoff at \( P < 0.01 \).

Eq. (11) was used to evaluate whether systematic changes in the growth rate occurred. For each culture, \( k_I \) was regressed vs. cell density. Since the experimental values for \( X_{\text{max}} \) were required for calculation of \( k_I \), this was the only time that \( k_I \) was used in a statistical analysis.

3. Results

3.1. Experimental growth curves

In order to ensure the likelihood that the major factor that reduced the growth rate was nutrient depletion, and in order to allow for accurate measurement of cell densities near \( X_{\text{max}} \) without dilution of the cultures, the initial glucose concentrations were low (0.4 g/l). The final OD for the cultures ranged from 0.45 for \( S. \) lactis to 0.65 for \( S. \) ruminantium. The growth media were highly buffered in order to avoid inhibition of growth by low pH. The lowest final pH for the anaerobic cultures was 6.6. For aerobic cultures, the final pH was 6.1.

3.2. Correlation of logistic function model with experimental data

A subjective comparison of the actual growth curves, with those generated from Eq. (4) is given by plotting both the actual data and the line resulting from the model. Data from the growth curve of \( S. \) ruminantium are used to illustrate application of the logistic function model to data. Analyses for the data from all of the bacterial growth curves are presented in Tables 1–4. Fig. 1 compares data for \( S. \) ruminantium with results from

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Table 1

<table>
<thead>
<tr>
<th>Culture</th>
<th>( r^2 )</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli W3110</td>
<td>0.948</td>
<td>0.981</td>
</tr>
<tr>
<td>E. limosum ATCC 10825</td>
<td>0.980</td>
<td>0.987</td>
</tr>
<tr>
<td>S. lactis C2</td>
<td>0.973</td>
<td>1.070</td>
</tr>
<tr>
<td>S. marcescens ATCC 8195</td>
<td>0.984</td>
<td>0.965</td>
</tr>
<tr>
<td>S. ruminantium HD4</td>
<td>0.981</td>
<td>0.957</td>
</tr>
<tr>
<td>B. polymyxa ATCC 12321&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.993</td>
<td>0.992</td>
</tr>
<tr>
<td>B. polymyxa ATCC 12321&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.997</td>
<td>0.965</td>
</tr>
<tr>
<td>Yeast&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.995</td>
<td>0.999</td>
</tr>
</tbody>
</table>

<sup>a</sup>Statistics were calculated using least squares linear regression, with model-generated data as the independent variable, and the actual data as the dependent variable.

<sup>b</sup>From the published data of de Mas et al. (1988), grown at 500 rpm.

<sup>c</sup>From the published data of de Mas et al. (1988), grown at 800 rpm.

<sup>d</sup>From the published data of Carlson (1913), species not given.
Table 2
Evaluation of Eq. (8) for estimation $X_{\text{max}}$ at three stages of the growth curves

<table>
<thead>
<tr>
<th>Culture</th>
<th>Actual $X_{\text{max}}$</th>
<th>Estimated $X_{\text{max}}$^b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\lambda$</td>
<td>1/2</td>
</tr>
<tr>
<td>$E. coli$ W3110</td>
<td>0.518</td>
<td>0.268^c</td>
</tr>
<tr>
<td>$E. limosus$ ATCC 10825</td>
<td>0.530</td>
<td>0.423^d</td>
</tr>
<tr>
<td>$S. lactis$ C2</td>
<td>0.447</td>
<td>0.256^e</td>
</tr>
<tr>
<td>$S. marcescens$ ATCC 8195</td>
<td>0.650</td>
<td>0.258^f</td>
</tr>
<tr>
<td>$S. ruminantium$ HD4</td>
<td>0.654</td>
<td>0.653^g</td>
</tr>
<tr>
<td>$B. polymyxa$ ATCC 12321^f</td>
<td>3.167</td>
<td>1.591^f</td>
</tr>
<tr>
<td>$B. polymyxa$ ATCC 12321^h</td>
<td>6.767</td>
<td></td>
</tr>
<tr>
<td>Yeast^h</td>
<td>0.670</td>
<td>0.934^i</td>
</tr>
</tbody>
</table>

^a For all data shown, the regression of Eq. (8) was significant ($P < 0.01$ for $H_0$; intercept = 0).

^b Logistic model $X_{\text{max}}$ was calculated from the reciprocal of the intercept, using Eq. (8) for data from the first 1/2 or 3/4 of the growth curves, or the entire growth curves, as indicated by the column headings. Units for $X_{\text{max}}$ were OD_{660} for $E. limosus$, $E. coli$, $S. marcescens$, $S. ruminantium$, and $S. lactis$. Units for $X_{\text{max}}$ for $B. polymyxa$ were grams per liter, and units of $X_{\text{max}}$ for yeast were grams/100 ml.

^c $r^2$ of the regression of Eq. (8): $\geq 0.95$.

^d $r^2$ of the regression of Eq. (8): $\geq 0.90$.

^e $r^2$ of the regression of Eq. (8): $\geq 0.80$.

^f From the published data of de Mas et al. (1988), grown at 500 rpm.

^g From the published data of de Mas et al. (1988), grown at 800 rpm.

^h From the published data of Carlson (1913) species not given.

^i $r^2$ of the regression of Eq. (8): $\geq 0.99$.

Table 3
Evaluation of Eq. (9) for estimation $X_{\text{max}}$ at three stages of the growth curves

<table>
<thead>
<tr>
<th>Culture</th>
<th>Actual $X_{\text{max}}$</th>
<th>Estimated $X_{\text{max}}$^b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\lambda$</td>
<td>1/2</td>
</tr>
<tr>
<td>$E. coli$ W3110</td>
<td>0.518</td>
<td>0.306^c</td>
</tr>
<tr>
<td>$E. limosus$ ATCC 10825</td>
<td>0.520</td>
<td>0.574^c</td>
</tr>
<tr>
<td>$S. lactis$ C2</td>
<td>0.447</td>
<td>0.333^c</td>
</tr>
<tr>
<td>$S. marcescens$ ATCC 8195</td>
<td>0.650</td>
<td>0.326^c</td>
</tr>
<tr>
<td>$S. ruminantium$ HD4</td>
<td>0.654</td>
<td>0.523^c</td>
</tr>
<tr>
<td>$B. polymyxa$ ATCC 12321^f</td>
<td>3.167</td>
<td>2.651^f</td>
</tr>
<tr>
<td>$B. polymyxa$ ATCC 12321^h</td>
<td>6.767</td>
<td>4.780^g</td>
</tr>
<tr>
<td>Yeast^h</td>
<td>0.670</td>
<td>1.038^c</td>
</tr>
</tbody>
</table>

^a For all data shown, the regression was significant ($P < 0.01$ for $H_0$: slope = 0).

^b Logistic model $X_{\text{max}}$ was calculated from the reciprocal of the slope, using Eq. (9) for data from the first 1/2 or 3/4 of the growth curves, or the entire growth curves, as indicated by the column headings. Units for $X_{\text{max}}$ were OD_{660} for $E. limosus$, $E. coli$, $S. marcescens$, $S. ruminantium$, and $S. lactis$. Units of $X_{\text{max}}$ for $B. polymyxa$ were grams per liter, and units of $X_{\text{max}}$ for yeast were grams/100 ml.

^c $r^2$ of the regression of Eq. (9): $\geq 0.95$.

^d $r^2$ of the regression of Eq. (9): $\geq 0.99$.

^e $r^2$ of the regression of Eq. (9): $\geq 0.80$.

^f From the published data of de Mas et al. (1988), grown at 500 rpm.

^g From the published data of de Mas et al. (1988), grown at 800 rpm.

^h From the published data of Carlson (1913) species not given.

Eq. (4). The constants were $k = 1.57$, $X_{\text{tot},0} = 0.036$, and $X_{\text{max}} = 0.654$.

Table 4
Evaluation of rate constant ($k$), calculated by Eqs. (10) and (11)

<table>
<thead>
<tr>
<th>Culture</th>
<th>$k_\text{a}$</th>
<th>$k_\text{b}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E. coli$ W3110</td>
<td>1.852</td>
<td>1.338</td>
</tr>
<tr>
<td>$E. limosus$ ATCC 10825</td>
<td>1.843</td>
<td>1.831</td>
</tr>
<tr>
<td>$S. lactis$ C2</td>
<td>1.816</td>
<td>1.657</td>
</tr>
<tr>
<td>$S. marcescens$ ATCC 8195</td>
<td>1.604</td>
<td>1.106</td>
</tr>
<tr>
<td>$S. ruminantium$ HD4</td>
<td>1.624</td>
<td>1.572</td>
</tr>
<tr>
<td>$B. polymyxa$ ATCC 12321^f</td>
<td>0.470</td>
<td>0.356</td>
</tr>
<tr>
<td>$B. polymyxa$ ATCC 12321^h</td>
<td>0.593</td>
<td>0.439</td>
</tr>
<tr>
<td>Yeast^h</td>
<td>0.487</td>
<td>0.506</td>
</tr>
</tbody>
</table>

^a $k_\text{a}$ was calculated using Eq. (10). Values are given as means from triplicate tubes. Units for $k_\text{a}$ are $t^{-1}$.

^b $k_\text{b}$ calculated using Eq. (11). Values are given as means, using the entire growth curves. Units for $k_\text{b}$ are $t^{-1}$.

^c From the published data of de Mas et al. (1988), grown at 500 rpm.

^d From the published data of de Mas et al. (1988), grown at 800 rpm.

^e From the published data of Carlson (1913) species not given.

Fig. 1. Growth curve for $S. ruminantium$. Plot of cell density (OD) vs. hours. Values for cell density were determined for each of three tubes at each time point. Some values cannot be seen because they overlap others. The line represents the logistic function model for the growth curve.

Statistical comparison allows for a more objective analysis. If the data can be described by the model, then regression of these data as the dependent variable, with the model as the independent variable, will result in $r^2$ and slope near unity. For the enteric species ($E. coli$ and $S. marcescens$), correlation with the model was lower than that seen for the other growth curves, possibly due to the effect of the pH drop. For the remaining organisms, the $r^2$ was greater than 0.97, and the slope for all of the regression was between 0.95 and 1.1 (Table 1).

3.3. Estimation of $X_{\text{max}}$ using Eq. (8)

When double reciprocal plots are employed for data which span several orders of magnitude, as occurs with the values for $X_{\text{r}}$, statistical evaluation is needed for a
complete analysis of the data. Compression of the graphical scale occurs for low values (high \( X_t \) and high \( X_{\text{max}} \)), relative to high values (low \( X_t \) and low \( X_{\text{max}} \)) (Fig. 2). Therefore, the regression may be strongly influenced by the first few points in the growth curve, when effects of nutrient limitation are minor.

Determination of \( X_{\text{max}} \) may be inaccurate. When Eq. (8) was used for estimation of \( X_{\text{max}} \), based only on values for cell density that were less than \( 1/2 \) of \( X_{\text{max}} \), only four of the eight transformed growth curves were statistically significant, and the estimates for \( X_{\text{max}} \) were poor (Table 2). Inclusion of data up to \( (3/4)X_{\text{max}} \) improved the estimates for \( X_{\text{max}} \) only slightly. When the entire growth curves were analyzed using Eq. (8), the regressions were significant for all of the growth curves, except for that of \( B. \) polymyxa, grown at 800 rpm. In general, Eq. (8) provided only a rough approximation of the \( X_{\text{max}} \) with an error of 10–30% from the actual values.

3.4. Estimation of \( X_{\text{max}} \) using Eq. (9)

As occurs with Eq. (8), this linear transformation results in compression of scale at low values, and expansion of scale for high values (Fig. 3). However, Eq. (9) is more highly influenced by data points that occur later in the growth curve when nutrient depletion is more significant. When data from less than \( (1/2)X_{\text{max}} \) were analyzed using Eq. (9), the regression was significant for all of the cultures, except for \( B. \) polymyxa, grown at 500 rpm (Table 3). Correlation coefficients varied, however, from 0.977 for \( E. \) limosum, to 0.977 for \( E. \) coli. The error rate was variable, ranging from 8% for \( E. \) limosum to 50% for the yeast culture. When cell densities up to \( (3/4)X_{\text{max}} \) were used, estimates for \( X_{\text{max}} \) improved and were better than when Eq. (8) was used.

When the entire growth curves were analyzed using Eq. (9), calculation of \( X_{\text{max}} \) from the slope was more reliable. Except for \( S. \) ruminantium, all of the estimates were within 2% of the actual \( X_{\text{max}} \), for \( S. \) ruminantium, the estimate was within 11% of the actual \( X_{\text{max}} \) (Table 3).

Regression of \( k_1 \) vs. cell density resulted in small but statistically significant trends for the enteric species, and for \( S. \) lactis, and \( E. \) limosum (\( P < 0.01 \)). These slight trends could also be observed in the plots of \( k_1 \) vs. OD/\( X_{\text{max}} \) (Fig. 4) and may have been due to pH effect, or accumulation of fermentation products. However, these effects were not well correlated with cell density (\( r^2 < 0.3 \)) and the values for \( k_1 \) did not change to such a
degree that the correlation of the models with the experimental data was influenced. Since the greatest variability in \( k_L \) occurred after most of the growth curve was completed, the late values for \( k_L \) were not included in the graphic evaluation. The mean values for \( k_L \), taken for the entire growth curves, were generally close to the values for \( k_F \) (Table 4), indicating that \( k_F \) was a reasonable estimate for \( k \). This analysis confirmed that the logistic function model is fairly robust, and these small changes in \( k_L \) did not significantly hamper the utility of the model.

4. Discussion

Historically, Eq. (6) has been used to describe the growth of microbial cultures, however the latter parts of the growth curve are not considered with this model. The logistic function model is increasingly being used empirically to describe growth of microbial cultures. If nutrients become depleted before the inhibitory effects of fermentation product accumulation are seen, the logistic function model seems appropriate. It also seems appropriate to derive parameters that have biological meaning instead of simply fitting growth curves empirically.

For cultures that were grown for the present investigation, a low initial nutrient concentration was used so that logistic growth would be more likely to occur. Although the media were also buffered to avoid a large drop in pH, there may still have been a small pH effect on the enteric species. The pH of the \( B. polyryx \) culture was reported to be controlled (de Mas et al., 1988), and limitation of growth of \( B. polyryx \) appeared to be related primarily to nutrient (oxygen and constituents of yeast extract) depletion. While statistical analyses demonstrated good general correlation of the growth curve data with the model, the logistic function model was demonstrated to be appropriate for batch growth under these low nutrient conditions. Although Carlson (1913) did not describe the growth conditions for the yeast data, the shape of the yeast growth curve was typical for logistic growth and the yeast data were also well correlated with the logistic function model.

The estimates for \( X_{\text{max}} \), as calculated by Eq. (9), appeared to be more reliable than those obtained from Eq. (8). As would be expected, the closer the growth curves were to their completion, the more accurate were the estimates for \( X_{\text{max}} \).

The results for Eq. (9) validate the model as a description of batch growth. Early in the growth curve, when OD is less than \((1/2)X_{\text{max}}\), purely exponential growth is usually expected to occur. This is also the most stringent test of the model, because the effects of nutrient depletion will be small, and experimental error may be greater than the difference in cell density that results from lack of nutrients. However, if growth was exponential, with no effects due to nutrient depletion, then the slope for Eq. (9) would be zero, and the estimate for \( X_{\text{max}} \) would be undefined (infinite). For the growth curves that were examined in the present work, the estimates for \( X_{\text{max}} \) were fairly close to the actual \( X_{\text{max}} \) (and far from infinite). Even in the worst case, the estimates for \( X_{\text{max}} \) were of some value. For example, using Eq. (9) when OD < \((1/2)X_{\text{max}}\), the estimate for \( X_{\text{max}} \) was 0.326 for \( S. marcescens \). Using values of \( X_{\text{tot},0} = 0.039 \) and \( k_F = 1.604 \) to estimate OD at 4 hours, the usual exponential method \((X_T)\) would result in OD = 24, while Eq. (4) results in OD = 0.322; the actual value was 0.567.

The logistic function model, even in the worst case, was much closer to the actual data than the exponential model given by Eq. (6). Therefore, the logistic function model was an appropriate description for the growth of these cultures, even during the period of the growth curve that is generally considered to be exponential.

The utility of the logistic function model lies in several aspects of analyzing growth data. If growth is logistic, the logistic function model can be used to predict when a given cell density will be reached, more accurately than is the case when the exponential model is used. This would be especially true for nutrient optimization experiments, in which it is desired to clearly correlate the growth rate, or the final cell density, to the concentration of a particular nutrient, and the nutrient in question is at a low concentration. Microbial growth in natural habitats (soils, aquatic environments, and gastrointestinal tracts of animals) may be a clear function of the concentration of a particular nutrient, and the logistic function model may describe the growth cycles in these environments more reliably than the exponential model. The linear transformations of the model allow for inclusion of a greater proportion of the growth curves, from the early exponential through the stationary phases of growth, in simple linear regression analysis. The model allows for calculation of the rate constant \((k_L)\) at discrete points of time, when nutrient concentration limits growth; this is not true for the exponential model. Analysis of \( k_L \) as a function of cell density may reveal the times at which fermentation product accumulation influences growth, as discussed earlier. If a fermentation product is produced as a direct function of growth, and its concentration is proportional to cell density then the accumulation of the fermentation product is also described by the logistic function model. Conversely, the concentration of the limiting nutrient may be predicted by the model, as the difference between initial concentration of the nutrient, and the quotient of cell density divided by the yield of cells per unit substrate.

Although this derivation of the logistic function model as a function of nutrient depletion is different
from most other derivations which describe "inhibition" as a function of the square of cell density (Freedman, 1980), the form of the model is the same. The logistic function model has been employed as a purely empirical (best-fit) description of population increase (Freedman, 1980; Mulchandani et al., 1988). Therefore, the linear transformations, especially Eq. (9), may be useful when the pattern of growth appears to be logistic, even when the nutrient concentrations are known to limit growth.

By deriving the logistic function model as a property of cell density, in which the difference between potential biomass and existing biomass substitutes for substrate concentrations, a foundation is laid on which other, more complete models can be built. For example, if a property can be directly related to cell mass, then a mathematical function that describes that property can be substituted for \( k \) in Eq. (1). The effects of various properties can be examined by simulating growth curves from the equation, and comparing the relative effects of the property in question, nutrient depletion, and experimental error. Such simulations may help in planning experiments. Used with experimental data, these derivations may be used to give relatively rapid and early indications of inhibition. These derivations may also be useful in interpreting data for microscale, high throughput microbial screens. Properties may include the effects of inhibitory fermentation products, changes in viscosity, or changes in fermentation pathways.

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References


