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Features of the Development of the Fungus *Medusomyces gisevi* at the Initial Stage of Cultivation and its Mathematical Description

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Abstract

In experimental and theoretical studies of the cultivation of the tea mushroom *Medusomyces gisevi*, the features of the kinetics of the development of the tea mushroom at the initial stage of cultivation are of particular interest. This approach to the study of kinetic patterns of mushroom development is due to several reasons. Firstly, at this time, the formation of the general chemical composition of the mushroom infusion begins; secondly, it is important to find the time of transition from one stage to another; thirdly, information about such studies in the literature is extremely rare.

Based on previously performed experimental studies, the selection of equations describing the kinetics of acid formation in the culture medium was carried out. The analysis of equation (3), as well as its derivatives (4), (5), made it possible to establish three periods of mushroom development in the initial stage of cultivation and determine their time limits. According to the location of characteristic points on the graphs, an explanation of the features of the physical and biological processes occurring in the culture medium during each of the periods is given.

According to the location of characteristic points on the graphs, an explanation of the features of the physical and biological processes occurring in the culture medium during each of the periods is given. The main reason for the three-period development of the fungus is the presence of oxygen dissolved in the culture medium. It is in the first 2 - 3 hours of cultivation that there is a sharp drop in the concentration of oxygen and the pH of the medium from the maximum values to the minimum, with the simultaneous formation of high-activity acids.

Keywords: *Cultivation; Fungi Medusomyces gisevi; Fungi Rice; Kinetics; Mathematical Model*

Introduction

Drinks from infusions of tea mushroom *Medusomyces gisevi* are distinguished by high healing and taste qualities due to the presence of various biologically active substances in them - organic acids, alkaloids, antibiotics, vitamins, etc. Such a variety of composition is explained by the fact that the mushroom is a polyculture of microorganisms consisting of yeast, acetic acid and lactic acid bacteria, the products of metabolism of which are listed above the substance.

Many scientists and practitioners have been studying the biological value for human health, morphological and physiological properties of kombucha, and the chemical composition of its infusions. In works [1-4], a detailed analysis of the possibility of using tea infusions in medicine, animal husbandry, and the production of general and functional beverages is given. It is noted that infusions of "tea" mushroom differ not only in their high taste qualities, but also in their usefulness for human health. They can be successfully used to improve the health of people suffering from diseases of the gastrointestinal tract, atherosclerosis, and other diseases.

There is a lot of information in the literature about the use of tea mushroom infusions in medicine [1-5], animal husbandry [1]. various industries: dairy [5,6], bakery [7-10], microbiological in the production of bacterial cellulose [11]. In [12,13], a broad analysis of the literature data on the morphology, physiology, and practical use of kombucha producers in many industries is given.

Thus, a lot of work has been accumulated on the study of the cultivation and properties of tea. But, strange as it may seem, mycologists have not paid enough attention to studies of the kinetics of biological processes and their mathematical description. This can be explained by the lack of need for such studies, since until now the preparation of drinks from mushroom infusions has been widespread at the household level.

Currently, in order to meet the increased needs for the products of the metabolism of the fungus, there is a need to establish the release of its infusions on an industrial scale. However, the design of industrial plants, the calculation of technological equipment and parameters that determine the conditions of their operation require more and more in-depth knowledge about the physico-chemical and microbiological features in which the fungus develops, about the kinetics of metabolic processes in culture media during various periods of its development. Therefore, more attention has recently been paid to the problem of revealing the kinetic laws of mushroom development and searching for their mathematical models (hereinafter MM) [8,14-16].

The need to uncover the kinetic laws of mushroom development and search for mathematical models describing them is dictated by the following reasons:

- High rates of development of microbiological industries, increased requirements for calculation and design work, considering a wide degree of automation and computer control of technological processes.
- The need for a deeper disclosure of the essence of material and energy exchange between the cultural environment, which opens the possibility of predicting this exchange and determining ways to intensify processes.
- The possibility of using MM in conducting computational experiments in order to determine the dependence of the speed of biological processes on changes in parameters important for cell development (temperature and pH of the medium in which they develop, the amount of initial seeding of pure culture, etc).

The complete MM of any microbiological process, as a rule, is a system of empirical, at best semi-empirical, equations describing a number of particular physical, biological and biochemical processes (cell division, their consumption of substrate and oxygen, their release of metabolic products). And the more parameters affecting the course of biological processes the full MM will take into account, the more partial equations there will be in it.

But before proceeding to the search for mathematical models, it is necessary to find out whether any empirical equation, even if describing experimental results with one hundred percent accuracy, can be considered a mathematical model of the process under study. The fact is that any experimental data with the same accuracy can be approximated by several equations. The question arises, which one to choose, what requirements should the model meet? The opinions of various authors on this matter largely coincide, although the formulations are different [17-24].

The general opinion is that MM should satisfy several requirements. Firstly, it is adequate to reflect the nature of the course of a particular process. Secondly, the model should be able to predict the course of the biological process outside the experimental conditions. I must say that fulfilling the second requirement may be more difficult than the first. A lot depends on how deeply the experimenter understands the problem he is working to solve. He must himself, even intuitively, foresee the development of the process under study outside of the experiment and on the basis of this choose one or another equation. Thirdly, the equations of mathematical models, on the one hand, should be as simple as possible, but on the other hand, they should correspond to the results of the experiment with a certain degree of accuracy. Fourthly, in order to delve more deeply into the essence of the processes occurring in the culture medium and the cell, to give all the empirical coefficients included in the MM equations a certain physico-bi.

The authors of [14,15], based on experimental studies of changes in the infusion density, acidity and concentration of oxygen dissolved in it, hypothesized a two-stage development of the fungus. The first stage lasts, from the moment of introduction of the seed culture into the nutrient medium to the formation of a solid film on the surface of the liquid, 4 - 5 days. But, since oxygen is necessary for the course of biological processes, it was assumed that at the first stage the fungus would consume oxygen, which was initially in the dissolved state of the culture fluid.

The second stage begins after the formation of a solid film of a new mushroom on the surface of the liquid. By this time, oxygen in the liquid medium will decrease to a certain critical value, after which the cells of microorganisms will not be able to consume it (this will be discussed later). Next, the fungus will consume oxygen from the outside surface of the air, i.e. the process of mushroom development becomes aerobic. A similar point of view is expressed by the author of the work [13]. The authors of the works also adhere to the hypothesis of two stages of development [6,16].

In this article, the main attention is paid to the study of the kinetics of the development of kombucha in the initial stage. This approach to the study of kinetic patterns of mushroom development is due to several reasons. Firstly, during this period, the formation of the general chemical composition of the mushroom infusion begins; secondly, it is important to find the time of transition from one stage to another; thirdly, no information about such studies has been found in the literature.

The kinetics of the flow of biological processes can be traced by various methods:

- Firstly, by changing the total titrated acidity K and pH of the infusions [1,8,14-16];
- Secondly, according to the kinetics of changes in the concentrations of individual acids in infusions (the method is expensive, but gives very useful information about the formation of the acid composition of infusions as a whole);
- Thirdly, by biomass growth - a method, in the study of kombucha, rarely used because of the difficulties in determining the weight gain with sufficient accuracy [8];
- Fourthly, by changing the content of oxygen dissolved in the culture liquid (this option is especially necessary at the initial stage of cultivation, when there is still dissolved oxygen in the culture medium) [14,15].
- Fifthly, by changing the physical properties of the medium (in particular, density); the method is simple, but rarely used [14-16].

Methods for measuring the main parameters and conducting experiments are described in detail in [1,5,8,15,16].The kinetics of the flow of biological processes can be traced by various methods:

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Search for mathematical models of the kinetics of the development of kombucha

The search was carried out on the basis of experimental data on the first and fourth methods. The search for MM is based on experimental data from works [14,15] performed on media with a sugar content of S_0 = 0.05, 0.1, 0.18, 0.27 mass fractions, with 2% infusion of black tea at a temperature of 18 - 20°C. In addition, the data of the work [16] performed at S0 = 0.10 were used (hereafter the index "0" means the initial values of the parameters, without the index - the current values.). A certain moist body mass of an already existing mushroom containing metabolic products was used as a seed crop. Since the initial values of pH, titrated acidity and oxygen concentration in the culture fluid were different, in order to bring the experimental results to some kind of uniformity, further processing of experimental data was mainly carried out in dimensionless coordinates: pHb = pH/pH_0, Ob = O/O_0. This method of processing experimental data is also convenient because we become independent of the quantities.

In experiments, the density of the infusion, its titrated acidity, and were determined by standard methods. The concentration of oxygen dissolved in the culture fluid was measured directly in the cultivator by the MARK-4 device [15].

The results of the experimental data that formed the basis for the search for MM equations are presented in figure 1A in dimensional form and 1B dimensionless. Explanations to the figures will be given further in the text.

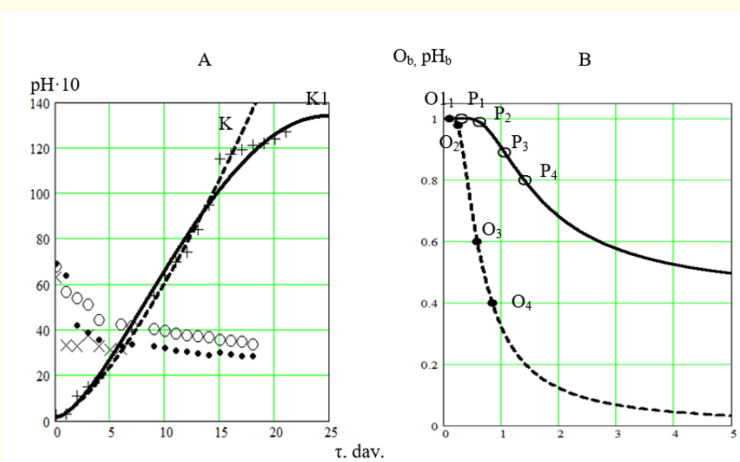

Figure 1: *Dynamics of changes in acidity values.*

Figure 1A: Lines of equations: functions: K1(τ) ▬ (1), K(τ) ▬ (1a).

Experience. meaning. K - + S_0 = 0,1; pH - ●– S_0= 0,1; × - S_0 = 0,27; ○ - S_0 = 0,1 [16].

Figure 1B: The lines: ▬ pH_b, ▬ ▬ O_b correspond to equation (3)., at S_0= 0.1.

The icons indicate the characteristic points of equations (4) and (5).

Figure 1A draws attention to the difference in the characters of the rates of change of active and activated acidity. The active acidity of the culture fluid for the first 4 - 5 days sharply decreases from a value close to neutral to pH = 3 - 2.8, remaining constant at this level until the end of the cultivation process. This dependence on time is confirmed by the authors of the work [5,6,16]. At the same time, the titrated acidity gradually increases during 16 - 17 days. It is quite possible that after reaching the maximum, it will begin to decline.

The peculiarities in the characters of changes in the titrated acidity and the concentration of oxygen dissolved in the medium allowed the authors of works [14,15] to make an assumption about the production of acids with a high degree of dissociation by the fungus in the initial stage, first of all. Such as pyruvic acid with a dissociation constant Cd = 5.6×10-1, oxalic acid - Cd = 3.8 ×10-2, fumaric acid - Cd =1·10-3 [25,26]. Acetic acid with Cd = 1,7 • 10-5 and other acids with lower Cd values, constantly produced by the fungus throughout the cultivation process, have a lesser effect on the pH value, but affect the overall acidity of the culture fluid.

An increase in the sugar concentration in the initial tea solution leads to a faster decrease in active acidity. The titrated acidity depends weakly on the initial sugar content in the culture medium.

Mathematical description of the kinetics of changes in titrated acidity

The kinetics of changes in titrated acidity over a wide range of time changes can be expressed by equations of various types [17-24]. As a result of processing experimental data, the equation that most adequately reflects the change in function over a wide range of cultivation time was selected:

$K1(\tau)=K_0 + (\gamma_1 \cdot \tau)^{n1} - Kr(\tau)$, (1).

The function Kr(τ) reflects the appearance of factors in the cultivation process that negatively affect the development of microorganisms (a decrease in sugar in the culture fluid, the formation of metabolites in it that interfere with the further development of fungi). Its type will be determined by its tendency either to a constant value, or, after reaching the maximum, to decrease.

In particular, for the curve of the function K(τ) shown in figure 1A, and its expected decrease in the future, it is accepted $Kr(\tau) = (\mu_1 \tau)^2$. In this case, when $K_0 = 1.5$, we get $\gamma_1=2.05$, $n1 = 1.8$, $\mu_1 = 1.9$. With such values of coefficients, according to equation (1), the function reaches its maximum value after about 29 days. Further cultivation should lead to a decrease in total acidity. Practice shows that cultivating kombucha for more than 12 - 15 days leads to a deterioration of its taste qualities [13]. There is no information about whether an increase in cultivation time will be accompanied by a decrease in acidity or the death of microorganisms in the literature for kombucha.

If we limit the cultivation time to 12 - 15 days, then the influence of the function can be neglected, In this case equation (1), for the variant presented in figure 1, will take the form:

$K1(\tau)=1.5 + (1.85 \cdot \tau)^{1.4.}$ (1a).

The discrepancy in the calculations of titrated acidity according to equations (1) and (1a), within the specified limits of cultivation time, does not exceed 5%.

Denoting in equation (1) the product (1/K0)1/n·γ1 by γ and reducing it to a dimensionless form, we present equation (1a) in the following entry: $K_b(\tau)=1 + (\gamma\tau)^n$ (2).

As a result, the equation of the well-known power law of the development of the biological process is obtained [21,22]. The value γ can be called the specific rate of increase of acidity; n - the rate of increase of acidity, determines the shape of the curve of the function $K_b(\tau)$.

The values γ and n are given in table 1. The ratio $1/\gamma=\tau_2$, where τ_2 is the doubling time of titrated acidity. For equation (1a) $\tau_2 \approx 0.72$ day. Such a rapid increase in the total acidity of the infusion may occur due to the receipt of part of the acids from the seed culture, which is a certain body weight of the fungus, located initially at the bottom of the cultivator.

Mathematical description of the kinetics of changes in active acidity and oxygen concentration

Figure 1B shows in dimensionless form the change in time of the active acidity of the infusion and the oxygen consumed by the fungus, where the similarity of these two processes is clearly visible. That is, the processes of oxygen consumption by the fungus and changes pH in the infusion should obey the same pattern and, therefore, their kinetics can be described by a general equation satisfying the condition - at $\tau \longrightarrow \infty$ the function O_b and pH_b should decrease and tend to const. Based on this fact, we will continue to search for MM equations.

According to figure 1B, at $\tau \to \infty$, the function $O_b(\tau)$ tends to a certain minimum constant value $O_{bmin}=O^*_{bmin}$, where $O^*_{bmin} = O_{min}/O_0$ is the dimensionless value of the minimum equilibrium oxygen concentration in the mushroom infusion at a given value of temperature and pressure in the cultivator, in the second stage of its development. The oxygen concentration in environments where biological objects develop never drops to zero, but decreases to a certain constant value depending on the type of biological object, the temperature of the habitat and pressure [27]. The studies carried out by the authors [14,15] only confirmed the previously established pattern.

The processing of experimental data has shown the possibility of using an equation of the form to describe the kinetics of mushroom development from several equations:

$Y_b=1-a\cdot\exp(-(b/\tau)^c$, (3)

Where $Y_b=Y/Y_0$, Y_0 - is the initial value of the function. The values of empirical coefficients and exponents, in equations (2), (3), are given in table 1.

	a	b, day	c	γ' 1/ day	n	Equation
$\overline{K}$				1,385	1,4	(2)
pH_b [12, 13] pH_b [14]	0,58 0,54	1,45 2.7	1,6 1.5			(3)
O_b [12, 13]	0,997-0,998	0,55 - 0,6	1,6			(3)

Table 1

According to equation (3), some explanations should be given, and the coefficients included in them should be given a certain physical and biological meaning. To this end, we analyze equation (3). For при $\tau \to\infty$ $\exp(-(b/\tau)^c \to 0$, the function $Y(\tau) \to 1$ and $Y_b \to Y_0$. Since the ratio b/ τ is a dimensionless quantity, it b must have the dimension of time. Table 1 shows that the coefficient b varies within 1.4 - 2.7 days for pH_b and 0.55 - 0.6 for O_b. In figure 1B this time, roughly, corresponds to the transition from sharp decline pH_b and O_b a smooth transition to a constant value.

When $\tau \to \infty$ the exponent tends to unity, and the function $Y_b(\tau)$ tends to the minimum value equal to $(1-a) = O^*_{bmin}$. For the example shown in figure 1B, it is equal to O^*_{bmin} = 0.002 - 0.003. The inconstancy of values O^*_b is associated with the inconstancy of concentration O^*

over time. The equilibrium concentration, as already noted, depends on the concentration of metabolic products in the infusion, fluctuations in temperature and atmospheric pressure during cultivation, lasting several days. Therefore, the values of the empirical coefficients in equations (2), (3) given in table 1 have somewhat averaged values.

For a deeper understanding of the essence of the biological processes occurring in mushroom infusions, we will analyze the first and second derivatives of equation (3) - $Y_b^{'}$ и $Y_b^{''}$, the graphical representation of which is given in figure 2A and 2B.

$Y_b^{'}(\tau)$= -a·(b/ τ)^c·(c/τ)·exp(- (b/ τ)^c) (4).

$Y_b^{''}(\tau)$=(a·(b/ τ)^c·(c/τ^2)·exp(- (b/ τ)^c))·(c+1-(c·(b/τ)^c)) (5).

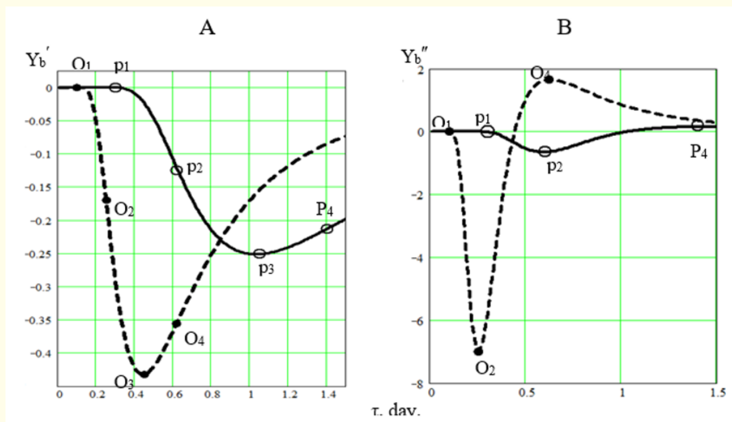

Figure 2: *Dynamics of changes in functions $Y_b'(\tau)$ and $Y_b''(\tau)$.*
Line: ▬ ▬ $O_b'(\tau)$ u $O_b''(\tau)$; ▬ $pH_b'(\tau)$ И pH_b.

Figure 1A and 1B serve as sources of useful information about interesting events in the development of the fungus that occur at the initial stage, or more precisely in the first one and pH_b a half to two days. Therefore, the analysis of the causes of changes in the concentration of oxygen and pH_b in the infusion makes it possible to more deeply understand the kinetics of the process of development of the fungus as a whole.

Graphs 1B, 2A and 2B show several characteristic points, the location of which traces the course of a biological process consisting of at least three periods, different in the rate of change in oxygen concentration and pH over time.

The first period: The duration of the first period is approximately 0.5 days. The points O_1 and p_1 correspond to the beginning to the beginning of the processes of reducing the concentration of oxygen dissolved in the mushroom infusion, and its pH, in fact, the beginning of the process of development of the fungus as a whole. Figure 2A and 2B show an earlier time ($\tau_1 \approx 0.1$ day) of the beginning of the decrease in oxygen concentration compared to the decrease pH ($\tau_2 \approx 0.31$ day). The question arises - why does the process of reducing the oxygen concentration begin before the decrease pH. This development of the cultivation process suggests that in the time interval $\tau \approx 0.3$ of a day, microorganisms have not yet begun to secrete a sufficient amount of metabolic products containing highly active acids. That is, an earlier decrease in oxygen concentration is largely due not to the vital activity of microorganisms, but rather to the course of the physical process of disturbing the equilibrium state of the system: the infusion is the air dissolved in it, due to the entry into the culture medium of acids from the mushroom seed culture. In addition, some of the acids can pass from the original tea solution. All this contributes to the displacement of not only oxygen from the mushroom infusion, but also other gases contained in the air.

The above does not mean the complete absence of a certain amount of oxygen and nutrients necessary for their development by cells during a period of 0.31 days. The resulting metabolic products, CO_2, organic acids, etc. begin to be released into the culture medium, i.e. biological and physical processes in the first period can occur simultaneously. This corresponds to figure 1A, according to which the total acidity of the liquid medium in which the fungus develops is constantly increasing. Consequently, the physical process of oxygen displacement from the liquid can take place throughout the entire time of mushroom cultivation. But the oxygen concentration in the medium will never become zero, regardless of the metabolites released by microorganisms. The oxygen concentration in the liquid medium, as already mentioned, will be equal to the minimum equilibrium O^*_{bmin} value corresponding to the cultivation conditions.

Let's pay attention to the point O_2 ($\tau \approx 0.24$ day, figure 2A), which determines the position of the inflection point of the function $O_b'(\tau)$ and in figure 2B corresponds to the maximum of the function $O_b''(\tau)$, i.e. the maximum acceleration of the decrease in the rate of oxygen concentration in the infusion. It can be assumed that in the time interval $\Delta\tau = \tau_2 - \tau_1 = 0.24 - 0.1 = 014$ days, the predominant role is played by the physical process of oxygen displacement from the infusion. The biological process of oxygen displacement mainly begins from the point O_2, here the main process of formation of active acids begins.

Thus, the first period of the initial stage of mushroom development continues from the moment of introduction ($\tau = 0$) into the initial tea infusion of the sowing culture to the point p_1 of figure 1B, 2A and 2B. It can be assumed that the physical process of oxygen displacement from the infusion prevails in the section of the curve O_1O_2.

Finishing the analysis of the first period of the initial one, we note a somewhat arbitrary choice of the location of the points O_1 and p_1. The fact is that the functions $Y_b(\tau)$, according to equation (3), will be equal to one only when $\tau = 0$. The choice of the start time of the process should be determined by the researcher, but in such a way that the deviation of functions $Y_b(\tau)$, from unity could be neglected. In principle, this does not matter much, because the essence of the analyses of equations (3), (4) and (5) does not change from this

The second period is limited to the points p_1 - $\tau \approx 0.31$ и p_4. - $\tau \approx 1{,}5$. At the point p_1, an accelerated drop in the function $pH'(\tau)$, begins, reflecting the rate of decrease in active acidity.

The point p_2 is the first inflection point of the function $pH_b'(\tau)$, (Figure 2A). In figure 2B it determines the time ($\tau \approx 0.62$ day) for the function $pH''(\tau)$ to reach the maximum value ($pH'' = -0.6$). At the point p_3 the rate of decrease in active acidity reaches a maximum ($pH' = 0.25$ and $\tau \approx 1.05$ day) and then decrease to the point p_4 (the second inflection point of the function $pH_b'(\tau)$ after that comes the period of its smooth tendency to zero (Figure 2A). The second derivative of the function $pH_b'(\tau)$ will also tend to zero.

Similarly, to the change in pH_b, the oxygen concentration in the infusion will also change. The drop in the function $O_b'(\tau)$, which characterizes the rate of decrease in the concentration of oxygen in the infusion, begins to increase (as mentioned earlier) already in the first period, it reaches a maximum at the point at, and then it sharply decreases to the point (the second inflection point) with its further smooth tendency to zero.

It can be said that changes in the functions of $pH_b'(\tau)$ and $O_b'(\tau)$, in the specified time interval, reflect the process of isolation of high-activity acids by microorganisms. The sections of the O_2 - O_4 and p1 - p4 lines correspond mainly to a biological process. It is quite possible that at the second inflection point of the function $O_b'(\tau)$ (point - O_4), the attenuation of the biological process of consumption by the fungus of oxygen dissolved in the infusion begins.

The third period starts from the point p4, followed by a gradual decrease to zero of the functions Y_b' and Y_b''. The end of the third period of the initial stage of the cultivation process can be considered the fourth or fifth day. This is followed by the second stage of the cultivation process, which proceeds at constant pH values and the concentration of dissolved oxygen in the infusion (Figure 1B). That is, going back to table 1, we can say that in equations (3), (4) and (5), parameter b approximately determines the duration of the third period of the initial stage of mushroom development. In other words, the third period is the time of attenuation of biological processes associated with a decrease in the concentration of oxygen dissolved in the infusion. One of these processes is a drop in the rate of release of highly active acids by microorganisms.

Thus, the analysis of equations (3), (4) and (5) gives grounds to assert that the formation of the acid composition of the tea mushroom infusion in the initial stage of cultivation occurs in three periods. The main factor determining this course of the biological process is the presence of oxygen dissolved in the culture medium at the initial stage of cultivation.

It should be borne in mind that all the previous arguments about the two stages and three periods of the development of the fungus in the first stage, for the most part, are of an assumed nature. They are mainly based only on experimental studies of the kinetics of changes in pH, titrated acidity and oxygen concentration in the infusion. Therefore, the equations (1) (2), (3), (4) and (5) allowed us to solve only particular problems of mathematical modeling of the kinetics of the development of kombucha at the first and second stages of its development: the consumption of oxygen by the fungus, changes in the active and titrated acidity of infusions.

The performed studies do not make it possible to fully reveal the kinetic patterns of the development of the tea mushroom. To search for a more complete MM, experimental data on changes in the chemical composition of the infusion over time are needed, confirming the release of high-activity acids (and not only acids) by microorganisms at the initial stage of the fungus. Moreover, such studies should be carried out both at the initial stage and throughout the entire cultivation time. This task cannot be avoided if infusions are used to extract certain metabolites from them. Why it is necessary to know the time of liquid extraction from the bioreactor for its further processing.

However, despite these shortcomings, the experimental and theoretical studies carried out and the kinetic equations obtained on their basis made it possible to explain the mathematical, physical, and biological meaning of the coefficients and exponents leading to them and to show the need for further research.

Conclusion

Conclusions analysis of equations (1), (2), (3), (4) and (5) allowed:

- To establish the presence of three periods of mushroom development in the initial stage of cultivation and to determine the time boundaries between them;
- By the location of characteristic points on the graphs of functions, the course of physical and biological processes occurring in the cultural environment is explained;
- A system of equations (1), (2), (3), (4) and (5) can be considered a particular mathematical model of the kinetics of changes in the concentration of oxygen and infusion and the associated formation of acids of high activity;
- Explain the physical and biological meaning of some empirical coefficients included in the MM equations;
- Mathematical processing of experimental results of studies of the kinetics of the development of the fungus *Medusomyces gisevi* allowed: to delve deeper into the essence of the physico-biological processes occurring in the culture medium; to detect shortcomings in experimental studies and outline ways to improve them.

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