STUDYING THE BIOKINETICS OF PIGMENTED YEAST BY STOCHASTIC METHODS

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Abstract: A country that owns high-performance computing facilities and mathematical modeling algorithms is able to provide its competitiveness in all the sectors of economy. The application of mathematical modeling is environmentally safe and cost effective and increases the technical and general culture of production. First of all, this concerns the development of new technologies. That is why the creation of new products in both the pharmaceutical and food industries is already impossible without the use of mathematical modeling. Today this is a necessity, the fulfilment of which has already been specified in relevant technical regulations. The simultaneous use of both mathematical methods and experiment provides not only the reduction of time, energy, and financial expenditures, but also the acquisition of additional information and the establishment of a direction of studies. This considerably reduces the time between the generation of an idea and its implementation in the form of a product. The technologies of the use of L-phenylalanine ammonia-lyase for the achievement of certain objectives in medicine, biotechnology, agriculture, and food industry have been developed by now. The insufficient application of algorithmic and mathematical approaches by researchers for development and analysis can be considered as a factor limiting the active use of biotechnological methods in the production of this enzyme. The description of microbial biosynthesis mechanisms by classical mathematical methods encounter some difficulties due to the combined effect of numerous chemical, physical, biological, engineering, and other factors. Another important thing is the more profound study of the kinetics of microbiological synthesis susceptible to both internal and external effects. The batch cultivation of pigmented yeast has been studied by probabilistic methods. A stochastic model providing the system study of the biosynthesis of L-phenylalanine ammonia-lyase by pigmented yeast has been formulated. The cultivation of microorganisms is described by the birth-and-death process. The mathematical expectation and dispersion of the number of population members are proposed as efficiency characteristics. The dependence between the amount of synthesized enzyme and the birth and death rates of a cultivated population is derived through the concentration of cultivated microorganism biomass and the birth and death rates of its members.

Key words: cultivation of microorganisms, biosynthesis of enzyme, probability, stochastic model, mathematical expectation, dispersion, differential equations

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INTRODUCTION

Microbiological synthesis products (enzymes) find wide application in various branches of petrochemical, food, and processing industries, medicine, pharmacology, etc. The principal stages of fermentation are the selection of a producing strain and the determination of conditions for its cultivation, during which the microbiosynthesis of a certain enzyme proceeds. Fermentation is most often implemented as a continuous process (maintaining the cultivation conditions throughout the entire period of its duration), but many metabolites can be obtained only via batch synthesis with the withdrawal of a product at the end of the process. The regularities governing the formation of an enzyme complex strongly depend on many process parameters (physicochemical, engineering, biological, and other factors [1–7]), the adjustment of which provides the efficient organization of this process. The technology of such a process usually ensures the simultaneous attainment of a maximum of both productivity and quality at minimum expenditures.

Since the synthesized enzyme is extracted from a cultivated biomass or a cultural liquid, it is necessary to perform a series of studies on the effect of the birth and death rates of cultivated population members on the amount of formed biomass. The consumption of time in this project will be reduced by constructing a mathematical model, which adequately describes the process of fermentation.

The objective of this work is to construct mathematical models providing the system description of the microbial cultivation and biosynthesis of the target enzyme and to study the kinetic regularities governing the duration of batch cultivation.

OBJECTS AND METHODS OF STUDY

Biokinetic regularities were studied using the cultivation of the *Escherichia coli* strain, a recombinant *Rhoodesporidium foruloides* L-phenylalanine ammonia-lyase producer, grown at the Research Institute of Bioengineering of the Kemerovo Institute

of Food Science and Technology [8–10]. The submerged batch cultivation of yeast in a fermenter was performed following the existing producer recommendations (in a culture medium containing (g/l): glucose, 20.0; peptone, 10.0; yeast extract, 5.0), at a temperature of 26°C for 24 h in the regime of non-controlled pH. Control measurements were performed every half hour beginning from the moment of pitching. The biomass and protein concentrations were determined from the absorbance in compliance with the manufacturer's manuals for an UV-1800 spectrophotometer (Shimadzu, Japan) [9].

Mathematical models were constructed using the tools of probability and stochastic process and queueing theories, in particular, the birth-and-death process [11–20] together with the differential equation generation method designed immediately for the derivation of the mathematical expectations and dispersions of random values [21–24].

The process of biokinetics is considered as a dynamic system of flows. This allows it to be described as a queueing system represented by a labeled graph of states (Fig. 1). The source of arrivals (birth of population members) is assumed to be inexhaustible and characterized by the parameter μ. The death of population members is considered to be the service of arrivals and characterized by the parameter λ. The state C_n is understood to mean a system state, in which the population size is equal to n. The transition from the state C_n to the state C_{n+1} means that the population size has increased by unity, and the transition from C_n to C_{n-1} means the death of one population member. It has been assumed that the fraction of cells dying per time unit is *averagingly* the same independently of the start moment of time counting [2, 25, 26]. The kinetics of the growth and death of a microorganism population is described with an exponential distribution [18, 19, 21].

Model. Let μ be the intensity of a flow of arrivals to a queueing system. The number k of arrivals to a system is a random value ξ obeying the Poisson law

$$P\{\xi = k\} = \frac{(\mu t)^k}{k!} e^{-\mu t},$$

where the intensity μ is determined as the average number of population members born per unit time, $t \in [0,\infty)$, $k = 0, 1, 2, \ldots$.

An arrival that has just entered the system is immediately served. The service time is a random value η distributed in compliance with the exponential law

$$P(\eta < t) = 1 - e^{-\lambda t},$$

where $\lambda = 1/t_{\text{av}}$ is the intensity of service, and t_{av} is the average arrival service time determined as the average life of a population member.

It is required to find the mathematical expectation $M_i(t)$ (average value) of the random parameter characterizing the number of cultivated population members at a time moment t under the condition that their number is $M_i(0) = i$ at an initial time moment and the dispersion is $D(t)$, $D(0) = 0$, $t \in [0,\infty)$, $i = 0, 1, 2, \ldots$.

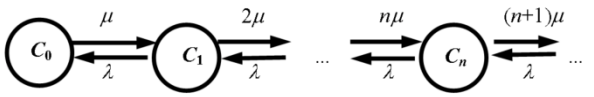

Fig. 1. Labelled graph of states C_n, n = 0,1,2,…, , for a system S, where μ and λ are the parameters characterizing the process.

The formulated model is described by the birth-and-death process formalized with the cognominal set of equations, which correspond to the labelled graph of states in Fig. 1 [11, 22]. Let us introduce the generating function $F(z,i,t) = \sum_{k=0}^{i} z^k P_k(t)$, where $P_k(t)$ is the probability that the system is in the state C_n, $n = 0, 1, 2, \ldots$, at a time moment $t \in [0,\infty)$, and apply it to the set of birth-and-death equations. Performing necessary rearrangements, we obtain the partial differential equation [11, 21, 22, 24]

$$\frac{\partial}{\partial t} F(z,t,i) + (z-1)n\lambda \frac{\partial}{\partial z} F(z,t,i) = \mu(z-1)F(z,t,i), \quad (1)$$

with the initial condition

$$F(0,t,i) = z^i$$

(z is the complex variable, and $|z|<1$), from which we obtain the set

$$\begin{cases} \dfrac{dM_i(t)}{dt} + \lambda M_i(t) = \mu, \\ \dfrac{dD(t)}{dt} + 2\lambda D(t) = \dfrac{d}{dt}(M_i^2(t) - M_i(t)) - \\ -2\lambda(M_i^2(t) - M_i(t)) + 2\mu M_i(t). \end{cases} \quad (2)$$

in compliance with the definition and method [21–24] for the generation of equations just for momenta.

The solution of set (2) with consideration for the initial conditions

$$M_i(0) = i,\ D(0) = 0$$

has the form [21, 23, 24]:

$$M_i(t) = \frac{\mu}{\lambda} + \left(i - \frac{\mu}{\lambda} \right) \cdot e^{\lambda t}, \quad (3)$$

$$D(t) = (1 - e^{-\lambda t}) \cdot \left(\frac{\mu}{\lambda} + i \cdot e^{-\lambda t} \right). \quad (4)$$

The amount of the target microorganism cultivation product is determined from the dependence [25, 26]

$$\frac{dP(t)}{dt} = \alpha \frac{dX(t)}{dt} + \beta X(t), \quad (5)$$

where $X(t)$ is the biomass concentration, $P(t)$ is the product concentration, and α and β are the size constants of growing and non-growing associates in g/g and g/(g h), respectively.

RESULTS AND DISCUSSION

The batch cultivation of pigmented yeast has been

modelled. A microorganism population member is understood to mean 1 gram of biomass in a unit cultural solution volume at each time moment t, $t \in [0,\infty)$. Since μ and λ are the average population growth and death rates per unit time, respectively, they can be determined by considering the studied process as pure birth [11, 12]. From Eq. (1) at $\lambda = 0$ we similarly obtain

$$\frac{\partial}{\partial t} F(z,t,i) = \mu(z-1)F(z,t,i) \qquad (6)$$

From Eq. (6) we obtain the differential equation

$$\frac{dM_i(t)}{dt} = \mu,$$

the solution of which at the initial condition

$$M_i(t_0) = M_0$$

has the form

$$M_0(t) = \mu \cdot t + M_0, \qquad (7)$$

The growth of a population during the batch cultivation of microorganisms has a complicated character: the growth of the amount of biomass is intensively accelerated within a short time period (exponential growth phase) after initial relative constancy [1–6]. For this reason, the death of population members during this process period was neglected. Denoting the initial and finite time moments characterizing the exponential growth phase (with a maximum population growth rate) as t_0 and t_1, respectively, we determined this interval for our sampling as (3, 3.5). The further increase in the duration of cultivation is accompanied by the death of population members, thus decelerating the growth of the biomass concentration (Fig. 2a). The average growth rate of population members μ =0.4094g/(l h) was determined using Eq. (7) $M_0(t)$ = 0.4094·t+1.2876 and the equation of a line passing through two points (Fig. 2b).

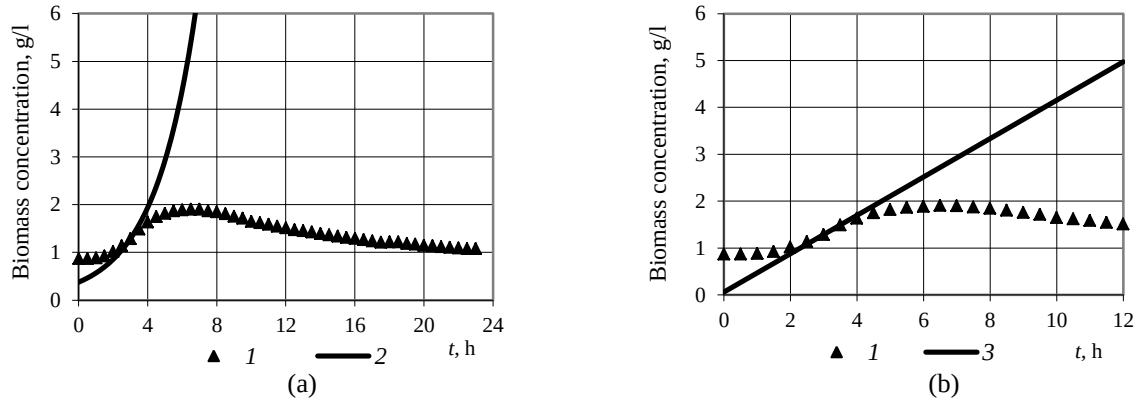

Fig. 2. Biomass concentration versus cultivation duration: (*1*) experimental data, (*2*) exponential growth curve $M_0 \exp(\mu(t-t_0))$, (*3*) $M_0(t)$.

The comparison of experimental and theoretical values of $M_0(t)$ enable us to estimate the average deviation between them on the interval from t_1 to t_2 h, where t_2 is the time moment, at which the death of population members begin to prevail over their birth, and to calculate $\lambda = 0.112$ g/(l h) from it as the average number of dead population members per unit time.

Let $M(t)$ be the average concentration of the biomass of cultivated microorganisms at a time moment t, $t \in [t_0,\infty)$; then, using the solution of set (2) with the initial conditions

$$M(t_0) = M_0,\ D(t_0) = 0,$$

we obtain [21, 23, 24]

$$M(t) = \frac{\mu}{\lambda} + \left(M_0 - \frac{\mu}{\lambda}\right) \cdot e^{\lambda(t-t_0)}, \qquad (8)$$

$$D(t) = \left(1 - e^{-\lambda(t-t_0)}\right) \cdot \left(\frac{\mu}{\lambda} + M_0 \cdot e^{-\lambda(t-t_0)}\right), \qquad (9)$$

The found parameters μ, λ, and t_0 were substituted into Eqs. (8) and (9). The obtained results of modeling with consideration for the mean square deviation $\sqrt{D(t)}$ are plotted in Fig. 3.

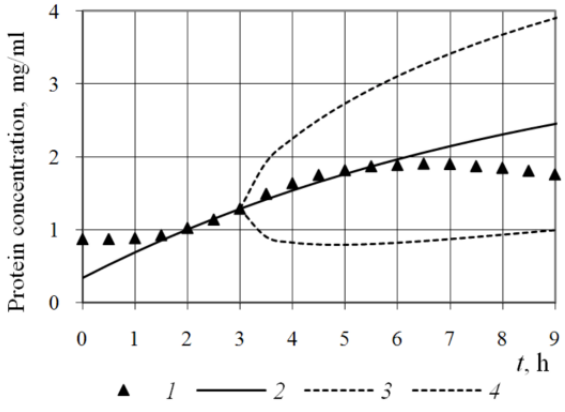

Fig. 3. Protein concentration versus cultivation duration: (*1*) experimental data, (*2*) $M(t)$, (*3*, *4*) $M(t) \pm \sqrt{D(t)}$.

In the considered variant, cultivation for more than 8 h is unreasonable and leads to additional time and energy expenditures. This is explained by a high death rate of population members, as the deviation between theoretical and experimental values in this case is more

than half the theoretical "ideal" biomass concentration. Moreover, it is desirable to restrict the process of cultivation by 6.0 ± 0.5 h for our strain characterized by the synthesis of a higher target product fraction with respect to the cultivated biomass [8–10]. A more precise value of t_2 can be obtained after analyzing the quality and amount of the target fermentation product. The proposed model gives a rather adequate description up to 12 h of cultivation (calculation error does not exceed 10%). The use of the standard deviation alone enables the estimation of the interval, into which experimental biomass concentrations almost reliably (with a probability > 0.95) fall in the case of cultivating the considered microorganism strain under the earlier defined conditions.

The biosynthesis of the target product (enzyme) was analyzed from the viewpoint of the dependence between the concentrations of the yeast biomass and the product of its activity (produced enzyme). Let us use Eq. (5) and modify it taking into account Eq. (3) as

$$\frac{dP(t)}{dt} = \gamma_P \frac{dM(t)}{dt} + \delta_P, P(t_0) = P_0 , \qquad (10)$$

where γ_P is the product yield with respect to the formed biomass, mg/g, and δ_P is the correction coefficient, mg/(ml h).

The solution of Eq. (10) has the form

$$P(t)=\gamma_P (M(t)-X_0)+\delta_P (t-t_0)+P_0, \qquad (11)$$

The average fraction of synthesized protein per unit weight of the formed biomass of the pigmented yeast strain under the earlier selected cultivation conditions is $\gamma_P = 0.27$ mg/g (corresponding to a nearly 33.5% expression for cultivation without induction [8, 10]). Our studies show that the averaged parameter $\delta_P = 0.11$ mg/(ml h) for different pigmented yeast strains, including the strain considered in this work. Note that the calculation error is up to 9.0% for the considered strain (from 5 to 12% for other strains), if the parameter δ_P is considered to be nonsignificant. The use of the averaged value of this parameter has allowed the calculation error to be decreased, in some cases, by three times. We defined this parameter as the fraction of the target product (protein), which passes into solution from the biomass of dead species (microorganisms both accumulate the enzyme inside and secret it into a cultivation solution during their life activity). It appears that this parameter directly depends on both the intensity of the death of population members (λ) and the amount of enzyme accumulated inside a microorganism. The experimental and theoretical dependences $P(t)=0.27(M(t)-M_0)+0.11(t-t_0)+P_0$ of the concentration of formed enzyme (L-phenylalanine ammonia-lyase) in the cultivation of pigmented yeast on the interval $[t_0, t_2]$ are plotted in Fig. 4. An increase in the cultivation duration above 6.0 h reduces the amount of both biomass and enzyme. The target product loss is more than 5% of the maximum amount for 8 h of cultivation and nearly 15% for 10 h of cultivation in addition to energetic and material expenditures. It is obvious that the duration must not exceed 5.5–6.0 h for our strain. Further refinement is possible during the activity studies of the produced enzyme. The parameter t_2, at which not only the maximum amount of the target microorganism cultivation product, but also its highest quality is attained, is uniquely determined in this case.

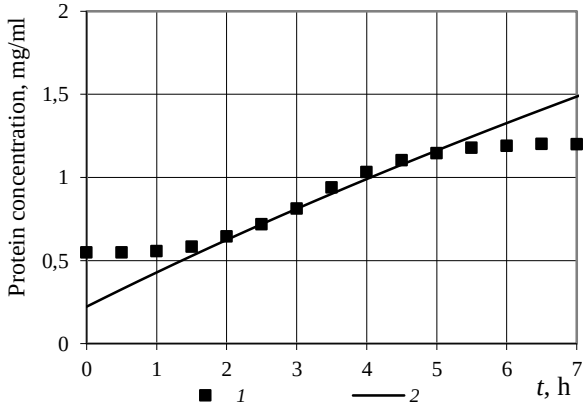

Fig. 4. Target biosynthesis product concentration versus cultivation duration: (*1*) experimental data, (*2*) $P(t)$.

The constructed stochastic model has allowed us to find the mathematical expectation and dispersion of the number of microorganism population members at an arbitrary moment of cultivation, to determine the recommended duration of fermentation, and to describe the amount of the synthesized enzyme. A method of estimating the parameters of this model has also been developed.

The batch cultivation of pigmented yeast was modeled using the well-known Markovian "birth-and-death" process. However, the Markovian process proved to be insufficient for the system study of the process of cultivation, as its characteristic property, such as the absence of aftereffects, was violated [24]. The use of heuristic equation (5) transformed into Eq. (10) after modernization has allowed us to solve the formulated problem. Naturally, the proposed model has some shortcomings, e.g., very high dispersion, and is sewed of two processes, but the application of numerical method in this case gives only a quantitative results and does not reflect the depth of study.

Hence, the system study of the cultivation of pigmented yeast has been performed. The relationship between the synthesized enzyme amount and the birth and death rates of a cultivated population has been derived through the concentration of the biomass of cultivated microorganisms and the birth and death rates of its members. The proposed model has provided the quantitative description of the biosynthesis of L-phenylalanine ammonia-lyase and the selection of a recommended duration for the cultivation of a producing strain for the efficient organization of biosynthesis.

REFERENCES

1. Varfolomeev, S. D. and Kalyuzhnyi, S.V., *Biotekhnologiya: Kineticheskie osnovy mikrobiologicheskikh protsessov* (Biotechnology: Kinetic Principles of Microbiological Processes), Moscow: Vysshaya shkola, 1990.
2. Rubin, A.B., Pyr'eva, N.F., and Riznichenko, G.Yu., *Kinetika biologicheskikh protsessov* (Kinetics of Biological Processes), Moscow: Izd. MGU, 1987.
3. Pirt, S.J., *Principles of Microbial and Cell Cultivation*, Oxford: Blackwell, 1975.
4. Tsiperovich, A.S., *Fermenty. Osnovy khimii i tekhnologii* (Enzymes. Principles of Chemistry and Technology), Kiev: Tekhnika, 1971.
5. Nikitin, G.A., *Biokhimicheskie osnovy mikrobiologicheskikh proizvodstv* (Biochemical Principles of Microbiological Production), Kiev: Vysshaya shkola, 1992.
6. Bisswanger, H., *Enzyme Kinetics. Principles and Methods*, Weinheim: Wiley-VCH, 2008.
7. Glick, B.R. and Pasternak, J.J., *Molecular Biotechnology. Principles and Application of Recombinant DNA*, Washington, DC: ASM Press, 2000.
8. Babich, O.O., Soldatova, L.S., and Razumnikova. I.S., Klonirovanie i ekspressiya gena L-fenilalanin-ammonii-liazy v *Escherichia Coli* i vydelenie rekombinantnogo belka (Cloning and expression of the L-phenylalanine-ammonium-lyase gene in *Escherichia Coli* and extraction of recombinant protein), *Tekhnika i tekhnologiya pishchevykh proizvodstv* (Technics and Technology of Food Industry), 2011, no. 1, pp. 8–13.
9. Babich, O.O., *Biotekhnologiya L-fenilalanin-ammonii-liazy* (Biotechnology of L-Phenylalanine-ammonium-lyase), Novosibirsk: Nauka, 2013.
10. Babich, O.O., Dyshlyuk, L.S., and Milent'eva, I.S., Expression of recombinant L-phenylalanine ammonia-lyase in *Escherichia Coli*, *Food and Raw Materials*, 2013, vol. 1, no. 1, pp. 48–53.
11. Feller, W. *An Introduction to Probability Theory and Its Applications*, New York: Wiley, 1968, vol. 1.
12. Venttsel', E.S. and Ovcharov, L.A., *Teoriya sluchainykh protsessov i ee inzhenernye prilozheniya* (Theory of Stochastic Processes and Its Engineering Applications), Moscow: Vysshaya shkola, 2000.
13. Kleinrock, L. *Queueing Systems. Theory*, New York: Wiley, 1975, vol. 1.
14. Saaty, T.L., *Elements of Queueing Theory with Application*, New York: McGraw Hill, 1961.
15. Haefner, J.W. *Modeling Biological Systems: Principles and Applications*, New York: Springer, 2005.
16. Zhu, G.-Y., Zamamiri, A.M., Henson, M.A., and Hjortso, M.A., Model predictive control of continuous yeast bioreactors using cell population models, *Chemical Engineering Science*, 2000, vol. 55, no. 24, pp. 6155–6167.
17. Zhang, Y., Henson, M.A., and Kevrekidis, Y.G., Nonlinear model reduction for dynamic analysis of cell population models, *Chemical Engineering Science*, 2003, vol. 58, no. 2, pp. 429–445.
18. Xiong, R., Xie, G., Edmondson, A.E., and Sheard, M.A., A mathematical model for bacterial inactivation, *International Journal of Food Microbiology*, 1999, vol. 46, no. 1, pp. 45–55.
19. Gordeeva, Yu.L., Ivashkin, Yu.A., and Gordeev, L.S., Modeling the continuous biotechnological process of lactic acid production, *Theoretical Foundations of Chemical Engineering*, 2012, vol. 46, no. 3, pp. 279–283.
20. Skichko, A.S. and Kol'tsova, E.M., Mathematical model for describing oscillations of bacterial biomass, *Theoretical Foundations of Chemical Engineering*, 2006, vol. 40, no. 5, pp. 503–513.
21. Khoroshevsky, V.G. and Pavsky, V.A., Calculating the efficiency indices of distributed computer system functioning, *Optoelectronics, Instrumentation and Data Processing*, 2008, vol. 44, no. 2, pp. 95–104.
22. Yustratov, V.P., Pavskii, V.A., Krasnova, T.A., and Ivanova, S.A., Mathematical modeling of electrodialysis demineralization using a stochastic model, *Theoretical Foundations of Chemical Engineering*, 2005, vol. 39, no. 3, pp. 259–262.
23. Ivanova, S.A. *Stokhasticheskie modeli tekhnologicheskikh protsessov pererebotki dispersnykh system obezzhirennogo moloka* (Stochastic Models of the Processing of Dispersed Skim Milk Systems), Kemerovo, KemTIPP, 2010.
24. Pavsky, V.A., Pavsky, K.V., and Khoroshevsky, V.G., Vychisleniye pokazatelei zhivuchesti raspredelennykh vychislitel'nykh sistem i osushchestvimosti resheniya zadachi (Calculating the characteristics of the robustness of distributed computational systems and the feasibility of problem solutions), *Iskusstvennyi intellect* (Artificial Intelligence), 2006, no. 4, pp. 28–34.
25. Riznichenko, G.Yu., *Matematicheskie modeli v biofizike i ekologii* (Mathematical Models in Biophysics and Ecology), Moscow–Izhevsk: Institut komp'yuternykh issledovanii, 2003.
26. Bozhkov, A.I., *Biotekhnologiya. Fundamental'nye i promyshlennye aspekty* (Biotechnology. Fundamental and Industrial Aspects), Kharkiv: Fedorko, 2008.