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A SEGMENTED REGRESSION MODEL FOR DESCRIPTION OF MICROBIAL GROWTH

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Abstract

A segmented regression model for the description of microbial growth has been suggested. The model is able to predict the exponential growth, logistic growth, logistic growth with a phase of decline, diauxic growth, microbial growth in synchronous cultures and the oscillatory growth.

Introduction

During the last few decades of this century numerous mathematical expressions for microbial growth rate have been proposed and published in the literatures. [1-3] Some of them are used for better understanding of the microbial growth rate, and others are used for bioprocess modelling.

These models are interesting, but generally speaking, when there are many plateaus and extremes in the response curve, among the unstructured models, the segmented regression models [4] are the most appropriate ones. So far such models have not been suggested for description of microbial growth. In this work a segmented regression model containing exponential terms is proposed which, with its increased complexity, gives a good description of microbial growth in a variety of situations.

The Model

Simple mathematical expressions of growth rate can be elaborated upon and gradually made more complex. As they become more complex they come to resemble reality more. One of these simple expressions is the logistic equation (Verhulst Equation) which is actually a Riccati Equation [1] with constant coefficients. To elaborate, the coefficients may be assumed to be functions of time.

Integration of a Riccati Equation of the following form

$$\frac{dx}{dt} = k_1 x - \frac{x^2}{b_1} . e^{-k_1 t} . \frac{df}{dt} \qquad (1)$$

results in the equation

$$x = \frac{x_0 e^{k_1 t}}{1 - x_0 ((f_0 - f)/b_1} \qquad (2)$$

which may be used to describe the microbial growth at different conditions. Where x_0 and x are the initial cell concentration and cell concentration at time t respectively. k_1 and b_1 are constants and f is an arbitrary function of time. As t increases, x approaches $K(t) = k_1.b_1.\exp(k_1 t)/(df/dt)$, so f is a function which determines the pattern of the growth. f_0 is the value of this function at time zero.

Choosing a function of the following form for f

$$f = (e^{k_1 t} - 1) \prod_{i=2}^{n} \left[\frac{1 + e^{k_1 (t - t_{mi})}}{1 + b_1 e^{k_1 (t - t_{mi})}} \right] \qquad (3)$$

and substituting for f from this equation into Equation (1) results in the general explicit algebraic equation

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$$x = \frac{x_0 e^{k_1 t}}{1 - \frac{-x_0(1-e^{k_1 t})}{b_1} \cdot \prod_{i=2}^{n} \left[\frac{1+e^{k_1(t-t_{mi})}}{1+b_1 e^{k_1(t-t_{mi})}}\right]} \qquad (4)$$

which is able to describe the microbial growth in a variety of the situations as described below. Later in this work it will be shown that by function f (Equation (3)) the cell cycle concept is introduced into the modified logistic equation (Equation (1)). In Equation (4), k_i, b_i, and t_{mi} (i= 1,2,3,...,n) are constants. As t approaches infinity, x tends towards the stationary value, $x= b_1.b_2.b_3...b_n$. The initial value of x is x_0 and depending on the values of b_i, x from Equation (4) is able to follow different patterns between its initial and stationary values. As can be seen f is segmented, i.e. it may be written in the form: $f= f_1.f_2.f_3...f_n$. Each segment in f (i.e. f_1, f_2,...etc.) makes x adaptable to a specific segment of experimental data.

Results and Discussion

1) When $b_1=\infty$, Equation (4) results in the well-known exponential growth equation

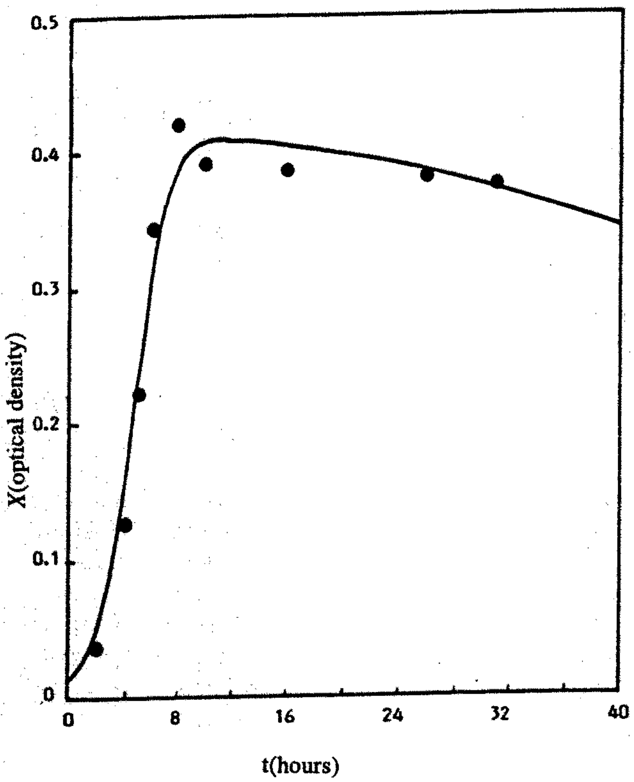

Figure 1. Growth curve as predicted by Equation (7).
● Experimental data obtained by author and A. Nimasa in growing *Escherichia coli* 026 in a medium containing glucose and mineral salts. $k_1=0.78$, $k_2=0.047$, $b_1=0.442$, $t_{m2}=66.46$, $x_0=$ 0.011.

$$x= x_0 e^{k_1 t} \qquad (5)$$

2) When $b_i= t_{mi}= 0$, i = 2,3,4,...,n, Equation (4) results in the logistic curve.

$$x = \frac{x_0 e^{k_1 t}}{1-x_0 (1-e^{k_1 t})/b_1} \qquad (6)$$

The logistic curve is sigmoidal and leads to a stationary growth $x_s= b_1$. The shortcoming in logistic equation is its failure to predict a phase of decline after the stationary growth.

3) When $b_i= 0$ for i >1, and $t_{mi}= 0$ for i >2, Equation (4) results in the modified logistic curve,

$$x = \frac{x_0 e^{k_1 t}}{1-x_0 (1-e^{k_1 t}) [1+e^{k_2(t-t_{m2})}] / b_1} \qquad (7)$$

which is able to predict a phase of decline. This has been shown in Figure 1. Here t_{m2} is the time at which cell concentration approaches the value $b_1/2$, i.e. half the stationary growth, $x_s= b_1$ as predicted by the logistic curve, i.e. Equation (6).

4) When $t_{mi}= b_i= 0$ for i >2, Equation (4) results in the equation

$$x = \frac{x_0 e^{k_1 t}}{1- \frac{x_0(1-e^{k_1 t}) [1+e^{k_2(t-t_{m2})}]}{b_1 [1+b_2 . e^{k_2(t-t_{m2})}]}} \qquad (8)$$

As illustrated in Figure 2 this equation successfully describes the diauxic batch growth. The equation is able to predict the lag time between growth in two substrates in diauxic growth. In this case the maximum stationary growth is $x_s=b_1.b_2$ and t_{m2} is the time at which x is almost equal to $(b_1+b_1.b_2)/2$, i.e. half the sum of the stationary values of the first and second plateaus. As can be seen, b_2 is equal to the ratio of the two stationary values. i.e. $b_2= b_1.b_2/b_1$. In general b_i is equal to the ratio of the stationary values of the two successive (i and i-1) stages (in this case plateaus). This knowledge makes the parameter estimation easier.

5) When $b_1= x_0$, $k_1=k_2= .. =k_i=k$. $b_2=b_3=...= b_i=2$. $t_{mi}=$ (i-1) t_d-t_c for i >1. and $t_{m1}=0$ (where t_d is the doubling time, and t_c is the age of the cell at the time of inoculation). Equation (4) results in

$$x = \frac{x_0 e^{k_1 t}}{1- (1-e^{kt}) . \prod_{i=2}^{n} \left(\frac{1+e^{k[t-(i-1) t_d + t_c]}}{1+2e^{k[t-(i-1) t_d + t_c]}}\right)} \qquad (9)$$

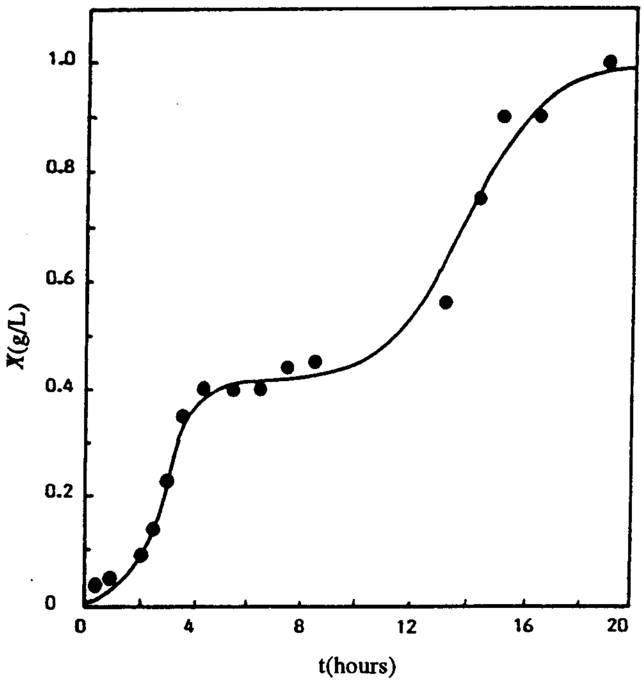

Figure 2. Diauxic growth curve as predicted by Equation (8). ● Experimental data is for *Klebsiella oxyloca* from reference [3]. k_1=1.67. k_2= 0.73, b_1= 0.42, b_2= 2.38, t_{m2}= 12.62, x_0= 0.0038.

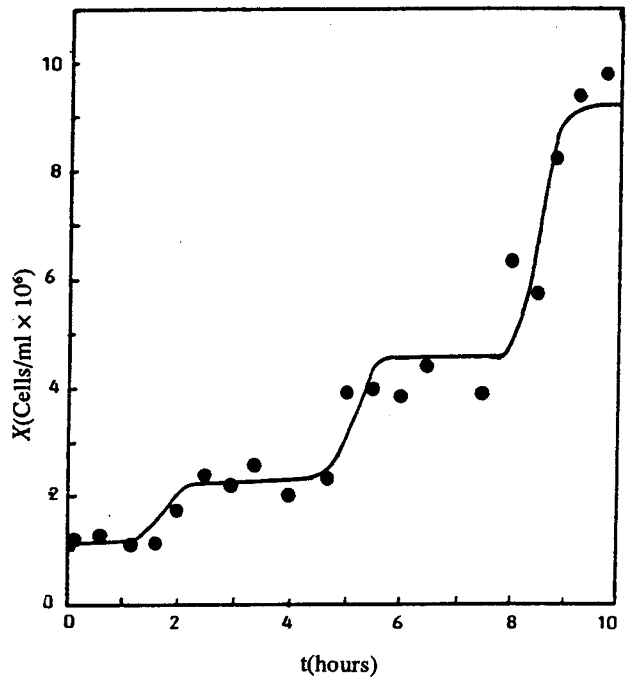

Figure 3. Growth curve in synchronous culture as predicted by Equation (6).
●Experimental data for the yeast *Schizosaccaromyces pombe* from reference [1], page 359. k= 6, $t_c=t_d/2=1.7$, $t_{mi}=(i-1)t_d-t_c$ for i>1, t_{m1}= 0.

which is able to describe the microbial growth in synchronous cultures. Synchronous cultures in which all the cells are the same age and state of development are used for studying the cell cycle.[1]. In Equation (9), t_d represents the length of the life cycle of the cell. In Figure 3, Equation 9 has been used to describe the stepwise increase in cell number in a synchronous culture of the yeast *Schizosaccharomyces pombe*. In Figure 3 the value of k is equal to 6. In Figure 4 the curve has been drawn for different values of k. As seen, when k=∞, Equation 9 predicts an ideal synchronous culture, and Equation 9 reduces to,

$$x=\sum_{i=1}^{n}(2)^{i-1}.\ x_0\ .\ u\ (t-t_{mi}) \qquad (10)$$

where $u(t-t_{mi})$ is a step unit function [6] and $t_{mi}=(i-1)t_d+t_c$ for i >1 and tm_1=0. Equation (10) may be written in the differential form.

$$\frac{dx}{dt}=\sum_{i=1}^{n}(2)^{i-1}.\ x_0\ .\ \delta\ (t-t_{mi}) \qquad (11)$$

where $\delta(t-t_{mi})$ is the Dirac delta function [6]. t_{mi} may be

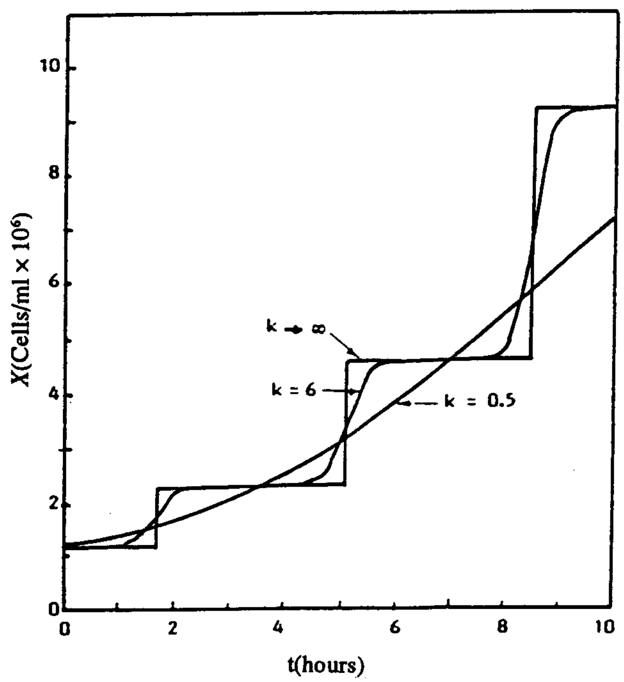

Figure 4. Growth curve in synchronous culture as predicted by Equation (9) for different values of k. Parameters are the same as in Figure 3.

divided into the durations of the four main phases: G_1, S(DNA replication), G_2. and M (mitosis) and therefore is under the influence of the factors that regulate the cell cycle [7]. Models based on cell cycle concepts have been constructed to study the effects of the external perturbations on cell proliferation [8] and tumour growth [9]. The model presented in this work (with only one parameter, t_d) may be used to study the effects of the molecular components that control the cell cycle progression. When the inoculum consists of a heterogenous population of the cells with different age, synchrony is not observed and the growth can be described by the equation.

$$x = \sum_{j=1}^{p} \frac{x_{0j}\, e^{kt}}{1-(1-e^{kt}) \cdot \prod_{i=2}^{n} \left\{\frac{1+e^{k[t-(i-1)t_d + t_{cj}]}}{1+2e^{k[t-(i-1)t_d + t_{cj}]}}\right\}} \qquad (12)$$

where x_{0j} is the number of cells that have an age of t_{cj} at time zero in the inoculum. There are p age groups at time

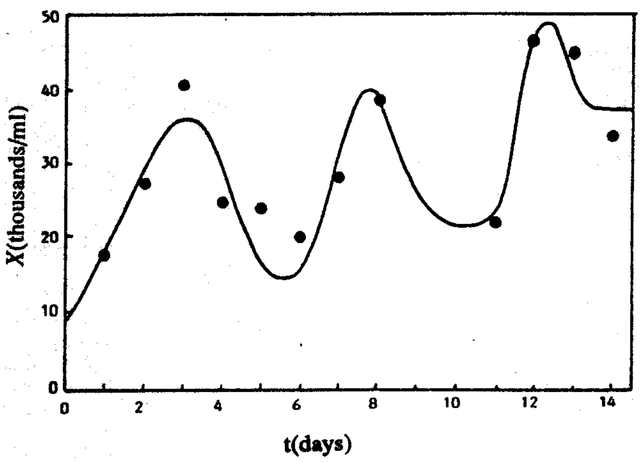

Figure 5. Oscillatory growth curve as predicted by Equation (4).
● Experimental data for the protozoan *Tetrahymena pyriformis* in a mixed culture from reference [1], page 873. $k_1= 0.95$, $k_2= 1.7$, $k_3= 1.96$, $k_4= 2.49$, $k_5= 4.8$, $k_6= 6.1$, $b_1= 50.2$, $b_2= 0.147$, $b_3= 7.32$, $b_4= 0.385$, $b_5= 2.4$, $b_6= 0.75$, $t_{m1}= 0$, $t_{m2}= 4.06$, $t_{m3}= 7$, $t_{m4}= 8.33$, $t_{m5}= 11.51$, $t_{m6}= 12.83$.
For $i > 6$ all of the terms have been cancelled.

zero in the inoculum. With $k= \infty$ this equation reduces to.

$$x = \sum_{i=1}^{n} \sum_{j=1}^{p} (2)^{i-1} \cdot x_{0j} \cdot u(t-t_{mij}) \qquad (13)$$

where $t_{mij}= (i-1)\, t_d + t_{cj}$ for $i>1$ and $t_{m1j}= 0$

6) When $b_i \neq 0$, and $t_{mi} \neq 0$, by choosing these parameters properly, Equation (4) may be used to describe the oscillation in growth of a mixed population of microorganisms. In Figure 5, Equation (4) has been adopted to an oscillatory growth. $t_{m,i+2} - t_{mi}$ is equal to the period of oscillation which may not be necessarily constant. In the case of oscillatory growth the value of b_i is alternately less or greater than one. In Figure 5 the values of b_1, b_3, and b_5 are greater than one and the values of b_2, b_4, and b_6 are less than one. But it seems that as the time passes, both series approach unity.

In conclusion, Equation (4) is a general one and by choosing proper values for b_i, k_i, and t_{mi} ($i=1,2,3,\ldots,n$) it can be used to describe the microbial growth in a variety of the situations. The main feature in Equation (4) is the appearance of a growth phase duration parameter, t_{mi}.

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