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# Mathematical modelling of biosystems exemplified by investigation of microbial population growth

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This paper has arisen as a result of teaching Models in Biology to undergraduates of Bioengineering at the Gediminas Technical University of Vilnius. The aim was to teach the students to use a fresh approach to the problems they are familiar with, to come up with an articulate verbal model after a mental effort, to express it in rigorous mathematical terms, to solve (with the aid of computers) corresponding equations, and, finally, to analyze and interpret experimental data in terms of their (mathematical) models. Investigation of microbial growth provides excellent possibilities to combine laboratory exercises, mathematical modelling and model-based data analysis. Application of mathematics in this field proved to be very fruitful in getting a deeper insight into the processes of microbial growth. The step-by-step modelling resulted in an extended model of the growth covering conventional "lag", "exponential" and "stationary" phases. In contrast to the known models (differential equations which can be solved only numerically), the present model is expressed symbolically as a finite combination of elementary functions. The approach can be applied in other areas of modern biology.

**Key words***:* Verhulst equation, logistic equation, growth rate, generation time, lag time

## INTRODUCTION

Both microbiology and microbiological techniques are widely used in modern biochemistry. In fact, microbiology is indispensable in biochemistry, molecular biology, gene engineering, biotechnology, biochemical education. Biochemistry students are expected to be computer-literate and are supposed to have the basic mathematical knowledge. In teaching biochemistry, investigation of microbial population growth provides excellent possibilities to combine laboratory exercises, mathematical modelling, qualitative and quantitative (model-based) data analysis. Such studies (not being highly demanding for material resources or skills, or special knowledge of mathematics) can be easily conducted while experience gained in these exercises and modelling will be very useful in other areas.

At the Gediminas Technical University of Vilnius, undergraduates of Bioengineering have a course of Models in Biology concurrently with other programmes. As a result of teaching (and learning), they are expected to use a fresh approach to the problems they are familiar with, to come up with an articulate verbal model after a mental effort, to express it in rigorous mathematical terms, to solve (with the aid of computers) corresponding equations, and, finally, to analyze and interpret experimental data in terms of their (mathematical) models.

Here, step-by-step modelling of microbial growth and model-based analysis of experimental data are presented. The known models are extended on the basis of reasonable assumptions. The extended model is new (never been published before) and rather simple. It can be extended further on. The way of getting such a model is of interest far beyond the area of teaching microbiology.

## MATHEMATICAL MODELLING

It is clear that at any moment, $t$, the (absolute) rate of population growth, $a$, is proportional to the population size, $A$, (number of cells, total mass, (optical) density, etc.), i.e.

$a = \alpha A$.

the coefficient of proportionality, $\alpha$, being a relative rate of population growth (or specific growth rate). The statement above (a verbal model of growth, as a matter of fact) can be expressed mathematically in the form of differential equation:

$$\frac{\mathrm{d}A}{\mathrm{d}t} = \alpha A, \qquad (1)$$

The specific growth rate, $\alpha$, depends on the environmental conditions, and it may be considered that

$\alpha = \alpha_0$ ($\alpha_0$ = const) (2)

as long as the conditions remain unchanged. With the growth of population size (density) the conditions may change because of the growth: first of all, the organisms may have to compete for available resources. Let the initial supply of the resources equal to 1; this supply may be supposed to be depleted proportionally to the population size, the availability of resources, the remaining $(1 - A/B)$ and, as a consequence, the relative rate of growth being, therefore,

$$\alpha = \alpha_0\left(1-\frac{A}{B}\right), \tag{3}$$

We can see that $\alpha = 0$ when $A = B$. For $A << B$, Eq. (3) is reduced to Eq. (2).

Solution of Eq. (1), taking into account Eq. (2) or Eq. (3), results either in the exponential or logistic (Verhulst) model. In defining the parameter $\alpha$ in Eq. (1), other reasonable considerations (beyond Eqs. (2) and (3)) can be taken into account, giving rise to new models.

## LABORATORY EXERCISES AND MODEL-BASED DATA ANALYSIS

Obtaining viable count data being time-consuming and expensive, we used a more rapid method of accumulating the data. We were measuring the optical density (OD) of microbial culture, OD being related to the turbidity or density of the culture. The culture (*Escherichia coli*) was grown in 100 ml of Luria–Bertani medium in 1 l flasks under conventional conditions (37 ˚C, agitated at 150 rpm).

Typical student-generated microbial growth data are presented in Fig. 1 (open circles). Conventionally, for analysis of such data the growth curve is divided into separate phases [1–3]; more promising seems using the above models directly. The exponential model (curves *a*)

$$A = A_0 e^{\alpha_0 t}, \tag{4}$$

(which is the solution of Eq. (1) taking into account Eq. (2) under the initial condition $A(0) = A_0$ ($A_0$ being the inoculum size)) is quite close to experimental data points at the beginning of growth and suggests the tendency which, however, may hardly be considered as a separate "exponential phase" since the model deviates considerably from further experimental data (see Fig. 1 *a*). However, before discarding the model, let us note that according to it the population doubles at a moment $T_0$, i.e.

$$A_0 e^{\alpha_0 T_0} = 2A_0, \tag{5}$$

from where (taking the natural logarithm of both sides of the equation)

$$T_0 = \frac{\ln 2}{\alpha_0}. \tag{6}$$

Parameter $T_0$ is called the generation time.

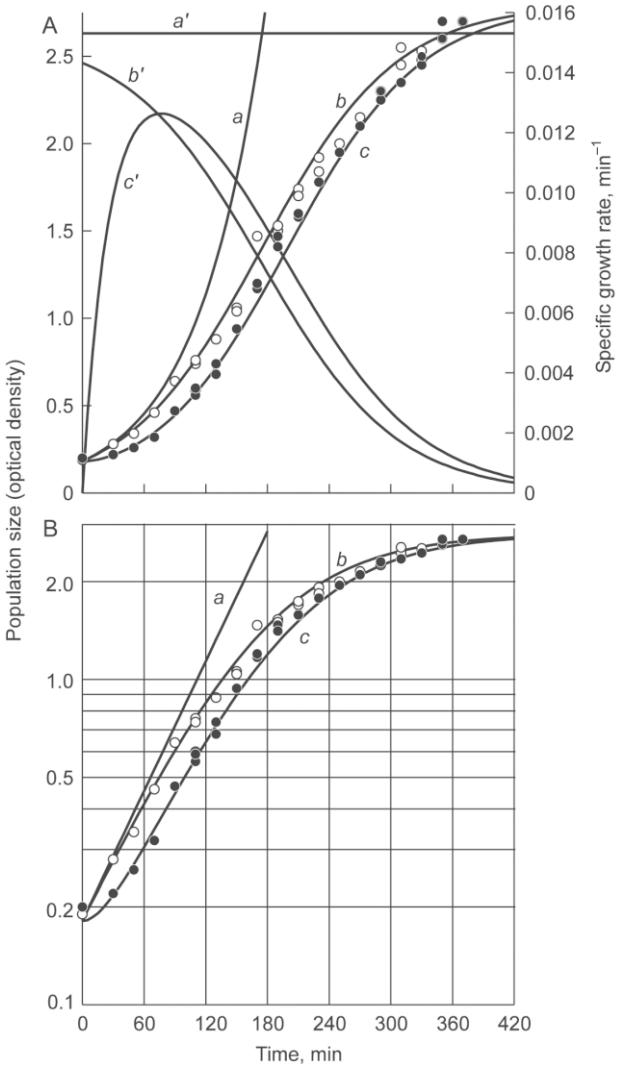


**Figure**. Time-course of microbial growth and growth rate in linear (A) and semi-logarithmic (B) scale. Circles symbolize experimental data points (the open ones correspond to routine experiment and the closed ones to an experiment performed after keeping bacteria in an ice bath for 2 h); *a* and *a'*, model of unrestricted growth (MUG), *b* and *b'*, model of limited growth (MLG), *c* and *c'*, extended model (EM). $A_0 = 0.18$, $B = 2.8$, $\alpha_0 = 0.0153$ min$^{-1}$ (shared by both models), $\beta = 0.040$ min$^{-1}$ (used in EM)

Solution of Eq. (3) under the initial conditions as above is

$$A = \frac{A_0 e^{\alpha_0 t}}{1+\frac{A_0}{B}\left(e^{\alpha_0 t}-1\right)} \tag{7}$$

(see Figure, *a*'. While appearing rather complicated, the model can be visualized directly in Maple worksheet as a graph of population size vs time). Note that the numerator of the model is the right-hand part of Eq. (4). One can see that $A \to B$ for $t \to \infty$, hence the meaning of parameter $B$: it is the asymptotic value of population size. It would be reasonable, therefore, to refer to Eq. (7) as a Model of Limited Growth (MLG), in contrast to Eq. (4) which might

be designated a Model of Unrestricted Growth (MUG). For $B >> A_0$, MLG is close to MUG and for $B \to \infty$ it is reduced to MUG (see also Fig. 1).

Once a model has been accepted as "good enough" to approximate experimental data, all the information contained in the data should be considered to be contained (in a generalized mathematical form) in the model, there being no need ever to come back to the data: everything of interest can be derived from the model.

The (absolute) rate of population growth, i.e. the rate of change in population size, is the first derivative of the latter (the right-hand part of Eq. (7)); this rate divided by the population size (the right-hand part of the same equation) yields the time-course of the relative (or specific) growth rate:

$$\alpha = \alpha_0 \frac{(B - A_0)\mathrm{e}^{-\alpha_0 t}}{(B - A_0)\mathrm{e}^{-\alpha_0 t} + A_0} \tag{8}$$

(see Figure, curve *b*').

Whereas several factors causing the decline in population growth are pointed out in textbooks [1–2], as mentioned above, only one has been taken into account in Eq. (3), namely, competition for available resources: this is sufficient to cause the decline.

As stated above, experimental data obtained by the students as a result of their laboratory exercises can be reasonably approximated by Eq. (7). The "lag" (if any) is supposed to reflect the time necessary for the microorganisms to adapt to the new environment after their transfer from the storage medium. It seems reasonable to assume that the period of time necessary for the adaptation depends on the contrast between the old and the new environments. The next experiment was based on this assumption.

## MODEL EXTENSION

To get an experimentally observable "lag", the microorganisms were kept before inoculation under 0 °C (in ice bath) for 2 h, the other conditions being the same. The data are presented in Fig. 1 (closed circles). To approximate these data, the MLG had to be extended.

Let us assume that the microbial population from the moment of change in the environment (i.e. the moment of inoculation) proceeds from state D ("dormant") to state G ("growing"),

$$\mathrm{D} \xrightarrow{\beta} \mathrm{G}; \tag{9}$$

let $d$ and $g$ denote the relative size of the population in respective state; $\beta$ is the rate constant (or relative rate) of the transit.

This transition is independent of the growth (the organisms undergoing the transition and those taking part in the growth are not the same), so the temporal increase in $g$ can be modelled independently. There are two unknowns, $d$ and $g$, and it is clear that $d + g = 1$. This makes one of the two equations of the system necessary to find these unknowns; another equation is based on the rate of the transit. So, the system is

$$\begin{cases} d + g = 1, \\ \dfrac{\mathrm{d}g}{\mathrm{d}t} = \beta d. \end{cases} \tag{10}$$

The solution of this system with respect to $g$, taking into account the initial condition (let $g(0) = 0$ (in general, $0 \le G \le 1$), i.e. at the moment of inoculation all the population is dormant) results in

$$g = (1 - \mathrm{e}^{-\beta t}). \tag{11}$$

Now Eq. (1) can be modified taking into account both Eq. (3) and Eq. (11):

$$\frac{\mathrm{d}A}{\mathrm{d}t} = \alpha_0 \left(1 - \mathrm{e}^{-\beta t}\right)\left(1 - \frac{A}{B}\right)A. \tag{12}$$

The solution of the above equation is

$$A = \frac{A_0 \, \mathrm{e}^{\alpha_0 t + \frac{\alpha_0}{\beta}\mathrm{e}^{-\beta t}}}{\mathrm{e}^{\frac{\alpha_0}{\beta}} + \frac{A_0}{B}\left(\mathrm{e}^{\alpha_0 t + \frac{\alpha_0}{\beta}\mathrm{e}^{-\beta t}} - \mathrm{e}^{\frac{\alpha_0}{\beta}}\right)}, \tag{13}$$

where $A_0$, as above, is inoculation size comprised of the cells residing in "dormant" state. As can be seen, for $\beta >> \alpha$ Eq. (12) is reduced to Eq. (7). Similarly, for $B \to \infty$ it is reduced to Eq. (4).

Equation (13) was fitted to the experimental data without any significant deviation (Fig. 1, curve *c* and closed circles), therefore it can be considered to approximate experimental data adequately. The extended model (EM), therefore, being more general than MLG, in addition to the latter covers also the "lag phase". By analogy with Eqs. (7) and (8), the dynamics of the relative growth rate can be determined for EM as well (Figure, curve *c*').

It is worth noting that the EM (Eq. (13)) is rather simple. First of all, it is expressed as a finite combination of elementary functions. The other features of the model will be discussed below.

## DISCUSSION

The modelling presented here is successful mainly because of a disregard of the conventional division of microbial growth into separate "phases". The "lag phase", however, cannot be just ignored, since it is of great importance in food microbiology. Estimation of the "lag", as well as its incorporation into growth equations, is bound, however, with serious difficulties. The reason for these difficulties, as Baty and Delignette-Muller put it,

"is the lack of physiological understanding of the lag phenomenon. In actual fact, little knowledge is available concerning this physiological stage and only few authors were able to put some biological information about λ [the "lag" duration] into model equations. The second reason is related to the first one and comes from the fact that the actual definition of λ is either purely geometric or purely mathematic" [4].

The "lag" is caused, presumably, by the inability of microorganisms residing in state D ("dormant") to take part in the growth of the population, the duration of the "lag", λ, being determined by the duration of residence of the organisms in state D. The transition D → G, is presumably a random process, the duration of residence of an individual organism in state D being a random value whose probability distribution can be found in the following way.

The relative number of the organisms, *d*, residing in state D in the course of time is the solution of Eq. (10) with respect to *d* taking into account the initial conditions. Let $d(0) = 1$ (i.e. at the moment of inoculation all the organisms are in state D). Thus,

$$d = e^{-\beta t}.$$

This solution, multiplied by β, may be therefore considered as a density of probability distribution of the duration of residence of the organisms in state D.

The mean duration of residence of the organisms in this state, therefore, is

$$\int_0^\infty t \cdot \beta e^{-\beta t} \, dt = \frac{1}{\beta}.$$

This mean can be expected to be equal to the "lag", i.e.

$$1/\beta = \lambda. \qquad (14)$$

Model parameters, including β, were estimated as a result of fitting the models (both MLG and EM) to the experimental data. There is a good agreement of the models with the data. The temporal shift of EM with respect to MLG, as expected (see Fig. 1 B), appears to be equal to parameter λ. Hence the geometrical definition: the "lag" is the shift of EM with respect to MLG. Equation (14) can be considered as a probabilistic definition of the "lag".

The known models developed to simulate the "lag" phase [5–7] are rather complicated, mainly because of the problems related to the nature of the "lag" phenomenon mentioned above. The corresponding equations can be solved only numerically.

## CONCLUSIONS

Investigation of microbial population growth provides excellent possibilities to combine laboratory exercises, mathematical modelling and model-based data analysis. Application of mathematics proved to be very fruitful in getting a deeper insight into the processes of microbial growth. The step-by-step modeling resulted in an extended model of the growth covering conventional "lag", "exponential" and "stationary" phases. In contrast to known models (differential equations which can be solved only numerically), the present model is expressed symbolically as a finite combination of elementary functions. The approach can be applied in other areas of modern biology.

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## BIOLOGINIŲ SISTEMŲ MATEMATINIS MODELIAVIMAS, ILIUSTRUOJAMAS MIKROORGANIZMŲ POPULIACIJOS AUGIMO TYRIMAIS

Santrauka

Šiame darbe bendrų samprotavimų pagrindu formuluojami žodiniai mikroorganizmų populiacijos augimo modeliai, kurie toliau išverčiami į matematinių sąvokų bei simbolių kalbą. Matematiniai modeliai, išreikšti diferencinėmis lygtimis, sprendžiami plačiai naudojamomis kompiuterinėmis programomis. Pirmasis pateiktas modelis (neriboto augimo) tik apytiksliai atspindi augimo tendenciją, ne visuomet suderinamas su bandymų duomenimis, tačiau pedagoginiu požiūriu yra paprastas ir aiškus. Antrajame (riboto augimo modelyje) atsižvelgiama į tikėtinas augimo greičio mažėjimo priežastis; šis modelis visiškai suderinamas su bandymų duomenimis, kai augimas nevėluoja. Trečiajame (išplėstiniame) modelyje atsižvelgiama į tikėtinas augimo vėlavimo priežastis; jis vienodai gerai suderinamas su bandymų duomenimis, gautais tiek augimo vėlavimą lemiančiomis sąlygomis, tiek ir kai pastebimo vėlavimo nėra. Šis modelis apima pirmuosius ir yra naujas, anksčiau neskelbtas. Jo kūrimo eiga yra visiškai prieinama studentams, tad jis gali būti įtrauktas į mokymo kursą, gali būti plečiamas toliau. Čia išdėstyta modeliavimo eiga taikytina ir kitose šiuolaikinės biologijos srityse.