A piecewise-linear growth model: comparison with competing forms in batch culture

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Abstract. A simplified one-dimensional growth model based on the mass conservation law is proposed. Mathematically, it represents an easiest special case of so called L-systems. The model developed being considered as a descriptive tool of the growth curves is compared with four other similar and widely used models, in application to numerical data of bacteria (taken from literature) and of algae (an original experiment) growing in batch. Identification of free constants of the five models in comparison is performed using a nonlinear least squares iterative procedure, for which special programs were written. Theoretical and practical (approximative) merits of the new model are shown and discussed.

Key words: Growth model — L-systems — *Dunaliella*

Introduction

A dynamic, deterministic model of a cell population growing under conditions of limited resources (batch culture in microbiology) can be presented in the following general form:

$$\frac{dx}{dt} = F(s_1, \ldots, s_M).$$ (A)

Here $x(t)$ = value of a size attribute of a whole cell population at time t (for instance, biomass, density, area, etc.), s_i = value of ith resource (quantity or concentration of ith substrate, etc.), and F = function to be formalized involving biological assumptions. Adding to (A) the mass conservation law in the form of quasichemical reaction,

$$dx = Y_1 \, ds_1 + \cdots + Y_M \, ds_M,$$ (B)

where Y_i = stoichiometrical (or the growth yield) coefficient of the ith resource, one can obtain a one-dimensional differential growth equation with respect to x.

Equation (A) gives a general framework, into which fall many well-known growth models. Recent success achieved in developing this class of models was

based on using a dynamical form of the ancient concept of the optima and limiting factors known as Blackman's (Blackman 1905), or Liebig's, or "bottleneck" (Poletaev 1974) principle. It implies that the current rate of growth is limited, at any moment of time, by the rate of assimilation of a single "slowest" factor. Thus, instead of (A), we have:

$$\frac{dx}{dt} = \min\{F_1(s_1), \ldots, F_M(s_M)\}, \tag{C}$$

where symbol min{ } implies that the minimum should be chosen from expressions within braces, at any time. Mathematical aspects of this formalism called L-systems (for limiting factors and for Liebig) were elaborated by the Novosibirsk group (Lyapunov 1972; Poletaev 1974, 1979). A model constructed below represents a special case of L-system and brings some practical support to this general approach.

When the chemical composition of the biomass synthesizing remains (or can be regarded, approximately) invariable, all Y_i in (B) become constant, and we can try to interpret (A) as an equation with constant coefficients. Efforts to describe cell population growth by means of such an equation were made over a few decades (McKendrick and Poi 1910; Blackman 1919; Robertson 1923; Lotka 1925; Pearl 1940; Monod 1942; Richards 1959; Lyapunov 1972; Poletaev 1974 and 1979) and led to the following four most known and widely used models (Causton and Venus 1981):

(i) $\dfrac{dx}{dt} = kx\left(1 - \dfrac{x}{g}\right)$ (the logistic equation)

(ii) $\dfrac{dx}{dt} = kx \ln \dfrac{g}{x}$ (the Gompertz model)

(iii) $\dfrac{dx}{dt} = \dfrac{kx(g-x)}{a + (g-x)}$ (the Monod model)

(iv) $\dfrac{dx}{dt} = \dfrac{kx}{m}\left(1 - \dfrac{x^m}{g^m}\right)$ (the Richards model)

Here x approaches asymptotically to its maximum value $x = g$ (the lower asymptote is $x = 0$); k in (i) and (iii) and k/m in (iv), are usually considered as maximum relative growth rate, whereas k in (ii) is merely a factor; then, in (iii), a is a product of Y and K where Y = yield coefficient and K = half-saturation (or effective Michaelis') constant, both with respect to a single limiting substrate; m in (iv) is an auxiliary parameter without clear biological meaning, $-1 \leqslant m < \infty$, $m \neq 0$. Constants k, g and a are essentially positive.

Model (iii) is used primarily in microbiology, models (ii) and (iv), in higher plant biometry where a main organ system or a whole plant is regarded as a cell population consuming the "substrate of fresh assimilates" of vegetation; model (i) (the logistic, or Verhulst–Pearl equation) is used in the mentioned fields as well as in the quantitative theory of animal or human population.

Equation (i)-(iv) were conceived as mechanistic, i.e. having parameters of some biological relevance and giving an insight into the process of growth in

non-singular environment. Historically, each of these equations, when they appeared, was claimed to be a fundamental law of biological growth: of a cell population, of a plant tissue, animal population or even of any living system. However, after Feller's classical work (1940) it became obvious that such a universal law presented in the form of a differential equation with constant coefficients could hardly exist; instead, several competing mathematical forms expressing growth phenomena on a theoretically acceptable basis should be considered together and compared in application to particular growing systems.

Equation (i), (ii) and (iv) possess explicit solutions with respect to x, whereas in Eq. (iii) one can only find an expression for t as a function of x

$$(a) \qquad x = g(1+(g/x_0-1)\exp(-kt))^{-1}$$

$$(b) \qquad x = g\exp(\ln(x_0/g)\exp(-kt))$$

$$(c) \qquad tk = (a/g+1)\ln(x/x_0)+(a/g)\ln((g-x_0)/(g-x)) \tag{1}$$

$$(d) \qquad x = g(1+(g^m/x_0^m-1)\exp(-kt))^{-1/m}$$

where x_0 = initial size. Notice that Eq. (iv) turns into Eq. (i) at $m=1$, and into Eq. (iii), at m approaching zero, so (i) and (ii) are merely particular cases of (iv).

An important advantage of Eq. (i) and (iii) is that they follow from the mass conservation law which, for the case of single substrate limitation, has the form of equality:

$$s = \frac{1}{Y}(g-x). \tag{2}$$

On the contrary, Eq. (ii) and (iv) cannot be derived through expression (2), which makes them semi-empirical, especially (iv), considering its artificial parameter m.

Equality (2) implies one-by-one correspondence between substrate consumption and new cell material formation. The fulfilment of (2) should be expected to remain fairly true in one-dimensional simplified systems like those in question. The fact that Eq. (ii) and (iv) do not satisfy (2) lessens their importance since it becomes impossible to put them at the base of continuous culture models (chemostat).

Piecewise-linear (PWL) model

To derive the growth equation, we distinguish two different regimes of growth: a) *free growth* in which the growth rate is constrained by some internal reaction, while the limiting substrate is available to the cell in a sufficient or excessive amount; and b) *exerted growth* in which external substrate level limits and controls the growth rate. Such a distinction is not itself new (Debes et al. 1973), but it needs some further formal justification.

A main model assumption accepted here states that the growth rate of a cell population is proportional to the number of cells which are not affected by substrate shortage, at any given time of the two regimes. Mathematically, this implies that, instead of the traditional assumption of proportionality of dx/dt to the product xs, we introduce proportionality of dx/dt to a logical product

(disjunction) $\{x\} \cap \{s\}$, where $\{x\}$ is the total number of cells and $\{s\}$ is an available number of all substrate "portions", each being equal to the substrate quantity consumed by a normally developing cell at unit time. Thus, one can write:

$$\frac{dx}{dt} = k \min(x, Ws), \qquad (3)$$

where the constant W, $W \leqslant Y$, has the same dimension as Y and can be interpreted as a yield coefficient of the exerted growth. Substituting (2) into (3) we have eventually:

(v) $$\frac{dx}{dt} = k \min(x, b(g-x)) \qquad \text{(PWL model)}$$

where $b = W/Y$. Solving (v), one obtains the following continuous and continuously differentiable function:

$$x(t) = \begin{cases} x_0 \exp(kt), & \text{when } t \leqslant t_p, \\ g - (g - x_p) \exp(-kb(t - t_p)), & \text{when } t \geqslant t_p. \end{cases} \qquad (4)$$

where

$$x_p = \frac{bg}{1+b}, \qquad t_p = \frac{1}{k} \ln \frac{x_p}{x_0}. \qquad (5)$$

Function (4) being plotted represents a sigmoid (S-shaped) concave-convex curve, skew-symmetrical with regard to point (5) at $W = Y$, and asymmetrical at $W \neq Y$. Point (5) is similar to point of inflexion $d^2x/dt^2 = 0$ of models (i)-(iv). The concave part of (4) (when $t \leqslant t_p$) corresponds to the free growth whereas the convex one (when $t \geqslant t_p$), to the exerted growth.

Experimental data

(1) Thornton's data. These six-point data demonstrate the increase of the area occupied by a growing bacterial colony (*B. dendroides*) and are taken from Feller (1940) who referred to Lotka (1925). Time was measured in days and the area, in square centimetres.

(2) Dunaliella data. Our own three experiments made with the halotolerant green unicellular alga *Dunaliella parva* (Lerche strain, No 19/9) in batch were performed in parallel (under identical conditions, differing one from another only by initial concentration (inoculum)) and lasted two weeks. The numbers obtained are presented in Table 1, time (t) given in hours, and concentration of the culture, (x), in Klett Units (KU), for it was measured by means of the Klett photometer. The composition of the medium and the environments used in the experiments are described elsewhere (Ginzburg and Ginzburg 1981).

Analysis and comparison

A nonlinear least squares iterative procedure was used to determine the constants of best fit for the five forms tested against the data. The quantity being minimized

Table 1. Growth of *Dunaliella*

(t) Hours	(x) Optical density Klett units		
	Experiment I	Experiment II	Experiment III
0	1.0	6.0	14.0
24	10.5	26.0	38.7
43	26.2	48.0	57.5
96	52.7	65.0	61.5
120	62.7	74.5	65.0
144	67.7	82.5	65.0
168	71.5	84.5	
192	75.2	87.7	
264	75.0	91.7	
336	78.7	100.2	

was the sum of the squares of errors:

$$Q = \sum_{i=1}^{N} (x_i - f(t_i))^2, \tag{6}$$

where N = number of observed data points (t_i, x_i), and f is the fitting function. Since t_i and x_i are known constants, then Q becomes a function of growth parameters $(k, q, $ etc.$)$ which are to be found in the process of minimization. Denoting those parameters p_i (i.e., $p_i = k$, $p_2 = q$, p_3 = third parameter if it exists; $p_4 = x_0$), one can reduce the problem of minimization (6) to numerical solution of the following set of transcendental equations:

$$\frac{\partial Q}{\partial p_i} = 0, \qquad i = 1, 2, (3,) 4 \tag{7}$$

This classical technique was applied, but proved to yield negative values of p_1 and p_3 for Monod's model. Furthermore, since for the Monod case one has to solve transcendental equation (1c) with respect to x on each iteration and for each observed point, the results have to be substituted into (6), then this numerical procedure becomes exceptionally tedious and the set of Q obtained can hardly be regarded as reliable in the comparison, especially because of probable accumulated errors. Therefore, the problem of minimization of (6) was reformulated as a problem of dynamic programming with constraints $p_i > 0$, $i = 1, 2, 3, 4$ (except for $i = 3$, for the Richards form) and a special algorithm similar to the simplified gradient method was developed and used (see Appendix).

Table 2 shows the results corresponding to the best fit to each of the five models for four sets of data. The arbitrary constants (k, g, third parameter, and x_0) are given along with the sum of the squares of errors, Q. The columns headed (t_p) and (x_p) represent coordinates of point of inflexion of the growth curve for the first four models, and the coordinates given by equalities (5), for the PWL model, so that d^2x/dt^2 in any case changes its sign at $t = t_p$. It should be kept

Table 2. Comparison of the models. Best fit of the four data sets against the logistic, Gompertz, Monod, Richards, and piecewise-linear (PWL) models

Thornton's data	k div/day	g cm^2	Third constant[a]	x_0 cm^2	t_p day	x_p cm^2	Q cm^4
Logistic	1.9593	49.88	–	0.370	2.499	24.940	0.1275
Gompertz	1.2040	52.15	–	4.10	2.197	19.186	7.8078
Monod	2.7434	49.05	43.36	0.850	2.711	29.111	1.3870
Richards	1.9811	49.83	1.016	0.370	2.502	24.991	0.1171
PWL	1.5078	51.80	0.691	0.649	2.310	21.167	1.8191
Dunaliella data	div/h	KU		KU	hour	KU	KU^2
(I)							
Logistic	0.03339	75.78	–	6.40	71.38	37.89	75.858
Gompertz	0.02275	77.15	–	3.16	51.06	28.38	24.609
Monod	0.08915	75.33	164.4	7.29	78.72	41.21	98.840
Richards	0.01815	78.27	−0.489	0.26	35.92	19.83	13.154
PWL	0.07745	79.34	0.188	1.54	27.08	12.54	15.145
(II)							
Logistic	0.02500	93.37	–	16.97	60.18	46.68	315.01
Gompertz	0.01832	94.85	–	13.23	37.01	34.89	191.84
Monod	0.21901	93.40	789.2	17.60	63.08	48.01	330.05
Richards	0.01126	99.10	−1.000	6.64	0.00	6.64	70.41
PWL	0.15685	99.10	0.072	6.64	0.00	6.64	70.41
(III)							
Logistic	0.07581	65.01	–	13.18	18.06	32.50	13.824
Gompertz	0.05221	64.55	–	13.18	8.87	23.75	20.959
Monod	0.07010	63.73	29.59	14.76	26.24	40.77	9.684
Richards	0.09448	65.00	1.788	13.96	22.26	36.63	11.866
PWL	0.04244	63.95	1.658	13.99	24.69	39.89	7.562

[a] i.e., $a = KY$, for the Monod form: cm^2, KU; m, for the Richards form: divisions; $b = W/Y$, for the PWL form: divisions

in mind that Richards' "flexible form" will always produce a fit not worse than the logistic and Gompertz ones.

(1) Thornton's data. These data were often quoted as a support for the logistic form, the agreement being considered as the best available or even possible (Lotka 1925; Feller 1940). Quite naturally, the Richards form fits the data better (see Table 2). But it must be emphasized here that four out of the five models, namely (i) and (iii)–(v), fit these data extraordinarily well. This result does not seem too unexpected, since, firstly, Thornton's data actually lie very accurately along the S-shaped concave-convex symmetrical curve and, secondly, they consist of only six experimental points whereas each model contains three or even four arbitrary constants to be varied. And even the Gompertz model which showed the worst fit (being the only one asymmetrical in principle) can be regarded as

appropriate and applicable to Thornton's data. Indeed, an average daily increase (DI) of the bacteria colony was 8.19 cm^2, whereas an average difference between an observed and a computed point,

$$d = \frac{1}{N} \sum |x_i - f(t_i)|,$$ (8)

was, for the Gompertz form, much less: $d = 1.00$ cm^2, so it can be considered acceptable. Comparing values of d for the logistic and the Richards forms (0.11 and 0.13 cm^2) and, then, for the Monod and the PWL ones (0.41 and 0.49 cm^2), we should conclude that differences between the pairs hardly exceed errors of measurement.

(2) Dunaliella data. In our first biological experiment, an S-shaped growth curve was obtained. It has a very short (some 30 h) concave part (free growth) and a long (about 300 h) convex part (exerted growth). Average daily increase was 5.55 KU, and both concave and convex parts were relatively slightly sloping, so the computations according to the models with fast saturation, namely the Monod and the logistic ones, produced worse agreement with the observed data. (Notice that the greater $a = KY$, the closer Monod's model to the logistic.) The best fit with the experimental data was obtained for the Richards and the PWL models, which must be considered here as yielding undistinguishably good approximation, since d for them equals to 0.92 and 0.98 KU, correspondingly.

In our second biological experiment, a purely convex curve was obtained, i.e. a curve with $x_0 = x_p$, $t_p = 0$. Obviously, the first three models should not be expected to describe this pattern of growth since they inevitably possess some concave part. This prediction was quite well confirmed by computations which produced t_p unequal to zero and large Q, for the logistic, Gompertz and Monod forms. In this experiment, DI = 6.73 KU, i.e. it has the same order as d which was equal to 4.01, 3.29 and 4.01 KU, respectively, so all the three forms should be regarded here as inapplicable. The best agreement was found for the Richards and PWL models. Not surprisingly, the computed curves for them coincided, since at $m = -1$ the Richards model gives the same mathematical form as the PWL one.

In our third biological experiment, a steep concave-convex curve was obtained. Since DI = 8.50 for it and, therefore, saturation was fast, the computations along logistic and Monod models gave comparatively good approximation. In this particular case, the fit obtained for each model should be considered as acceptable but the best agreement was exhibited by the PWL model.

Discussion and conclusion

Proceeding from the above comparison and analysis, one can at least assert that the PWL model proved to be a good approximation tool for some research and practical needs. For two out of the four data sets involved, the PWL model showed an agreement with the experiments which was not inferior to the Richards form which is usually regarded as the most flexible one-dimensional growth

model. We have also shown that only the Richards and PWL models, out of the five models analyzed, can describe purely convex growth curves. And when choosing between them, one should give preference to the PWL model, even if Q corresponding to it is a little greater, since it is just the biologically unreliable parameter m which yields flexibility to the Richards form. On the other hand, the PWL model has the same number of arbitrary parameters which have very transparent biological meanings. Moreover, the PWL model was derived directly from the mass conservation law, therefore it can be readily used as a basis of the chemostat theory. At last, mathematically it is simpler than the Richards form.

As concerns two other forms based on the mass conservation law (2), i.e. the logistic and the Monod ones, both were found to be inapplicable to the second *Dunaliella* experiment (purely convex curve) and fitted the two remaining sets of *Dunaliella* data worse than the PWL model. Notice that in the three *Dunaliella* experiments, a probable limiting factor was carbon (from $NaHCO_3$ of the medium or/and from atmospheric CO_2). Since carbon constitutes about 50% of dry weight of the cell, and optical density of 1 KU corresponds roughly to 2.5 mg/litre of the dry weight (Ginzburg and Ginzburg 1985), one can estimate magnitudes of the effective Michaelis constant, K, as some 205, 986 and 36 mg/litre, correspondingly to $a = 164.4$, $=789.2$, $=29.59$ KU listed in Table 2. In contrast, $K = 12$ mg/litre for *Escherichia coli* in Monod's experiment (1942) (according to Dabes *et al.* 1973). In any case, since the constant $a = KY$ is expected to be much less than g, the Monod model can hardly be regarded as applicable to *Dunaliella* data I, II and III. At a so large, the constants k and K obviously lose their usual biological meaning.

The Gompertz form produced a worse fit to all the four data sets as compared with the PWL and Richards forms. It has also shown worse agreement than the logistic and Monod forms in describing steep symmetrical curves (Thornton's data, *Dunaliella* data III), but, on the other hand, it had an advantage over those two forms in the interpretation of asymmetrical and slightly sloped curves (*Dunaliella* I and II).

Appendix

For minimization of (6), the following numerical procedure was used. To initiate the iterative process, a feasible solution $p_{10}, \ldots, p_{40}$ (usually, that found as a solution of the classical approach (7)) is to be chosen, $Q_0(p_{10}, \ldots, p_{40})$ computed and steps h_i, each with respect to each parameter p_i, assigned. Then the following values,

$$\min Q_0(p_{10} \pm n_1 h_1, p_{i0}), \ldots, \min Q_0(p_{i0}, p_{40} \pm n_4 h_4)$$

are to be computed, where $n_i = 0, 1, 2, \ldots$, and the minimum among them is assumed to be $Q_1(p_{11}, \ldots, p_{41})$. This process continues until $n_1 = \cdots = n_4 = 0$, at some sth iteration, when Q_s is considered as $Q_{\min}$ for the model in question, and $p_{1s}, \ldots, p_{4s}$, as the optimal set of parameters. In fact, this iterative algorithm examines the neighborhood of a global feasible minimum, which function (6) always possesses. A corresponding program was written for the personal computer Apple II (in Basic).

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