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Review Article

Features of the Search for Mathematical Models of the Kinetics of Microorganism Cultivation

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Received: October 22, 2020; **Published:** September 30, 2021

Abstract

Approaches to modeling the kinetics of cultivation of microorganisms in modern processes of food biotechnology of products from plant raw materials are presented. The necessity and urgency of mathematical modeling of biotechnological processes in modern conditions of maximum automation and computerization of production is substantiated. Physical, chemical and biological processes are described in detail on the example of the cultivation of microorganisms in a real fermentation plant. Difficulties and peculiarities that can be encountered when constructing mathematical models are noted. Separately at the initial stage of modeling - when setting the problem - and in the process of solving. Several examples of searching for mathematical models for describing the kinetics of biological processes, both empirical and semi-empirical, are considered. Discussion of the issue, conclusions and recommendations can be useful not only for researchers, but also for specialists in the field of design of technological processes.

Keywords: *Experimental Data; Biological Processes; Empirical and Semi-Empirical Methods; Mathematical Model*

Introduction

The great scientist Academician of the USSR Academy of Sciences A.A. Samarsky once very accurately formulated the following: "Construction of mathematical models is a kind of art where knowledge of theory, experience, and intuition are closely intertwined" [1, p. 28]. The search for mathematical models of the kinetic regularities of the development of cells of microorganisms is a difficult, interesting and necessary task. Already when setting this problem, many have questions that require an answer. The first question sounds like this: why do we need a mathematical model, what does it give? Second, what should be a mathematical model of a biological process and what is needed to find it? And the third, perhaps the most important question: what is the complexity of the task? It is rather difficult to give any unambiguous and short answers to the questions posed, so we will try to answer them in more detail.

The first question most often arises either among young people who are just starting their way in scientific activity, or among people whose scientific work was little connected with the mathematical processing of experimental data. But here an additional question arises: what is science? The answer to it can be found in any encyclopedic dictionary, roughly in the following form - this is the sphere of people's activity in the accumulation, systematization and analysis of the knowledge gained. But knowledge can be different. Specifically for the biological field of knowledge, the definition of the outstanding Russian biologist K.A. Timiryazeva: "Knowledge as a means is an art; knowledge as a goal is science" [2, p. 38]. And further, developing his thought, Timiryazev gives an example of the development of medicine from "crude empiricism" to the knowledge of the laws of development of living organisms.

Thus, the main task of science is to understand the laws of the world around us. Scientists should be interested not only in the questions - what and how (of course, very important), but the main question - why this or that phenomenon occurs. Knowing the answer to the question "why" makes it possible to predict the course of various processes.

Physical, chemical and biological processes during the cultivation of microorganisms

In the case of food biotechnology, an example is from the field of brewing. Beer was brewed several centuries ago without any science. And its quality depended on the skill of the brewer who knew how to brew beer. He received knowledge by inheritance, not wondering why beer is obtained of this quality, and not of another, and even more so he could not predict its sensory properties. I must say that in recent years, attempts have been made to solve this problem. An example of this is the doctoral dissertation of A.T. Dedegkaev [3].

Since all biological processes occur in time, their kinetic regularities are of interest, first of all. To predict the course of a biological process in time, one must have either a graphical representation of the kinetics of the studied process, or equations (equation) of kinetics, which can become a mathematical model of the studied biological process.

So, what is a mathematical model for? The need to search for mathematical models is dictated by several reasons:

- Firstly, the high rates of development of microbiological industries, increased requirements for the calculation and design work of technological processes and equipment for their implementation, taking into account the high degree of automation and computer control. It will be difficult to comply with them without data on the kinetics of the development of the cell population and mathematical models that adequately reflect the course of biological processes;
- Secondly, the involvement of the mathematical apparatus in the study of biological processes will allow us to delve deeper into the essence of material and energy exchange between the culture medium and the cell, predict this exchange and outline ways to intensify processes;
- Thirdly, a well-developed mathematical model can serve as a tool for a computational experiment in order to determine the dependence of the rate of a biological process on changes in certain parameters that are important for the development of cells (temperature and pH of the environment in which they develop, the value of the initial inoculation of a pure culture, and etc).

Before answering the second question, what should be a mathematical model and how to search for it, let's return to the beginning of the article. The expression belongs to Academician of the USSR Academy of Sciences A.A. Samarsky, working in the field of nuclear energy. The word "intuition" attracts attention in it. If nuclear physicists, for whom everything is subject to the strict laws of internuclear and intranuclear interactions, started talking about intuition, then what is left for biologists to do?

In biological sciences, in our opinion, the word "intuition" should be put in the first place. The fact is that when studying the kinetics of development of a cell or a population of cells of microorganisms in any branch of the industry related to microbiology, one has to deal with many biological processes occurring in time and simultaneously. These include processes taking place both inside the cell and in the surrounding culture medium. For example, the transport of nutrients from the culture liquid to the cell surface and further into it; transport of metabolic products from cells to their environment; removal of biological heat released as a result of cell activity; during aerobic cultivation of microorganisms, the processes of oxygen dissolution in the culture liquid and its transport into the cell, etc. are added.

The presence in any specific large biological process (fermentation of beer wort or the cultivation of baker's yeast) of many private, functionally interconnected biological processes, predetermines the general form of the mathematical model and its complexity. Each of the particular processes will be described by its own equation of a certain type, which should adequately reflect the flow and the influence on its speed of certain factors noted earlier. Thus, the general mathematical model will be a system of equations, the complexity of which will be determined by the number of technological parameters included in it.

One more circumstance must be borne in mind: any general biological process takes place in a certain apparatus, therefore, the general model should reflect the features of its design. The task, as you can see, is extremely difficult. Here we move on to answering the third difficulty question.

The first difficulty lies in the fact that the rates of biological processes depend on many factors, the functional relationship between which should be established by a mathematical model. Here is the state of the environment in which the cells develop (temperature, pH, the presence of necessary nutrients in it, etc.), and the state of the cells themselves, and the type of strain, and the hydrodynamic environment in the cultivator, etc. But the most important, in our opinion, is the multiphase factor of the environment in which the cells develop. Now we will restrict ourselves only to the conditions of anaerobic and aerobic development of cells in a liquid medium on the examples of the development of the yeast *Saccharomyces cerevisiae*, clarifying what is meant by the concept of "anaerobic fermentation" and "aerobic".

The fermentation process can be anaerobic in two ways. The first is when oxygen does not get into the fermenter from the outside in any way. This is possible when it is fully grooved and carbon dioxide is dumped out through the water seal.

The second option is feasible in the case when the apparatus is connected to the atmosphere, and atmospheric oxygen is natural, i.e. without forced feeding, due to molecular diffusion it can enter the wort through its free surface. Since the rate of oxygen diffusion is less than the rate of its consumption by cells, only the upper layers of the wort will be aerated. If in these layers the oxygen concentration is higher than a certain critical value, below which oxygen ceases to penetrate into the cell, then the process in the upper layers can be considered aerobic. In the layers below, the fermentation process will be anaerobic. According to previous studies, for various strains of the yeast *Saccharomyces cerevisiae*, this critical concentration is below 0.1 mg/l [4].

The physical model of the environment in which the cells of microorganisms develop, depending on the problem being solved, can be reproduced in different ways.

In anaerobic cultivation, the culture medium can, in some cases, be presented as a single-phase suspension with certain thermophysical properties, consisting of liquid and solid particles (cells). It is assumed that there is no interaction between them. Such a model is applicable in calculating and researching the energy costs for transporting a suspension through pipes and heat transfer between it and the heat transfer surfaces of heat exchange elements.

When solving other problems, the medium is considered to be two-phase - the culture liquid-cell, where the liquid and the cell interact. This model is required in studies of heat or mass transfer between a cell and a culture liquid, which has its own thermophysical properties, and cells are a conditionally solid phase [4].

In aerobic cultivation with forced aeration, the physical model is a three phase system: liquid - cell – air.

The second difficulty in the search for mat models of bioprocesses is, in most cases, in the absence of differential equations describing the course of biochemical, heat and mass transfer processes inside the cell and the plasma membrane. There are only differential equations describing the kinetics of cell division. These include various kinds of equations of exponential [5,6] and power-law form [7].

In connection with these circumstances, the main material for the search for mathematical models of biological processes is the results of experimental studies. The use of modern computer programs in order to search for equations of mathematical models allows the experiment itself to be carried out more carefully and purposefully.

Physical processes (transfer of momentum, heat and mass) in devices of various technologies are modeled based on differential equations of Navier-Stokes, Reynolds and Fourier-Kirchhoff [8]. The equations describing the course of biological and biochemical processes inside the cell have not yet been obtained, therefore the search for mathematical models of microbiological processes is based on the last two options. The specified differential equations of Navier-Stokes, Reynolds and Fourier-Kirchhoff can be used with certain reservations to model energy and material exchange between a cell and its surrounding culture medium [4].

It should be borne in mind that differential equations are rarely solved up to the numerical value of constant coefficients. Most often, the coefficients included in the final equations are found or refined experimentally, that is, these solutions are not analytical, but semi-empirical.

Before starting the search for mathematical models, it is necessary to find out whether any empirical equation, which, even with one hundred percent accuracy, describes experimental results, can be considered a mathematical model of the studied process. The point is that any experimental data can be approximated with the same accuracy by several equations. The question arises which of the equations should be chosen. What requirements should a mathematical model meet? The opinions of various authors on this matter largely coincide, although the wording is different.

In our opinion, the model should meet several requirements. First, to adequately reflect the nature of the course of one or another process, which does not mean the exact coincidence of the experimental data with the values of the studied parameters calculated using the model equations. On the contrary, the absolute coincidence of the experimental data with the calculated ones raises doubts about the possibility of using the obtained equations as a mathematical model. The reason is that measurements cannot be error-free.

Secondly, the model must be able to predict the course of a biological process outside the experimental conditions. It must be said that meeting the second requirement can be more difficult than the first. In this case, much depends on how deeply the experimenter understands the problem, on the solution of which he is working. He must himself, even if intuitively, foresee the development of the process under study outside the experiment and, on the basis of this, choose one or another equation. Of course, there is a certain risk of being wrong here.

Thirdly, the equations of mathematical models, on the one hand, should be as simple as possible, but on the other, they should correspond to the experimental results with a certain degree of accuracy.

Fourthly, it is desirable to give all the empirical coefficients included in the equations of the mathematical model a certain physico-biological interpretation (sometimes it is difficult to do this), which will allow a deeper understanding of the processes taking place in the culture medium and the cell.

Let us consider several examples of the search for mathematical models for describing the kinetics of certain biological processes, both empirical and semi-empirical.

An empirical method for finding a model of biological processes

Example 1: It is necessary to select an equation that adequately reflects experimental studies on the change in the biomass concentration of the yeast *Saccharomyces cerevisiae* when cultivated on molasses solutions under oxygen deficiency. An example is taken from a variety of variants of work [7], for the case x_o = 3.7 kg/m^3 of yeast with 75% moisture content, the initial concentration of carbohydrates S_0 = 0.115 mass fractions and is shown in figure 1.

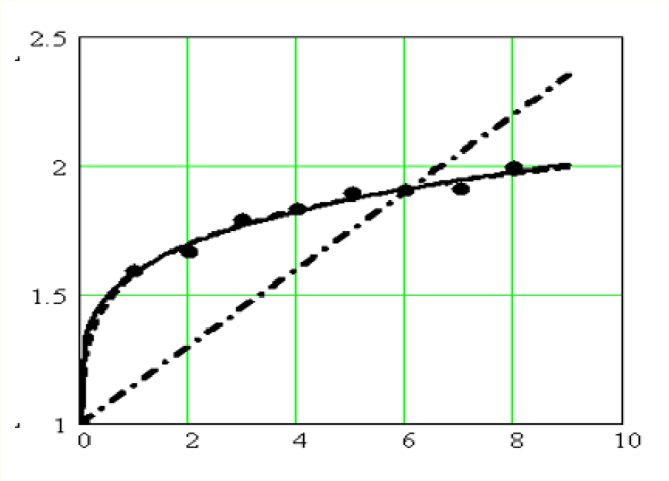

Figure 1: *Change in biomass concentration over time with oxygen deficiency: points correspond sto the experience; lines correspond to equations (1), (11), (12).*

As a supposed mathematical model, let us take, for example, an equation of a linear form that occurs in various versions and see if it meets our requirements:

$$x(\tau) = x_o + k\tau + a_1x_1 + a_2x_2 + a_3x_3 + a_4x_1x_2 + a_5x_1x_3 \dots, \quad (1)$$

Where x - current concentration of biomass:

τ - Cultivation time;

x_o - value of initial seeding,

k - a_1, a_2, a_3, a_4, a_5... - empirical coefficients,

x_1, x_2, x_3 - predetermined parameters affecting the kinetics of the process, for example x_1- the temperature of the medium, x_2- the atmospheric pressure, x_3 - the initial content of carbohydrates. If it turns out that one of the coefficients is significantly less than the others, then the influence of the corresponding parameter on the development of cells can be neglected. For example, if a_2 is significantly less than the other coefficients, then in further studies the influence of atmospheric pressure can be neglected.

In principle, the experimental data can be approximated by equation (1) with a sufficiently high degree of accuracy. Figure 1 shows that the approximation accuracy is not great. But when answering the question of whether equation (1) can adequately reflect the course of a real biological process, we must understand that we must make some conclusions only on the basis of experimental data on the change in biomass over time, even with graphs of these changes or some kind of equation, even if it describes the experimental data with one hundred percent accuracy, there are no grounds, since we are ultimately interested not in the form of the curve in the figure or the form of the equation describing it, but in the rate of the process, in other words, the time derivative of the function (1).

With constant values of all parameters, after differentiating equation (1), we obtain $dx/d\tau = k$. Thus, the rate of biomass growth is constant. If this contradicts the course of the process in real conditions, then equation (1) cannot serve as a mathematical model.

To what has been said, it is necessary to add one important remark: neglect of some parameters when studying the development of living things, in our opinion, is generally not permissible, since various factors at different times can influence the development of a living organism in different ways. Therefore, this method of modeling biological objects is also not acceptable.

Example 2: The example is fairly typical. The change of a certain value over time during the development of microorganisms, for example, the concentration of oxygen in the culture medium, is considered. On the graph, the experimental points with a sufficient degree of accuracy fit around the curve corresponding to the equation:

$$C_0 = a - b\tau + c\tau^2 \quad (2)$$

According to equation (2), the concentration of oxygen C_o decreases until a certain time and after reaching the minimum begins to increase. In fact, C_o tends to a constant value, i.e. equation (2) cannot predict the course of the process outside the experimental conditions and therefore cannot be accepted as a mathematical model. The researcher should choose a different equation as a mathematical model. Equation (2) is just an empirical relationship, suitable only for the conditions of the experiment.

Semi-empirical search method for models of biological processes

Semi-empirical models are based on various hypotheses based on the assumption of the continuity of cell division and proportionality between the increase in biomass per unit time and its concentration in the culture liquid, the cultivation time and can be mathematically expressed as a generalized differential equation

$$dx = kx^{m}\tau^{n} \cdot d\tau \quad (3)$$

Where k is the proportionality coefficient, as well as the exponents m and n, is found experimentally.

Depending on the value of the exponents m and n, one can obtain equations for various mathematical models of the kinetics of certain biological processes, while the coefficient k will naturally change. Let's consider several options for models.

The exponential model is obtained from equation (3) with m = 1 and n = 0. Under these conditions, it will take the known form:

dx = x.μ.dτ (4)

Integrating equality (4) in the range from x_0 to x and from τ = 0 to τ, we obtain the exponential equation

$$x = x_0 e^{\mu\tau} \quad (5)$$

In order not to be dimensionally dependent when processing experimental data, it is better to express the concentration of substances in the culture liquid in a dimensionless form, as the ratio of the current concentrations to the initial ones. In particular for the concentration of biomass - $x_b = x/x_0 = e^{\mu\tau}$. In the new notation, equation (5) takes the form

$$x_b = x/x_0 = e^{\mu\tau} \quad (6)$$

Where μ is the coefficient of proportionality, called the local specific rate of growth of biomass, depending on the same parameters that are included in equation (1). Therefore, equations (5) or (6) will be supplemented with equations that establish a functional relationship of μ with certain parameters, and the exponential model will be a system of equations. Thus, equation (5) is a special case of equation (3), but it is impossible to approximate the experimental data presented in figure 1 with it. Therefore, it is necessary to choose other models.

Power-law models. Model 1. Let us take n = 0 in equation (3) and represent it in differential form:

$$\frac{dx}{d\tau} = kx^{m} \quad (7)$$

The coefficient k_1 is similar to the specific velocity μ in equation (6). After integrating equation (7), within the same limits as equation (4), we will have a power-law equation:

$$x_b^{1-m} = 1 + \frac{(1-m)k}{x_O^{1-m}}\tau \quad (8)$$

Denoting the fraction in front of the brackets on the right-hand side of equality (8) by δ and carrying out simple transformations, we write:

$$x_b = (1+\delta\tau)^{\frac{1}{1-m}} \quad (9)$$

Expressing the exponent on the right-hand side of equality (9) in terms of m_1, we write

$$m_1 = \frac{1}{1-m} \quad (10)$$

Taking into account equality (10), equation (9) takes the form:

$$x_b = (1 + \delta \cdot \tau)^{m_1} \quad (11)$$

Analysis of equation (10) is of interest. It has already been said that for m = 1 the integration of equation (4) gives an exponential dependence (5). But when substituting m = 1 into equation (10), the function m1 (m) undergoes a discontinuity and m1 =. It is in the discontinuity zone that the exponential dependence (5) takes place. For m < 1, m_1 will always be greater than zero, and we get an equation of power-law form (11).

According to figure 1, equation (11) approximates the experimental results with a high degree of accuracy.

Model 2: For m > 1, m_1 will have a negative value, and we get an equation of a different form

$$S_b = \frac{1}{(1 + \delta_s \tau)^{m_{1s}}} \quad (12)$$

In this case, S_b it means any component of the culture liquid, the concentration of which decreases during the course of the biological process. In particular, equation (12) with a fairly high degree of accuracy approximates the experimental data on the change in the concentration of dry substances in the beer wort during fermentation [9].

The proportionality coefficient δ and the exponent m or m_1 are found experimentally.

Model 3: Consider a slightly different option, assuming in equation (3) m = 0 and n = 1. In this case, we obtain a differential equation of the form:

$$\frac{dx}{d\tau} = k_2 \cdot \tau^{n_1} \quad (13)$$

After integrating this equation and dividing both sides of the equality by x_o, we write

$$x_b = 1 + \frac{k_2 n_1}{x_0 (n_1 + 1)} \tau^{n_1 + 1} \quad (14)$$

Let us denote in equation (14) the degree at τ by n, and the fraction by the quantity γ^n, we find

$$x_b = 1 + (\gamma \tau)^n \quad (15)$$

Where γ and n are parameters determined experimentally. Equation (13), as well as (11), corresponds with high accuracy to the experimental results (Figure 1).

The advantage of equation (15) over equation (11) is that it is easier to give the parameters included in equation (15) a well-defined physico-biological meaning. If in these equations the parameters m_1 and n_1 have the same meaning - the rate of change of the function x (τ), i.e. the change in the rate of biological processes in time, then with the parameters δ and γ the situation is more complicated. We can say that δ and γ represent the specific growth rate of biomass relative to the initial concentration in the time interval from τ = 0 to $\tau = \tau_i$.

However, unlike δ, from equation (15) it is easy to prove that the value 1/γ is the doubling time of the function x (τ) when its argument changes from τ = 0 to τ = τ_u, a value that is important in technological calculations.

A comparative assessment of the possibilities of using the considered mathematical models for describing experimental data on yeast cultivation showed that the results of these experiments cannot be approximated by a simple exponential dependence (6). Either equation (11) or (15) is quite suitable for this purpose. Both of them describe the results of experiments with the same degree of accuracy and satisfy the same boundary conditions. Which one to choose?

Let's try to do this by determining the speed of the process. Differentiating the equations, we obtain (11a) (15a). Graphically, the equations of the process rate (11a) and (15a) for one of the experiments are shown in figure 2.

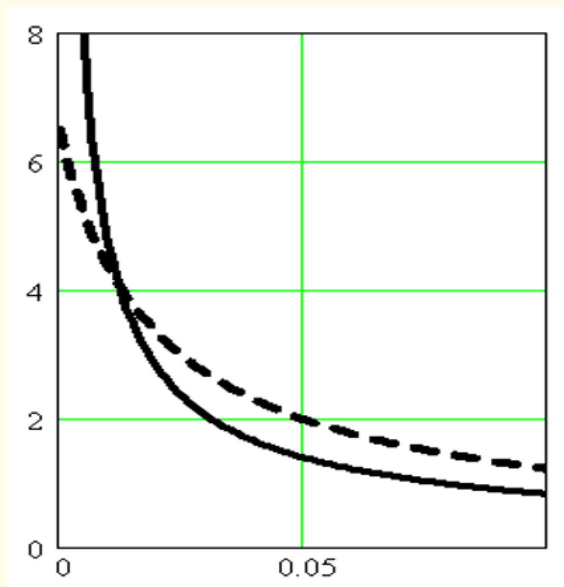

Figure 2: *Dependence of the rate of biomass growth on time: the lines correspond to equations (15a) and (11a).*

$$x'_b = (1 + \delta \cdot \tau)^{m_1 - 1} m_1 \cdot \delta \quad (11a)$$

$$x'_b = \gamma^{m_1} n \cdot \tau^{n_1 - 1} \quad (15a)$$

Figure 2 show that there are also significant differences in the change in the rate of increase in biomass. According to equation (11a) at tends to the maximum possible speed, 1 / h, and according to equation (15a) at.

The first option in real conditions is not possible in principle. The second requires giving it some kind of biological meaning. Perhaps this is the maximum biomass growth rate that can be for a given yeast strain in the absence of a lag phase. But this hypothesis requires a special experiment to confirm it. Third, if we do not take into account the first minutes of cultivation, then preference should be given to equation (15), as a simpler one, and from it the biomass doubling time is easily found. To calculate the values γ and n included in it, empirical equations of the following form were obtained:

$$\gamma(x_o) = a - b \cdot x_o^c, \ (16)$$

$$n(x_o) = \frac{a_1 \cdot x_o}{b_1 + x_o}. \ (17)$$

In equalities (16) and (17) the coefficients *a, a_1, b*, depend on the initial concentration of carbohydrates in the culture liquid – S_0. As a result of processing the experimental data, the following formulas were chosen S_0:

$$a = \frac{0{,}112}{1 + 3.36 \cdot e^{-66 \cdot S_o}} \ (18)$$

$$a_1 = \frac{0{,}710}{1 - e^{-24 \cdot S_o}}, \ (19)$$

$$b = \frac{0.000543}{1 + 12.4 \cdot e^{-107 \cdot S_o}} \ (20)$$

The coefficients b_1 and *c* turned out to be constant: b_1 = 8; c = 1, 3.

The system of equations (15) - (20) cannot be considered a complete mathematical model of the kinetics of biomass growth. We studied the effect of only the initial values of x_0 and S_0 on the change in the concentration of biomass in the culture medium (with other constant factors that can affect the development of cells), but x and S themselves constantly change during cultivation. Therefore, to build a complete mathematical model, equations are needed that establish the functional dependence of γ and n_1 in equation (15) on x and S. It is simply not possible to fit all this information into one article.

Conclusion

The authors of this work set the task only to consider examples of constructing mathematical models and draw the attention of researchers to the difficulties that they will have to face in constructing them.

There are three main options for searching for mathematical models of any technological processes: analytical, empirical and semi-empirical. The first requires the presence of appropriate differential equations, the second requires experimental research. The third is based on hypotheses, which, under certain conditions, make it possible to obtain differential equations of a particular process, and their input coefficients are found experimentally.

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Volume 16 Issue 10 October 2021