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### Abstract

In order to meet the increased needs for metabolic products of the zooglue mushrooms *Medusomyces gisevi* - "tea" mushroom and *Oryzamyces indicia* RSC - "rice" mushroom (hereinafter CHG and RG), it became necessary to establish their production on an industrial scale. However, the design, calculations of technological parameters and equipment require knowledge about the kinetics of the development of metabolic processes and the availability of mathematical models (hereinafter MM) that adequately reflect their course.

The search for MM is based on the results of research by various authors on the cultivation of two zooglea fungi, on the basis of which equations describing the kinetic patterns of their development are selected. It was confirmed that at the initial stage of cultivation there were three periods of development of CHH and RG, during which, due to the presence of oxygen dissolved in the culture medium, acids of high activity are formed. The time limits between the periods have been clarified. The essence of the processes occurring in cultural media at various stages and periods is revealed. The physical and biological meaning of some empirical coefficients included in the MM equations is explained.

Keywords: Kinetics; Cultivation; Mushroom; Medusomyces gisevi; Rice Mushroom; Mathematical Model

### Introduction

Infusions of zooglue mushrooms *Medusomyces gisevi* ("tea" mushroom - CHG) and *Oryzamyces indicia* RGC ("rice" mushroom - RG) are characterized by high healing and taste qualities, the reason for this is the ability of their release of various metabolites consisting of biologically active substances - organic acids, alkaloids, antibiotics, vitamins, etc. are constantly expanding and the areas of application of infusions. There is information about their use in medicine, animal husbandry, production of various kinds of beverages, dairy products, etc. [1-6].

The increased need for metabolic products of these fungi poses the task of establishing the production of infusions on an industrial scale, which in turn requires deeper knowledge in the field of physico-chemical and microbiological features of their development; studying the kinetic patterns of metabolic processes during various periods of cultivation and their mathematical description.

Since the problems of the practical use of HCG producers and the mathematical description of the kinetics of the development of its cell population have already been given in [2], this article will focus on a comparative analysis of the cultivation processes and equations describing two zooglue fungi - *Medusomyces gisevi* and *Oryzamyces indicia* RCC. It follows from the existing works that there are many

similarities and differences between the species of zooglue fungi considered in this article. We will not dwell on this in detail, but will highlight only those that are important for solving the tasks we have set.

Before proceeding to a comparative analysis of the development of two zooglea fungi, let's pay attention to the difficulties that may arise in this case. The fact is that the experimental data presented in the literature [1-7] have different dimensions. Therefore, in the future, in order to facilitate the solution of the task, we will mainly process experimental data in a dimensionless version in the form of ratios of the current values of the studied parameters to their initial values: concentrations of oxygen -  $O_0$ , substrate -  $S_0$ , acids -  $\hat{E}_0$ ,  $pH_0$  (hereafter, the index "0" means the initial values of the parameters). In the drawings. 1 and 2 the research data are presented in dimensional and dimensionless forms -  $p\overline{H} = pH/pH_0$ .  $\overline{K} = K/K_0$ ,  $\overline{O} = O/O_0$ ,  $\overline{G} = G/G_0$ .

However, dimensionless processing of experimental data requires knowledge of the values of the initial parameters. Unfortunately, such data are not always provided in the literature. Thus, when using the results of experimental studies performed with rice mushroom by the authors of [3,4], difficulty arose due to the lack of values of initial concentrations -  $K_0$ . The graphs of the functions  $K(\tau)$  (See figure 2A) were constructed starting from the data corresponding to the first days of cultivation. Therefore, the initial values were taken from pre-selected equations describing the dynamics of changes in acid concentrations in rice mushroom infusion over time. As a result, the following initial concentration values were adopted: acetic -  $Ku_0 = 0.05$ , lactic -  $Km_0 = 0.06$ , amber -  $K_{Y0} = 0.073$ , shikim - g/l.

It cannot be said that the accepted method of processing experimental data is highly accurate. But even if errors are made in determining the initial values, this will not affect the nature of the change in functions, but only the numerical values of the empirical coefficients included in the MM equations.

The initial mass of the seeded crop is given by the authors [1], -  $G_0$ =100r.

### Similarities and differences in the development of fungi

#### Similarities:

- The first similarity lies in the cultivation of mushrooms in open vessels without stirring. Consequently, the surfaces of culture fluids come into contact with air (for kombucha only at the initial stage of development);
- Secondly, the similarity of the composition of nutrient media aqueous solutions of sucrose;
- Thirdly, the concentrations of hydrogen ions in infusions are similar in values;
- Fourth, the rate of increase in acidity is determined by the rate of sugar consumption [1];
- Fifthly, both fungi equally possess the ability to suppress the development of other microorganisms in culture media, thereby maintaining sterile conditions of existence;
- Sixth, the same acids as CHH with dissociation constants were found in RG infusions: acetic 1.75×10-5, lactic 1.38 10-4, malic-3.5×10-4, citric 8.4×10-4, gluconic 1.38×10-2, oxalic 3.8×10-2, pyruvic -0.56.

#### **Indifference:**

- The first difference lies in the cultivation methods. Kombucha belongs to aerobes [1,2,8], rice mushroom belongs to anaerobes [1,3,4], which affects the mechanism of oxygen consumption by mushrooms.
- The second difference: During the cultivation of CHG, 4 5 days after the introduction of the seed culture into the nutrient medium, a continuous film begins to form on the surface of its infusion, the thickness of which constantly increases over time, forming the body of the fungus. Before the formation of a continuous film, in the initial stage of development, the microorganisms of the tea mushroom will consume oxygen in the infusion in a dissolved state [2,7]. After completely covering the surface of the infusion with a solid film, oxygen consumption by the tea mushroom will begin to occur on its surface from the air (an aerobic process).

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On the surface of the infusion of RG, no film is formed during the entire cultivation period. Colonies of RH microorganisms are mainly located in the bottom layer of the culture fluid, and therefore its development is considered anaerobic [1]. However, it will not be entirely accurate to talk about the development of RG in purely anaerobic conditions. It is quite possible for oxygen to reach the cells in two ways: firstly, already dissolved in the initial nutrient medium, and secondly, from the air through the free surface of the liquid due to molecular diffusion during the entire cultivation process. But since the diffusion rate is significantly lower than the rate of oxygen consumption by microorganisms, most of it from the infusion should be consumed by RG, as well as CHG, at the initial stage of cultivation. Diffusion transfer, if it affects the development of cells, then only in the uppermost layers of infusions. Unfortunately, no data has been found in the literature on studies of RG oxygen consumption. However, the results of experimental data on changes in the pH of mushroom infusions presented in figure 1A and 1B give reason to assert that the process of oxygen consumption by zooglue mushrooms is subordinated to one pattern.

• The third difference lies in the variety of plant additives used in nutrient media. For CHG, it is a tea leaf, for RG, the range of additives is much wider - raisins, figs, other berry and fruit crops [1,3-5].

• The fourth difference is most likely a consequence of the first two and consists in a slightly greater variety of the acid composition of RG infusions compared with CHG. The authors [3, 5], by chromatography, found high-activity acids in RG infusions, which are not present in CHG infusions: amber –  $K_{dY}$  = 7.4×10-5, tartaric -  $K_{dV}$  = 1.3×10-3, shikimic -  $K_{dh}$  = .3×10-3. The values of dissociation constants are taken from the literature [9,10].

The comparative analysis of experimental studies on the cultivation of two zooglea fungi allows us to state some features of their development. According to figure 1A and 1B, the pH of the infusions of both mushrooms slightly depends on; a sharp drop from pH= 6-7 to pH= 4-3 during cultivation occurs within 4-6 days, remaining constant thereafter.



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Similarly, the concentration of oxygen dissolved in infusions should also change - from maximum values 8 - 7.5 to minimum values  $O_{\min} = 0.016 - 0.024 \text{ mg/l}$ , which will undoubtedly affect the kinetics of acid formation and their chemical composition. This is confirmed by the studies of the authors [3,4], presented in figure 2A, which draw attention to the formation of acids with a high degree of dissociation at the initial stage in the infusion of RG.

Since the dynamics of changes in the infusions of both fungi are close, then in the infusions of CHG at the initial stage, during three periods of cultivation, first of all, acids of high activity should be formed. According to figure 2A it can be assumed that the duration of the first period of cultivation of RG can be assumed to be from 0 to 0.3 - 0.4 days. The second period is from 0.4 to 3.5 days, depending on the degree of dissociation; the third period is from 3.5 and beyond days. Thus, the duration of the periods of development of RG is somewhat longer than that of CHG. However, the assumptions made require experimental verification, since the dynamics of changes in oxygen concentration in RG infusions has not been studied.



B. - RG biomass,  $\overline{G}: S_0 = 0,04: \bullet \bullet \bullet (4); S_0 = 0,05 = (4); S_0 = 0,06 = (4)[1].$ 

Attention is drawn to the achievement of high activity  $\overline{K}_m(\tau)$   $\overline{E}_Y(\tau)$ ,  $\overline{K}_h(\tau)$  maximum values of the RG function of acids by 3 - 4 days of cultivation, and the infusion pH and the concentration of oxygen in them to a minimum. From the specified period of time, the concentration of highly active acids in the infusion of rice mushroom begins to drop. It follows from the above that the formation of highly active acids is quite possible in the infusions of both zooglea fungi, but such a process can occur mainly at the initial stage of cultivation with a sufficient amount of oxygen dissolved in the infusions.

The authors of the work [1], in addition to establishing the acid composition, conducted interesting studies on the increase in RG biomass G at various initial concentrations of sugars  $S_0$ , varying from 0.01 to 0.08 mass fractions. All experiments were characterized by the presence of maxima of the function  $\overline{G}(\tau)$  (Figure 2B). Moreover, the maximum value of the function depends on  $S_0$ , and the time to reach the maxima is constant -  $\tau_m \approx 30$  day.

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With kombucha, such studies have hardly been conducted. An exception is the work [12], in which the increase in the biomass of tea mushroom was determined by a change in the thickness of the mycelial film h during cultivation. The method is not accurate enough to look for some equations of kinetic regularities based on it, but the nature of the change in the function  $h(\tau)$  of the tea mushroom is similar to the change in the function of the RG. Therefore, it is likely that the maximum body weight of the tea mushroom will be reached in the same time frame (after about 25 - 30 days). But this assumption requires additional experimental verification.

Thus, based on a brief analysis of experimental work related to studies of the dynamics of the development of fungi *Medusomyces gisevi* and *Oryzamyces indicia*, the RSC showed that they differ in morphological properties and have much in common in the physiology of their development. But if a fairly extensive amount of experimental material has already been accumulated on the chemical composition and properties of infusions of zooglue mushrooms, then research on the kinetics of biological processes and their mathematical description is clearly insufficient.

### Mathematical models of developmental kinetics mushrooms-zooglean

The search for models will be carried out according to the methods described in [2], based on experimental studies presented in figure 1A, 1B and 2A, 2B. In this article, we will talk more about the development of the RG, since the development of the CHG is described in detail in [2]. Experimental data on the cultivation of RG were taken from the works [1,3,4].

### Kinetics of changes in mushroom infusions of pH and oxygen concentration

In figure 1B a sharp decrease in pH is clearly visible at the initial stage of cultivation. It is peculiar to both fungi and weakly depends on  $S_0$ . In this case, the concentration of oxygen dissolved in the infusions of both mushrooms should also change identically. But in any case, the oxygen concentration will not drop to zero, but will decrease to a minimum equilibrium value  $O = O_{\min}^*$ , depending on the type of fungus, temperature and pressure in its habitat. From all that has been said, it follows that the kinetic patterns of pH and Ochanges during the cultivation of both fungi will be described by an equation of the same type:

### $\overline{Y}(\tau) = 1 - a \exp((-(\mu/\tau)^c)) \quad (1)$

Where  $\overline{Y} = Y/Y_0$ ,  $Y_0$  - is the initial value of the function  $Y(\tau)$  of any of the parameters under study. According to equation (1) for  $\tau \to \infty \exp(-(\mu/\tau)^c \to 1)$ , and the function  $\overline{Y}(\tau) \to \overline{Y} \min = (1-a)$ . In this case, you can write: for oxygen  $a = 1 - \overline{O}_{\min}^*$  and *C* for active acidity  $a = 1 - p\overline{H}_{\min}$ . According to equation (1), the coefficients *a* and *C* are dimensionless quantities, as for the specific velocity  $\mu$ , it has the dimension of time. Taking into account the previous one, equation (1) implies the form of functions  $\overline{O}(\tau)$  and  $p\overline{H}(\tau)$ :

$$\overline{O}(\tau) = 1 - (1 - \overline{O}_{\min}^*) \exp((-(\mu_o / \tau)^c))$$
(2)

$$p\overline{H}(\tau) = 1 - (1 - p\overline{H}_{\min}) \exp((-(\mu_p / \tau)^c))$$
(3)

In equations (2) and (3), the dimensionless values are-  $\overline{O}_{\min}^* = 0,002 - 0,003$ ,  $p\overline{H}_{\min} = 0,42 - 0,46$ , and the exponent is c = 1,6. The coefficients  $\mu_o$  and  $\mu_p$  have the dimension of time. Their values may vary within: -  $\mu_o = 0.55 - 0.6$  days. and -  $\mu_p = 1.4 - 1.5$  days.

#### Kinetics of changes in acid concentrations and the mass of microorganisms in infusions of zooglue mushrooms

The kinetic patterns of formation of infusions of zooglue mushrooms of any acids, shown in figure 1A, 2A and 2B, can be described by an equation of the dimensionless form [2]:

## $\overline{K}(\tau) = 1 + (\gamma \cdot \tau)^N - (\mu_1 \tau)^n$ <sup>(4)</sup>.

The function (4) at the point corresponding to time  $\tau = \tau_m$  has a maximum value for all acids  $\overline{K} = \overline{K}_{max}$ . The time  $\tau_m$  is found from the condition that the first derivative of the function (4) is equal to zero -  $\overline{K}' = 0$ . If the description of the kinetics of cultivation is limited by experimental conditions, then the influence in equation (4) of the third term can be ignored, and it will appear in the form of a power law [2,13]:

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$$\overline{k}(\tau) = 1 + (\gamma \cdot \tau)^{n1} \quad (5).$$

In equations (4), (5) and, respectively, the specific rate of increase in the concentration of acidity and its decrease. To a greater extent, the kinetic patterns of the formation of low-activity acids are subordinated to equation (5).

As for acids with a higher degree of dissociation, the dynamics of their changes at the initial stage of development looks somewhat different. So, for example, in RG infusions, the kinetic patterns of formation of high-activity acids, shown in figure 2A, can be described both by equation (4) and others, with higher accuracy.

Experimental data on the formation of lactic acid in RG infusions can be approximated by an equation of the logistic form:

$$\overline{K}m(\tau) = \frac{a}{1 + be^{-c\tau}} \quad (6).$$

According to equation (6) when the  $\tau \rightarrow \infty$  coefficients  $a \rightarrow const=24,5$ , b = 23.5, 1/day. With less accuracy, the approximation can also be carried out using equations (4) and Gauss (7)

$$\overline{K}(\tau) = A \cdot \exp(\frac{-(\tau - B)^2}{2 \cdot c^2}) \quad (7).$$

The approximation of experimental data on changes in the concentrations of succinic and shikimic acids in infusions can be carried out both by equation (4) and (7). One feature of the Gauss equation should be noted. When processing experimental data in dimensionless form,  $\tau = 0$  the function  $\overline{K}(\tau)$  must be equal to one. Equations (4), (5), (6) this condition is satisfied, equation (7) is not always. It is difficult to say which equations should be preferred, since the authors of [3,4] limited the experiments to only six days. Therefore, it is only possible to speak with confidence about the kinetics of the formation of the acid composition of RG infusions beyond six days. The values of the coefficients included in the equations (4), (5), (6) and (7) are shown in table 1.

The kinetics of biomass  $\overline{G}_m$  growth by rice mushroom was studied by the authors [1] in a wide range of cultivation time, but with a wide range of experimental data. The best coincidence of the experimental and calculated values is given by equation (4). If we limit the processing of experimental data to six days, then equation (5) can also give good convergence.

Despite these inaccuracies and limitations, the data presented in figure 2A, 2B and table 1 allow us to make some generalizations and state some features of the development of fungi, which are quite difficult to explain.

Firstly, in table 1, attention is drawn to the increase with an increase in the dissociation coefficient –  $K_d$  of the specific rates of change in acid concentrations y in mushroom infusions. The processing of experimental data has shown the possibility of describing them with a slightly modified equation (1):

γ(Kd)=a-b(exp(-(f Kdc)) (8)

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The total acidity of the tinctures of CHG, Figure 1A.  $K_T( au)$  ,  $ar{k}( au)$  . Equat. (4): -  $\gamma = 1.651$ , N = 1.83, n = 2,  $\mu_1 = 1.14$ , n = 2.  $\overline{K}_{T \max} \approx 100$ ,  $\tau_m \approx 29$ . Equat. (5): -  $\gamma = 1.4$ ,  $n_1 = 1.4$ ,  $\overline{k}(\tau)$ Acetic acid, figure 2A.  $\overline{K}_{u}(\tau)$ ,  $K_{du} = 1.75 \cdot 10^{-5}$ . Equat. (4): -  $\gamma = 1.3$ , N = 2, n = 3,  $\mu_1 = 0.4$ ,  $\overline{K}_{\mu \max} \approx 175$ ,  $\tau_m \approx 28$ ; Equat. (5): -  $\gamma = 1.3$ , n1 = 2. Succinic acid, figure 2A.  $\overline{K}_{V}(\tau)$ ,  $K_{dV} = 7.4 \cdot 10^{-5}$ . Equat (4): -  $\gamma = 1.94$ , N = 2.2, n = 2.6,  $\mu_1 = 1.33$ ,  $\overline{K}_{Y \max} \approx 15$ ,  $\tau_m \approx 3.8$ ; Equat (7): A = 15.07, B = 3.82, c = 1.64. Lactic acid, figure 2A.  $\overline{K}_m(\tau)$ ,  $K_{dm} = 1.38 \ 10^{-4}$ . Equat. (4): -  $\gamma = 2.45$ , N = 1.56, n = 3,  $\mu_1 = 0.59$ ,  $\overline{K}_{m \max} \approx 25$ ,  $\tau_m \approx 4.8$ ; Equat. (6): - a = 24.5, b = 23.5, c = 1.5; Equat (7): A = 24.85, B = 4.4 сут., c = 1.89 сут. Shikimic acid, figure 2A.  $\overline{K}_h(\tau) \cdot 10$ ,  $K_{dh} = 3 \cdot 10^{-3}$ . Equat. (4): -  $\gamma = 3.37$ , N = 2.59, n = 3,  $\mu_1 = 2.28$ ,  $\overline{K}_{hmax} \approx 2..5$ ,  $\tau_m = 3.6$ ; Equat. (7): - A = 10.52, B = 3.5 сут., c = 1.016 сут. The biomass of the RG.  $\overline{G}$ : Figure 2B. For all values:  $S_0$  N = 0.9, n = 3. Equat. (4).  $S_0=0.04$  ,  $\gamma=0.14$  ,  $\mu_1=0.034$  ,  $\overline{G}_m$  =3.6,  $\tau_m pprox 30$  cyt.  $S_0=0.05$  ,  $\gamma=0.078$  ,  $\mu_1=0.028$  ,  $\overline{G}_m$  =2.6,  $\tau_mpprox$  30 cyr.  $S_0=0.06$  ,  $\gamma=0.06$  ,  $\mu_1=0.026$  ,  $\overline{G}_m$  =2.23,  $\tau_m\approx 30$  cyr. Table 1

Where a = 3.8, b = 2.7, f = 303, c = 0.6. In this case, with the dissociation coefficient –  $K_d = 0$ , the difference (a-b)=0.67, and the function  $\gamma(K_d) \rightarrow \text{const} = a$ . It is quite difficult to give all this some kind of physico-biological meaning. Most likely, this dependence is associated with a high rate of oxygen consumption by microbial cells, at the initial stage of cultivation, in the process of their production of highly active acids.

Secondly, the time  $\tau = \tau_m \approx \mathbf{B}$  of day during which the function  $\overline{G}(\tau)$  reaches its maximum value does not depend on  $S_0$ . At the same time, with an increase in  $S_0$  the maximum value of the function  $\overline{G}(\tau) - \overline{G}_m$ , the specific rates of biomass growth RG -  $\gamma$  and the specific rates of cell death  $\mu_1$  decrease.

IN general, studies have shown the dependence of the parameters included in equations (1) – (7) of the studied parameters on their initial values, acid dissociation constants, and sugar concentrations in the culture medium. In other words, it is necessary to establish explicit functional connections -  $\gamma(S_0, K_0, O_0, G_0, K_d)$ ,  $N(S_0, K_0, O_0, G_0, K_d)$ ,  $n(S_0, K_0, O_0, G_0, K_d)$ ,  $\mu_1(S_0, K_0, O_0, G_0, K_d)$ . However, this requires additional, more extensive and accurate experimental studies on the kinetics of

changes in the chemical composition of infusions throughout the cultivation process. This task cannot be avoided if infusions are used to extract certain metabolites from them. For this, it is necessary to know the time of selection of the culture liquid from the bioreactor for its further processing.

Without disclosing these problems, all previous discussions about the stages and periods of development of fungi, for the most part, are presumptive. Therefore, equations (1) - (7) can be taken as mathematical models of the kinetics of only particular processes that occur in culture media: biomass growth, oxygen consumption by fungi, changes in the active and titrated acidity of their infusions. However, despite the fact that the studies performed do not make it possible to fully reveal the kinetic patterns of fungal development, they are necessary to search for a more complete MM.

### Conclusion

- Based on a comparative analysis of theoretical and experimental studies of the cultivation of zooglue fungi *Medusomyces gisevi* and *Oryzamyces indicia* RSC, an analogy was established in the change and concentration of oxygen in their infusions, despite significant differences in morphological properties, which made it possible to describe the kinetic patterns of their development using unified equations.
- 2. It has been established that the formation of highly active acids during the cultivation of both fungi occurs at the initial stage of cultivation, for three periods, with the obligatory presence of oxygen dissolved in the initial culture medium. The time of the periods has been clarified.
- A complex functional relationship has been established between the acid dissociation constants and the specific velocities included in equations (4) (7), some proportionality coefficients and exponents. However, it turned out to be difficult to give a clear explanation of the discovered connections due to the lack of necessary experimental data.
- 4. Mathematical processing of experimental results of studies of the kinetics of the development of zooglue fungi allowed: to delve more deeply into the essence of the physico-biological processes taking place in the cultural environment; to detect shortcomings in experimental studies and outline ways to improve them.

Thus, a comparative analysis of the kinetic patterns of development of two fungi of the genus zooglea, presented in this article, showed that there are many similarities in their development processes, but much remains far from unexplained. The dependence of the parameters included in equations (1) - (7) of the studied parameters on their initial values, acid dissociation constants, in the nutrient medium is not entirely clear. In other words, it is necessary to establish explicit functional relationships –  $\gamma$  (S<sub>0</sub>, K<sub>0</sub>, O<sub>0</sub>, G<sub>0</sub>, K<sub>d</sub>), N (S<sub>0</sub>, K<sub>0</sub>, O<sub>0</sub>, G<sub>0</sub>, K<sub>d</sub>), N (S<sub>0</sub>, K<sub>0</sub>, O<sub>0</sub>, G<sub>0</sub>, K<sub>d</sub>), n (S<sub>0</sub>, K<sub>0</sub>, O<sub>0</sub>, G<sub>0</sub>, K<sub>d</sub>),  $\mu_1$  (S<sub>0</sub>, K<sub>0</sub>, O<sub>0</sub>, G<sub>0</sub>, K<sub>d</sub>). However, this requires additional, more extensive and accurate experimental studies of the kinetics of changes in the chemical composition of infusions throughout the cultivation process. This task cannot be avoided if infusions are used to extract certain metabolites from them. To do this, it is necessary to know the time of selection of the culture liquid from the bioreactor for its further processing.

Without answering the questions that have arisen, all previous discussions about the stages and periods of development of fungi, for the most part, are assumed. Consequently, equations (1) - (7) can be accepted as mathematical models of the kinetics of only specific processes: biomass growth, oxygen consumption by fungi, changes in the active and titrated acidity of their infusions. It is especially important to monitor the progress of these processes at the initial stages of cultivation, when not only the acidic, but also the general chemical composition of the infusions changes. However, despite the fact that the conducted studies do not allow us to fully reveal the kinetic patterns of fungal development, they will be necessary to search for a more complete MM.

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