Mathematical models for microbiological processes of rye broth maturation

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Abstract. One of the factors determining the quality of bakery products made of rye and mixed rye and wheat flour is broth, the parameters of its technology, and process regulation by varying the parameters of its technology. First of all, it is necessary to note the availability of the nutrient substrate and the nature of the interaction of the main microflora of broth cultures - yeast and lactic acid bacteria. The mathematical model of the process of cultivating microorganisms in liquid rye broth was suggested as applied to yeast and lactic acid bacteria based on the adapted equation of N.V. Stepanova and N.D. Jerusalem to describe the growth of lactic streptococci. Based on model systems, the interaction of yeast and lactic acid bacteria in liquid rye broth was studied. The symbiotic nature of this interaction was confirmed. It was justified that Lotka - Voltaire system is the most correct way for its mathematical description. The growth dynamics of the populations of yeast and lactic acid bacteria was described, and the adequacy of the obtained mathematical model was verified.

1. Introduction

Bread made from rye and mixed rye and wheat flour is traditionally included in the consumer basket of Russians [1]. Excellent consumer properties of rye, mainly including favorable amino acid and mineral composition, define the interest in these products existing in other countries. [1]. At the same time, rye flour is quite problematic in terms of processing and requires specific technological methods. Traditionally, the method of inactivation of α-amylase, necessary to reduce the accumulation of dextrines and prevent the effect of sticky crumb, is the use of broths [2, 3]. Russian scientific school is well-known, which allowed not only scientifically substantiating, but also widely introducing into practice the technology of liquid, thick rye broths [2–5]. However, standardized methods within production environment require constant adjustment related to the properties of flour - the presence of carbohydrate nutrition and other nutrients in it is necessary for the multiplication of microorganisms. Accordingly, it is of interest to formalize microbiological and biotechnological processes in the broth, which will allow development of certain regulatory actions in an automated mode.

2. Materials and methods

Given there is prevalence in production conditions, liquid rye broth was selected as the object of study. The production cycle was carried out using dry lactobacterin and pure culture of S. cerevisiae L-1 yeast in accordance with the recipe and technological parameters of the “Collection of technological instructions for the production of bread and bakery products” [6].
To develop a mathematical model of the process of cultivating microorganisms of liquid rye broth, the system was structured in the form of its breakdown into subsystems or smaller structures in accordance with the task set for the research. The rye broth preparation process can be split into certain blocks (table 1) [7].

| Block                                      | Input parameters Name | Indication | Output parameters Name | Indication |
|--------------------------------------------|-----------------------|------------|------------------------|------------|
| Kinetics of microorganism growth           | Concentration of:     | X          | Specific microorganism growth rate, µ | h⁻¹       |
|                                            | microorganisms,       |            |                        |            |
|                                            | mln/cm³               |            |                        |            |
|                                            | substrate, %          | S          |                        |            |
|                                            | metabolism products, %| P          |                        |            |
|                                            | hydrogen ions, pH     | pH         |                        |            |
|                                            | Temperature, °C        | t          |                        |            |
| Kinetics of substrate consumption          | Same                  | Same       | Specific substrate consumption rate, a | mg/(g mln) |
| Kinetics of metabolism product formation   | Same                  | Same       | Specific metabolism product formation rate, b | mg/(g mln) |
| Mass balance                               | Specific rate of:     | µ          | Concentration of:      |            |
|                                            | microorganism growth, |            | substrate, %           | S          |
|                                            | h⁻¹                   |            | metabolism product, %  | P          |
|                                            | substrate consumption,| a          | microorganisms,         |            |
|                                            | mg/(g mln)            |            | mln/cm³              |            |
|                                            | metabolism product    | b          | hydrogen ions, pH      | pH         |
|                                            | product formation,     |            |                        |            |
|                                            | mg/(g mln)            |            |                        |            |

Taking into account the specifics of liquid rye broth from the point of view of the joint coexistence of yeast cells and lactic acid bacteria in it, at this stage of the research, the mathematical model was built based on the same principle, but separately for these two groups of microorganisms.

The sub-processes were mathematically presented as equations connecting input and output variables. Specific microorganism growth rate was set as the output variable for the equation of kinetics of biomass growth (block 1). At the first stage its dependence on the medium acidity (pH), microorganism concentration (X), available sugars concentration (S), amine nitrogen concentration (A), organic acids (P), carbonic oxide (Y), and ethanol concentration (C) was evaluated. Each of these parameters in its turn was defined as f(τ).

3. Results
Reasoning from theoretical premises, the main consequence of nanopowder introduction into the melt should be refinement of the macro- and microstructure, as the powder particles must serve as nuclei of new grains. Figures 1 and 2 show photos of the microstructure of cast samples from the bronze of the lead-tin bronze grade, both modified by SDP of aluminium oxide and without modifier addition. The phase composition of the bronze under study represented in photos of the microstructure is a solid solution of tin in copper, lead inclusions and eutectoid inclusions based on electron compound.
Analysis of the obtained dependencies allowed identification of the key factors affecting microorganism growth, which are the concentration of substrate (digestible carbohydrates) and metabolism products (the total of all organic acids for lactic acid bacteria and carbonic oxide for yeast). The equation suggested by N.V. Stepanova and N.D. Ierusalimskiy for description of lactic streptococci growth [7] was customized for description of microorganism growth kinetics.

\[
\mu = \frac{\mu_m \cdot S \cdot k_p}{\{S + k_s(1 + (S/S_m)^2)\}(k_p + P)},
\]

where \(\mu_m, k_s, k_p, S_m\) – constant coefficients; \(\mu_m\) - maximum specific growth rate for \(S\rightarrow\infty\), h\(^{-1}\); \(k_s\) – coefficient equal to substrate concentration when \(\mu = \mu_m/2\); \(k_p\) – coefficient taking into account inhibiting effect of the metabolism products on the microorganisms; \(S_m\) – maximum substrate concentration in the medium, %.

The mathematical model was completed with the equations for mass balance for the bacteria mass (exclusively to its autolysis) (block 4), nutritious substrate (block 2), metabolism products (block 3).

The resulting mathematical model of the process of cultivating microorganisms in liquid rye broth can be represented as follows:

for lactic acid bacteria

\[
\begin{align*}
\mu_x &= \frac{cX}{\partial \tau}, \\
\mu_y &= \frac{Y S}{\partial \tau}, \\
\mu_s &= \frac{1}{b c P} \frac{1}{\partial \tau}, \\
\mu &= 0.14 \mu_m S K_p \frac{1}{\{S + K_s[1 + (S/S_m)^2]\}(K_p + P)},
\end{align*}
\]

\(\mu_m = 0.256\) h\(^{-1}\); \(Y = \frac{cX}{S} = 4.2 \cdot 10^7\) mln/g; \(b = \frac{c P}{c X} = 2.8 \cdot 10^{-9}\) g/mln;

\(S_m=4,79\%\); \(K_p=5,7\); \(K_s=-1,5\);

for yeast

\[
\begin{align*}
\mu_x &= \frac{cX}{\partial \tau}, \\
\mu_y &= \frac{Y S}{\partial \tau}, \\
\mu_s &= \frac{1}{b c P} \frac{1}{\partial \tau}, \\
\mu &= 0.15 \mu_m S K_p \frac{1}{\{S + K_s[1 + (S/S_m)^2]\}(K_p + P)},
\end{align*}
\]

\(\mu_m = 0.241\) h\(^{-1}\); \(Y = \frac{cX}{S} = 4.9 \cdot 10^6\) mln/g; \(b = \frac{c P}{c X} = 2.5 \cdot 10^{-8}\) g/mln,

\(S_m = 5,92\%\); \(K_p = 7,4\); \(K_s = -1,6\)

where \(b\) – amount of metabolism product, produced by a unit of microbial mass (g/mln); \(Y\) – microorganism growth as a result of digesting of a unit of substrate mass, mln/g.

The obtained mathematical models adequately describe the real process and can be used to optimize it. The studies used the graphoanalytical method based on the use of the obtained patterns of development of yeast cells and lactic acid bacteria in the broth. In relation to the process under study, it suggests graphic presentation of the dependencies like \(X=f(S)\) and \(\mu=f(S)\), definition of the curve maxima and corresponding \(S\) values for lactic acid bacteria and yeast cells.

To calculate the numerical values of the functions, the developed application software package was used. It has been found that the maximum specific growth rate of yeast is observed with substrate concentration, for which mono- and disaccharides can be taken quite naturally: 4.5% for yeast and 3.7% for lactic acid bacteria. The calculated value of the concentration of microorganisms themselves in this case satisfies the existing opinion on the ratio of different groups in liquid rye broth [8].
Considering that the average growth in digestible sugars during the preparation of the broth, which we found experimentally in the model series, is about 6.2%, their initial content in the nutrient mixture should be within 2%. This value corresponds to the mass fraction of moisture of 72%. Besides, using the regression equation $\mu = f(A)$ and the graphoanalytical method described above, the optimum amino nitrogen content in it was found to be 0.12%.

Thus, the cultivation process of lactic acid bacteria and yeast in liquid rye broth is quite adequately described by the mathematical model, which is based on the multiplicative equation. The resulting model was used as a mathematical description in the development of the application software package for determining the optimal parameters for preparing liquid rye broth. However, it does not take into account the interaction factor of various groups of microorganisms of the broth - yeast cells and lactic acid bacteria, and therefore raises a number of questions on the soundness of its use to develop regulatory effects on the system as a whole. The next stage of research was the development of a model of the resource interaction of yeast and LAB under the conditions existing in liquid rye broth.

To clarify the nature of the relationship between microorganisms introduced in the form of pure cultures in the diluting cycle and extraneous microflora, model experiments were performed.

During the first series (henceforth model system D1) of model experiments only pure culture of S. cerevisiae L-1 yeast was inserted in the diluting cycle. Process parameters corresponded unified instruction for malt broth. Broth yeast was prepared by adding 1 cm$^3$ of tap water with temperature of 28-30 °C to a test tube with colonies of microorganism cells on jams of wort-agar, shaking and washing off yeast cells from jams in the nutrient mixture with a temperature of 31-33 °C, consisting of 100 g flour, 300 g of water and 100 g of malt.

In the established production cycle (after 15 updates), the dynamics of the main biochemical and microbiological characteristics was recorded.

Despite introducing only pure yeast cultures into the dilution cycle, increase in acidity and, accordingly, concentration of hydrogen ions was found in the semi-finished product. At the same time, the general acidity level was more than 2 times lower compared with the broth according to the unified scheme. The increase in acidity can be directly related to the vital activity of yeast. Yeast cells during fermentation, in addition to alcohol and CO$_2$, form some amount of acid [9]. For three hours of fermentation of mixed rye flour and water with the addition of 6% yeast, the acidity level reaches 3 degrees.

The constant by-product of alcoholic fermentation is lactic and acetic acid. So, in the presence of 10% of the initial sugar in the fermented medium, 1 g / dm$^3$ of lactic acid is formed. However, the main role in acid formation is assigned to lactic acid bacteria.

Gas generation is fully provided by yeast: its level exceeded the control variant by 12%. In all likelihood, this is due to the large number of yeast cells. So, in the production broth by the end of fermentation there were 89 million/g of them, and in the model system D1 - 164 million/g.

The change in reducing sugars is similar to the dependence we found earlier, although at a lower level. On the one hand, this can be associated with less intensive hydrolysis of starch polysaccharides of flour under the influence of organic acids, and on the other hand, their significantly higher consumption by yeast cells. As for amine nitrogen, with the general tendency to increase during fermentation, its absolute growth in the model experiment is 0.022%, in the control one - 0.033%, which can also be explained by the lower active acidity of the medium in the latter case and, accordingly, the intensive hydrolysis of protein substances.

To study the patterns of the development of LAB, the second series of model experiments (henceforth model system LAB) was set without use of yeast. There the dilution cycle was fulfilled with inserting pure LAB cultures in the form of lactobacterin.

This model broth was described by acidity accumulation of 4 degrees and final pH value of 3.95 compared to control 4.9 and 3.33 with preservation of the general regularities of the process. Despite the absence of pure yeast cultures in the dilution cycle, fermentation is associated by active emission of CO$_2$ (52 % of the level typical for ordinary broth). This is due to the active life of heterozygotic LAB, as well as, of course, the presence of yeast cells that spontaneously fall into the broth with flour.
Similar information is given in the works of G. Spicher et al. [10]. S. cerevisiae and Pichia saitoi yeast cultures were found in the ‘pure culture’ broths. The number of yeast cells in model system LAB\textsubscript{1} by the end of fermentation process was 70 ml/g, which is 12.7 % less than in that the control broth. However, it is necessary to note a 4-fold increase in yeast cells - the highest in comparison with other systems. This can be attributed to a significantly smaller initial amount and absence of nutrient deficiency.

The nature of the microorganism growth in model experiments convincingly indicates facultative symbiosis between these microorganisms. Moreover, these are LAB who needs the presence of yeast, because the latter, as it is known, give them irreplaceable growth factors - vitamins, amino acids, purine and pyrimidine bases [11].

To qualitatively characterize the described situation, we attempted to use the interaction index, which for periodic cultivation has the following form [12]:

\[ I = \frac{\text{Species growth in the mixed culture}}{\text{Species growth in the pure culture}} \] \hspace{1cm} (4)

In our case the interaction index for yeast is \( I_1 = 0.54 \), for LAB \( I_2 = 1.13 \); for mutual cultivation \( I = 0.88 \). In theory, the interaction for which \( I_1 < I, I_2 > I \) is called commensalism – amensalism. In relation to liquid rye broth, it can be classified as an optional symbiosis, characterized by stimulation of the LAB by yeast. Thus, mathematical processing fully confirmed our experimental results.

In the model system LAB\textsubscript{1}, the general regularities of changes in the mixture of the content of amine nitrogen and digestible sugars are observed. The level and nature of nutrient consumption, which is greater than the control sample, is associated with their use for the growth of biomass of microorganisms.

Thus, the own amylolytic and proteolytic enzymes, as well as the natural microflora of rye flour, have significant impact on the growth, development and accumulation of metabolic products of yeast cells and lactic acid bacteria in liquid rye broth. The series of experiments made it possible to find the numerical characteristics of the “economic” and other coefficients as applied to yeast and LAB when constructing the mathematical model of the process.

The process of resource interaction of the two populations can be presented as system (MC) of the following general structure:

\[ \text{MC} = \{\text{MC}_1, \text{MC}_2\}, \] \hspace{1cm} (5)

where \( \text{MC}_1, \text{MC}_2 \) – subsystems, where \( \text{MC}_1 \) – subsystem of one population (of yeast); and \( \text{MC}_2 \) – subsystem of the other population (lactic acid bacteria).

In turn, subsystems \( \text{MC}_1 \) and \( \text{MC}_2 \) are also systems, i.e. include a number of subsystems, for example, for various species and genera of any particular population of microorganisms. Thus, for malt-free broth, subsystem \( \text{MC}_1 \) includes yeast \( \text{S.cerevisiae L-1 and S.minor Chernorechenskiy} \), for malt broth it includes only \( \text{S.cerevisiae L-1} \). Subsystem \( \text{MC}_2 \) in both cases suggests the total of lactic acid bacteria \( \text{L. plantarum - 30, L. brevis - 1, L. fermenti - 34, and L. casei - 26} \). However, in this study for the purpose of simplification and demonstrativeness, subsystems \( \text{MC}_1 \) and \( \text{MC}_2 \) should be viewed as indivisible, i.e. without further detalization, as within the population the presented species as very similar in terms of physiological requirements and, which is more important, their functionality.

The elements of one system are affected by various factors, both external and internal. In technological processes of microbiological basis, the most common relationships between elements of the same system are internal relationships arising from the use of a single food resource. It is this type of interaction that is typical of yeast cells and LAB in the conditions of liquid rye broth and can be structurally represented as Figure 1.
M1, M2 – functions describing inter-species interaction of the populations; B1, B2 – functions describing intra-species interaction of the populations.

An analysis of the works devoted to the study of issues related to the competitive interaction of two populations [13–15] shows that the mathematical description of this interaction is most correct by the Lotka–Volterra system. This model accurately describes the processes occurring between populations: growth, limited by the elements of intra-population and inter-population interactions.

We note that in the process of cultivating microorganisms under conditions of liquid rye broth, populations of microorganisms having sufficiently large number regardless of the period of the production cycle are considered. The rate of change in population size is determined by the instantaneous values of the numbers themselves without delay. Therefore, the growth dynamics of the populations of yeast and lactic acid bacteria can be described by the Lotka-Volterra system [14–16], adapted for the specific interaction situation, which in this case has the form:

\[
\begin{align*}
\frac{dN}{dt} &= \mu_1 \cdot N \cdot \left(1 - \frac{N}{N_m} + \frac{\alpha_1 \cdot M}{N_m}\right) - c_1 \cdot N \\
\frac{dM}{dt} &= \mu_2 \cdot M \cdot \left(1 - \frac{M}{M_m} + \frac{\alpha_2 \cdot N}{M_m}\right) - c_2 \cdot M
\end{align*}
\]

where N – one population size, yeast cells, mln/g; M – the other population size, LAB cells, mln/g; c1, c2 – coefficients of natural mortality of the corresponding populations (yeast and LAB), h–1; \(\alpha_1, \alpha_2\) – coefficients describing inter-species interaction of the populations (for yeast and LAB cultures correspondingly); \(N_m, M_m\) – maximum possible population sizes (for yeast and LAB cultures), mln/g; \(\mu_1, \mu_2\) – specific growth rates for the corresponding populations (for yeast and LAB cultures), h–1.

The first and the last terms of the equation system (6) describe the diminution between the multiplication and mortality in the microorganism populations, i.e. show the amplification of the corresponding culture. After assessment, we can get three possible options for the population behavior depending on the difference in parameters \(\mu_k\) and \(c_k\):

1) \(\mu_k > c_k\), k=1,2, i.e. \((\mu_k - c_k) > 0\) – stable amplification of the corresponding population is observed;
2) \(\mu_k = c_k\), i.e. \((\mu_k - c_k) = 0\) – multiplication equals mortality. The number of microorganisms remains stable;
3) \(\mu_k < c_k\), i.e. \((\mu_k - c_k) < 0\) – extinction (die-off) is observed in the corresponding population.

The terms \((\mu_1 \cdot N^2/N_m)\) and \((\mu_2 \cdot M^2/M_m)\) describe intra-species interaction of the corresponding populations of microorganisms. It is associated with intra-species link functions B1 and B2 in the structural scheme. This dependence is the typical population growth model by Verhulst-Gauze [14].

Figure 1. Structural scheme of interaction between two microorganism populations.

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where N – one population size, yeast cells, mln/g; M – the other population size, LAB cells, mln/g; c1, c2 – coefficients of natural mortality of the corresponding populations (yeast and LAB), h–1; \(\alpha_1, \alpha_2\) – coefficients describing inter-species interaction of the populations (for yeast and LAB cultures correspondingly); \(N_m, M_m\) – maximum possible population sizes (for yeast and LAB cultures), mln/g; \(\mu_1, \mu_2\) – specific growth rates for the corresponding populations (for yeast and LAB cultures), h–1.

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The calculation of the mathematical model (6) using the experimentally obtained values of the parameters and coefficients was carried out using Trax program for studying the trajectories of dynamic systems with the following values of empirically found coefficients: \( \mu_1 = 0.24 \text{ h}^{-1}, \mu_2 = 0.26 \text{ h}^{-1}, \alpha_1 = 0.54, \alpha_2 = 1.13, N_m = 164 \text{ mln/g}, M_m = 593 \text{ mln/g}, c_1 = 0.015 \text{ h}^{-1}, c_2 = 0.01 \text{ h}^{-1} \).

The maximum deviation of the calculated data from the experimental data does not exceed 10% and amounts to 8.62% in liquid rye broth with malt in the first hour of fermentation, which is a satisfactory result. The standard deviation for yeast is 2.677, for lactic acid bacteria of 14.2.

4. Conclusion

Based on the data obtained, it can be concluded that the model of resource interaction between two populations of microorganisms of the Lotka - Volterra type (6) adequately describes the process of preparing liquid rye broth.

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