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MODELING OF MICROBIAL SYNTHESIS PROCESSES CONSIDERING INOCULUMS IMMOBILIZATION IN POROUS NANOSTRUCTURED MEDIA

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Abstract. The results of analytical relationships refinement based on the system of differential equations application of material balance for microbiological synthesis batch process are presented. Improved technique for solving the system is based on the definition of *potential energy* batch of microbiological synthesis and *new formula of specific growth rate* of microorganisms' biomass concentration.

1. Introduction

The problem of modeling the production of microbial biomass is merely a special case of the more general problem of modeling any product production process. Indeed, the general problem of modeling the production process of a product expressly or implicitly contains two components: the modeling of the production process of the product itself and modeling of the profit dynamics on the production products sales (the economic component). The framework of the project didn't aim to model the process of microbial biomass sales, that is the economic component was ignored, which is generally a limiting assumption (but only from the point of view of the economy), as the economic component has a significant impact on production.

Results presented below allow simulating the process of production of biologicals in the idealized case, where no restrictions are placed on output. The approach implemented for solving the general problem of modeling the production process (including the economic component), is based on a *new concept in the interpretation of the system of differential equations of material balance* for periodic processes of microbiological synthesis. Typically, the economic component of the manufacturing process modeling for some products is studied within the *theory of production functions* course. Applying this theory is limited by constructing the product manufacturing empirical relationship of time and production predefined factors (see, e.g. [1]). In deeper studies optimization techniques taking into account the constraints on the production factors are used.

Mathematical model of the fermentation process can be written as a system of differential equations of material balance for basic measurement in the output variables experiment: the concentration of microbial biomass, substrates, and metabolic products. Of the material balance differential equations for the entire system, which we do not present here

biomass production (or optimizing the production of any other product) consists in constructing a mathematical model for the dependence of product production (in this case, the microorganisms' biomass) on time and the predefined industrial unit performance (fermenter). Of course, in the production of mathematical models physical constants can also be present and they have to be determined. However, this is clearly formulated purposeful mathematical problem, which is called the *inverse problem* of the mathematical model of production.

In modern mathematics, the solution of inverse problems received much attention, as many modern technologies in industrial production are based on their solution. It is important to mention that the mathematical model of the production of microbial biomass taking into account the time and performance of the industrial unit is much more difficult than simpler models (1) and (2) that depends only on time.

Onwards we present the technique for solving the simplest inverse problem for a system of differential equations (1) and (2). For solving the inverse problem, we rewrite the formula for the specific growth rate in the following form

$$\mu(t) = \frac{\sigma X'(t)}{X(t)V(t)}, \tag{3}$$

where a constant factor σ is to be determined.

Now the formulation of the inverse problem for equation (1) can be reduced to the classical one. Let the concentration of microbial biomass is known at certain points of time (or each point of time.) It is necessary to determine the volume of the culture liquid in the apparatus (potential energy) and the factor σ, i.e., *the ratio* in equation (1).

On the basis of a new concept in the interpretation of the basic material balance equation (1) the following physical characteristics of the fermentation process were introduced:

- Potential of biomass concentration;
- Deformation of the cultural liquid volume in the apparatus;
- Deformation volume tension of cultural liquid in the apparatus;
- Volume deformation change of the cultural liquid in the apparatus;
- Transport coefficient of the nutrient solution;
- Change in the density of the inoculum;
- *Rate of natural increase in population;*
- *Coefficient of internal struggle in the seed population.*

Some of these physical properties after the corresponding change in terminology can be transferred to any other production product.

As a graphic illustration, the results of a natural experiment regarding the concentration of microbial biomass, which were specially interpolated with a polynomial, are shown. The scale factors in graphic pictures were chosen arbitrarily. It is important to note that this interpretation of the proposed method of microbiological synthesis modeling batch is also possible for describing periodic processes of any product production.

2. Specific growth rate and the physical principles of the fermenter operation

The volume of the cultural liquid in the device V is the potential energy of the fermentation process. Changing the potential energy of a system when moving from an arbitrary position P_0 in an arbitrary position $P_{1\ \text{is}}$ gives the work

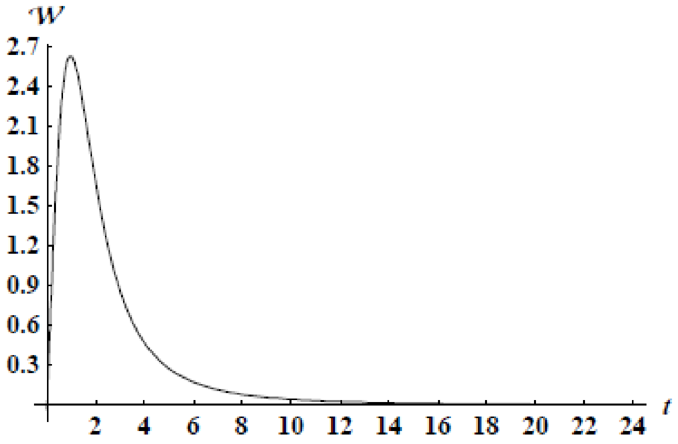
$$A = V(t_0) - V(t_1).$$

Solving the system of equations (1) and (2) relative to the volume of the cultural liquid in the

Potential biomass concentration *W* is the quantity characterizing the change (strain) state volume of cultural liquid in the apparatus, which is numerically equal to the consumption of nutrient medium with the opposite sign

$$W = \frac{C_0 C_1 (c_1 - c_0)}{C_0 - C_1} \frac{X'(t)}{X(t)^2}. \tag{7}$$

Graphical representation of potential biomass concentration is shown in Fig. 2.



Fig, 2. Potential biomass concentration.

Potential biomass concentration is the potential energy density a fermenter, and can be expressed as $\mathrm{A} = \int_{t_0}^{t_1} W(\xi)\mathrm{d}\xi$. Let's call the function $\frac{X'(t)}{X(t)^2} = \frac{C_0 - C_1}{C_0 C_1 (c_1 - c_0)} W(t)$ strain deformation volume of cultural liquid in the apparatus. Let's call the value $\frac{C_0 - C_1}{C_0 C_1 (c_1 - c_0)}$ change in the deformation volume of the cultural liquid in the apparatus. The energy that effects on changing the volume of the cultural liquid in the apparatus internally is spent by chemical reactions that are formed under the influence of external forces (stirrer speed, feed rate of oxygen, temperature, pH, etc.)

Let us denote the stock of chemical energy per unit mass of the cultural liquid by *Q(t)*. Internal forces chemical reactions energy can be spent both to increase biomass concentration (microorganisms particles detach - the growth of microorganisms "living tissue"), and to decrease the concentration of biomass (microorganisms accession particles - withering away microorganisms "living tissue"). Chemical energy is spent on the construction of microorganisms living tissue, and the external forces are spent on transportation nutrient medium to the microorganisms living tissue and under favorable conditions, there is a growth of biomass.

Thus, if the deformation strain is a negative value, we can talk about conditional accession of certain amount of biomass as a result of the aging process of microorganism healthy cells because of adverse growth conditions.

Time t_0 is the occurrence of this event corresponds to the solution of the equation

$$X'(t_0) = 0. \tag{8}$$

Loss of potentially possible biomass equals

$$X(t_0) - X(t),\ t \geq t_0. \tag{9}$$

4. Natural population increase and internal struggle coefficient in the seed population

In 1838 demographer P. Verhulst, based on the results of A.-L.-ZH. Ketle, his teacher and the famous Belgian statistics scientist, proposed a model for population growth, which was rediscovered in 1920 by Americans R. Perle and L. Reed. The model was named Verhulst-Pearl logistic growth model. The following is a description of *a general* Verhulst-Pearl law, and its output is based on the decision of the general Riccati equation with constant coefficients ($h \neq 0$):

$$y'(t) = fy(t)^2 + gy(t) + h.$$

Classical Verhulst-Pearl law is based on a solution of the simpler Riccati equation (h = 0):

$$y'(t) = fy(t)^2 + gy(t).$$

The solution of this equation was proposed by Verhulst [4-5], to describe *only* the microorganism-population-growth dynamics. As it was explained later (more than a hundred years later), this equation is of fundamental importance, and scenarios predicted within this equation were found in the description of some turbulent flow properties, as well as in research on laser physics, hydrodynamics and chemical reactions kinetics.

The essence of the Verhulst-Pearl law is that it expresses the concentration of microorganism biomass *X(t)* as a time function and only two physical values: the natural increase rate in population and the inner struggle coefficient in the seed culture population. That's enough to express all of the gained above dependences (e.g., the specific growth rate and the volume of cultural fluid in the apparatus) in terms of time, the natural increase rate in population and the coefficient of internal struggle in the seed population. Such representation simplifies qualitative study of the microorganism-biomass-concentration-behavior dynamics *X(t)* bringing it down to a foreseeable level. In some studies, the number of parameters is limited to one, i.e. to natural increase rate in population, considering it constant at a certain time interval. In the derivation of the *generalized*_Verhulst-Pearl law the number of living microorganisms *Y(t)* in the colony inoculum is taken proportional to the biomass concentration $Y(t) = \wp X(t)$.

It is assumed that the seed colony exists in the real natural conditions i.e. competition within a population, the lack of space and food, transmission of infection because of density, etc. Thus, it is necessary to find an expression for the law of living microorganisms' total number changing in the colony seed.

Let's assume first, that the colony lives in ideal conditions, has unlimited resources and power is not suppressed by other species. Due to the natural processes of birth and death, the number of colony's living organisms varies over time: the increase is proportional to the number of adult members. Let *Y(t)* is the number of organisms at time *t*, and *Y(t + Δt)* is the number of living microorganisms at time *t + Δt*. Then for a time interval *Δt*. *Y(t)* function increment will be

$$Y(t+\Delta t) - Y(t) = \Delta Y.$$

For time *Δt* all adult members of the colony (or part of them) produce offspring, and some members of the colony may die. Then

$$\Delta Y = N - M, \quad (10)$$

where *N* is the birth number during the time Δ*t*, *M* is the death number during the time Δ*t*.

The number of births *N* depends on the time length Δ*t* (the greater Δ*t*, the greater *N*) and on the number of parents (the more the adult organisms are, the more offspring they produce),

there are heat loses to the environment at a constant temperature U_1 = const.

It is required to solve the equation in the mathematical formulation

$$\partial_t u(r,t,z) = a^2(\partial_{r,r}u(r,t,z) + \frac{1}{r}\partial_r u(r,t,z) + \partial_{z,z}u(r,t,z)) + F(t),$$

with the following initial condition and boundary conditions on the lateral surface and the top of cylinder

$$u(r,0,z) = U_0, 0 \le r \le R, -h \le z \le h,$$

$$\partial_r u(r,t,z)_{r=R} = 0, -h \le z \le h, t > 0,$$

$$\partial_z u(r,t,z)_{z=h} = -q(u(r,t,h) - U_1), 0 \le r \le R, t > 0.$$

The presence of energy sources within the cylinder due to the fermenter with density *F(t)* is assumed.

The inverse problem is to determine the physical constants a^2, q and energy density *F(t)*, since the constants U_0 and U_1 we believe to be known. In calculating the coefficients a^2, q and energy density *F(t) sample values* obtained on the basis of *rational interpolation* are used. The average value of the direct problem (the variables *r and z*) as found by sampling the coefficients a^2, q and energy density *F(t)* is written below

$$mu(t) = 1.7620624\times10^{15} - 1.7620623\times10^{15}E^{-0.03622471t} - 6.386975\times10^{13}t + 1.1575347\times10^{12}t^2$$
$$-1.3979759\times10^{10}t^3 + 1.2588722\times10^8 t^4 - 862721.0397513t^5 + 3664.2611215t^6.$$

The average value of the *mu(t)* direct problem and sample data are shown in Fig. 5.

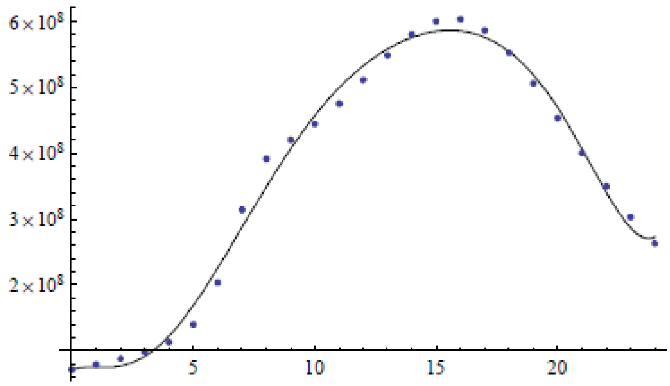


Fig. 5. Average value of the *mu(t)* direct problem and sample data.

Now we use the Verhulst-Pearl law to calculate the average specific rate of natural increase in population k. To do this, we remove the sample data with functions *mu(t)* with a time step of 1 hour and apply the least squares method to estimate parameters in a nonlinear Verhulst-Pearl model. Find average values $\lambda = 1.11018\times10^{-9}$ and $\Bbbk = 0.602537$. Then we write the Verhulst-Pearl law.

$$\frac{7.03781\times10^{15}E^{0.602537t}}{5.27863\times10^8 + 1.48746\times10^7 E^{0.602537t}}.$$

Apparent discrepancies between Verhulst-Pearl law and sample values in Fig. 6 may indicate two main reasons: first, the coefficient of specific rate of natural increase in the population

the smallness of the coefficients λ, ℘) and then the coefficient of specific rate of natural increase in population approximately coincides with the *classical* definition of specific growth rate. In this interpretation of the coefficients of equation

$$X'(t) = \Bbbk(t)X(t) - \lambda \wp X(t)^2$$

sample values of *mu(t)* and Verhulst-Pearl law will meet each other with sufficient accuracy. We do not give graphic factor of specific rate of natural increase in the population depending on the time as *visually* its graphic will coincide with the Fig. 1 for the specific growth rate in our definition (3).

Let's consider the same task, but under different fermenter operating characteristics: *Q* = 0.3, *n* = 89, where *Q* is an oxygen supply rate, and *n* is a stirrer rotational speed. There is no sample data for the mentioned performance values. There are only the following samples

KOE(*time*)={7.1×10^7, 1.1×10^8, $2.\times10^8$, 4.1×10^8, 4.2×10^8, 5.25×10^8, 6.3×10^8, 4.4×10^8, 2.8×10^8},
KOE(*time*)={1.7×10^7, 2.7×10^7, 6.5×10^7, 2.3×10^8, 2.4×10^8, 3.1×10^8, 3.4×10^8, 3.1×10^8, 4.2×10^8},
KOE(*time*)={1.5×10^7, 1.9×10^7, 4.6×10^7, 1.9×10^8, 1.9×10^8, 2.8×10^8, 2.9×10^8, 2.9×10^8, 2.9×10^8},
KOE(*time*)={1.3×10^7, 2.1×10^7, 5.1×10^7, 7.4×10^7, 7.5×10^7, 8.9×10^7, 9.5×10^7, 1.2×10^8, 3.6×10^8},
KOE(*time*)={1.5×10^7, 2×10^7, 6.2×10^7, 3.1×10^8, 3.2×10^8, 3×10^8, 3.2×10^8, 3×10^8, 3×10^8},
KOE(*time*)={4.7×10^7, 7.5×10^7, 9.5×10^7, 1.3×10^8, 1.4×10^8, 1.5×10^8, 4.1×10^8, 5×10^8, 4.4×10^8},

obtained for the time points 0, 4, 6, 8 , 10, 12, 16, 20, and 24 for six values performance equals {*Q*, *n*} = {1,50}, {0.7,50}, {0.5,50} {0,3,50}, {0.5,70} , {0.5,100}.

KOE concentration dependence as a function of time and performance *Q*, *n* should be found with the usage of special polynomial interpolation using the Maclaurin expansion, as the solution of the *inverse problem* for the physical and mathematical KOE model depending on the time, and performance of *Q*, *n* is quite difficult (even in the direct problem original formulation). Result of interpolation is denoted by the letter *g (t, Q, n).* Using the function *g (t, Q, n),* we form a set of sample values in the time interval [0, 24] with a time step of 1 hour: Sample data=(*I*, *g*(*I*,0.3/89)), *I* = 0,1,2,…,24 .

KOE initial value is equal to 6.82913×10^6. We use the sample data and the Verhulst-Pearl law in its simplest form to calculate the average value of the specific rate of natural increase in population $\Bbbk$. Parameter estimation in Verhulst-Pearl nonlinear model leads to the following results (see Fig. 8)

$$\Bbbk = 0.3358649,\ \lambda = 5.2084522\times10^{-6},\ \rho = 0.0005167\,.$$

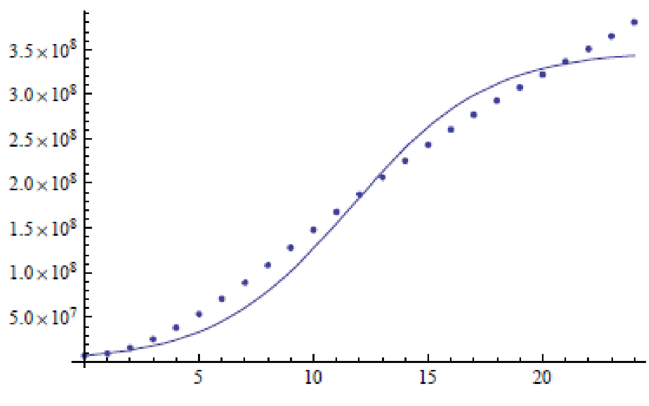


Fig. 8. Graph for Verhulst-Pearl law and sample values.

From the differential equation (11) for η = 0, as above, we find the coefficient of self-poisoning or internal struggle in the seed population.

$$\lambda(t) = \frac{(0.0001706(-4.45222+t)(-0.547357+t)(1442.84+(-68.6843+t)t))}{((1241.94+(-61.9011+t)t)^2(2.79385+t(0.126719+t))^2)}.$$

The results are presented in Fig. 9.

In this example the assumption of a constant specific rate of natural increase in population does not lead to some distortions in the interpretation of experimental data by Verhulst-Pearl law. This fact means that periodic fermentation process is still in its formative stages.

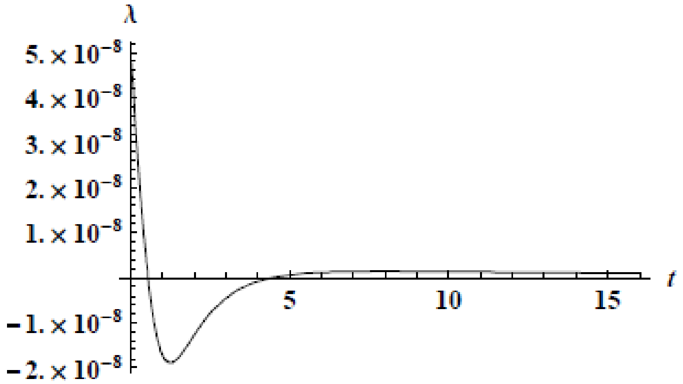


Fig. 9. Internal *struggle coefficient.*

4. Conclusions

To increase the efficiency and versatility of developed fermentation processes physical and mathematical modeling, following enhancements are included in the models developed:

1. The concepts of potential energy and kinetic energy of the periodic production processes have been introduced; developed and implemented software methods of mathematical modeling.

2. Analytical dependences between the main observable variables in the system of differential equations of material balance have been obtained.

3. A generalization of the Verhulst-Pearl law has been produced. This branch of research may even enter into the development framework of *nanotechnologies* to increase industrial and a decrease in microorganism biomass output and fermentation time decrease, since the change in the organizational structure of nano-objects causes a change in the coefficient of internal struggle and the specific rate of natural increase in the concentration of microorganism biomass.

It is important to note that the results obtained are of general nature and can be used not only in the description of the microbiological processes , but also in industry (see [2<1]), or in baking (see [2]), yet in various fields of natural sciences, even in the economy (see [1<3], for example.) In relation to the economy the main conclusion is the following: *the speed of capital movement* is the main component of earnings growth (*ceteris paribus*).

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