UDC 663.533

OPTIMIZATION OF THE YEAST CELL BIOSYNTHESIS PROCESS FOR THE FERMENTATION OF HIGH CONCENTRATION WORT IN BIOETHANOL PRODUCTION

DOI: https://doi.org/10.15673/fst.v16i4.2547

S. Kovalchuk[1], PhD, Assistant
Yu. Dolomakin[2], PhD, Associate Professor
[1]Department of hotel-restaurant business
[2]Department of Machines and Apparatuses for Food and Pharmaceutical Productions
National University of Food Technology
street Volodymyrska 68, Kyiv, Ukraine, 01601

Abstract. To prevent a decrease in the speed of the biotechnological process and achieve an optimal yield of the target product of biosynthesis (biomass), it is necessary to develop optimal technological parameters for the cultivation of production yeast Saccharomyces cerevisiae for fermentation of high-concentration grain wort. An increase in the concentration of dry substances of fermented wort and alcohol in brews leads to a slowdown in the processes of yeast cell synthesis and fermentation. Therefore, it is necessary to investigate the influence of the technological parameters of the cultivation of production yeast for the synthesis of the required concentration of yeast cells with high biochemical activity. Cultivation of industrial yeast was performed by an osmophilic thermotolerant race of alcoholic yeast *Saccharomyces cerevisiae* DO-16 (IMB Y - 5099) at a temperature of 30, 32, 35 and 37°C. The initial dry matter concentration was 20, 24 and 28%, the amine nitrogen concentration was 0.5, 0.7 and 0.9 g/dm³. To select the technological parameters of the process of cultivation of industrial yeast for fermentation of high concentrations of wort and to establish the patterns of change, laboratory experimental studies of samples according to the developed technology were conducted. This paper investigates the degree of influence on the synthesis of yeast cells of various process parameters in their interaction, the establishment of multifactor dependence, using the methods of mathematical planning of the experiment. Cultivation of industrial alcoholic yeast *Saccharomyces cerevisiae* DO-16 was performed in periodic culture. As factors of variation are being used: the initial concentration of dry matter of the wort, the concentration of amine nitrogen, the cultivation temperature. The created mathematical model makes it possible to calculate the concentration of production yeast depending on the initial wort concentration, amine nitrogen concentration and fermentation temperature. According to the equations of the mathematical model, the process of fermentation of high-concentration wort for bioethanol production was optimized. Using Origin software, we found the optimal values of technological parameters of yeast cell biosynthesis: dry matter concentration of grain wort 28%, cultivation temperature 30–32°C, amine nitrogen concentration 0.7 g/dm³.

Keywords: bioethanol, fermentation, biosynthesis of yeast cells, *Saccharomyces cerevisiae*, optimization, high-gravity wort.

Correspondence:
S. Kovalchuk
E-mail: kovalchuksvs@gmail.com

Cite as Vancouver style citation

Kovalchuk S, Dolomakin Y. Optimization of the yeast cell biosynthesis process for the fermentation of high concentration wort in bioethanol production. Food science and technology. 2022;16(4):4-14. https://doi.org/10.15673/fst.v16i4.2547

Цитування згідно ДСТУ 8302:2015

Kovalchuk S., Dolomakin Y. Optimization of the yeast cell biosynthesis process for the fermentation of high concentration wort in bioethanol production / Food science and technology. 2022. Vol. 16, Issue 4. P. 4-14 https://doi.org/10.15673/fst.v16i4.2547

Introduction. Formulation of the problem

In order to prevent a decrease in the speed of the biotechnological process and to achieve an optimal yield of the target product of biosynthesis (biomass), it is necessary to develop optimal technological parameters for the cultivation of production yeast Saccharomyces cerevisiae for the fermentation of grain wort of high concentrations.

Analysis of recent research and publications

In most of the developed countries of the world, the use of renewable energy sources and resources is being promoted to achieve the following main goals: ensuring access to energy, mitigating the effects of climate change, supporting agricultural activities and ensuring food safety. Affordable energy, climate change and social stability as the three pillars of sustainability are directly related to the above-mentioned main goals [1,2].

In the last decades of the 20th century, there was a huge interest in the production and use of liquid biofuels (biodiesel or bioethanol) as promising substitutes for fossil fuels. Biofuels produced from plant biomass are renewable energy sources. The use of this raw material

will reduce the consumption of fossil fuels and, therefore, reduce the negative impact on the environment [1,3].

The biggest problem remains the reduction of the cost of bioethanol production. Therefore, it is necessary to develop a new concept of biorefineries, which is based on a more complete use of renewable raw materials and for the production of by-products with added value, which will reduce the cost of bioethanol production. This will make bioethanol more affordable and economically competitive than fossil fuels [4,5].

One of the areas of implementation in the production of bioethanol is the development of a resource- and energy-saving technology of high-concentration wort fermentation from grain raw materials by osmophilic strains of alcoholic yeast [6].

To develop the technology of highly concentrated brews from grain raw materials, it is necessary to choose the technological parameters of the cultivation of industrial yeast and the fermentation of wort.

The biochemical activity of yeast plays an important role in alcohol production. The use of osmophilic strains of alcoholic yeast, and optimization of their cultivation parameters allows to increase the yield of alcohol with minimal costs [7].

For distilleries that process starch-containing raw materials, yeast must have the following characteristics:

- withstand high concentrations of dry substances and alcohol;
- completely ferment the carbohydrates of the wort;
- accumulate the maximum amount of alcohol and minimum biomass;
- resistance to foreign microflora and increased acidity [7,8].

The influence of various factors on the vital activity of alcoholic yeast

Temperature. Changing the culture temperature affects the RNA, protein and lipid composition of the yeast cell. At the same growth rate, the RNA content in yeast increases several times when the temperature drops, and the content of unsaturated fatty acids in the lipid composition of the cell increases [8,9].

Alcoholic yeasts belong to the group of mesophilic microorganisms and do not lose their vital activity at temperatures from -5 to 40°C, but the temperature optimum for their growth and reproduction is 28–30°C. At a temperature of 20–32°C, the specific growth rate of yeast and fermentation activity increase in direct proportion to its increase, at a temperature above 34°C, yeast activity decreases sharply, at a temperature above 40 °C, growth and cell reproduction practically stops, and at 45–50°C Yeast activity stops [9,10]. There are heat-tolerant strains of alcoholic yeast that are capable of actively fermenting raw materials at temperatures of 35–37°C, which is economically beneficial for alcohol production. There are certain defined cultivation parameters for each yeast race. For example, studies have shown that industrial strains of alcohol yeast can be divided into several groups in relation to temperature. For some yeast races, the optimum temperature is 29–30°C, for another group of yeasts – 32–34°C, and for thermotolerant races – 35–37°C. Depending on the yeast race and its thermal stability, increasing the fermentation temperature to 35–37°C has different effects on the metabolism of yeast cells, their physiological, morphological and cultural properties [8-10].

Therefore, the process of intensification of alcoholic fermentation can be carried out with the help of thermotolerant yeast races.

The concentration of dry matters. Osmotic pressure depends on the concentration of soluble solids in the nutrient medium. The value of osmotic pressure, which is created by a solution, depends on the concentration, and not on the chemical nature of the substances dissolved in it, the greater the concentration of the solution, the greater the osmotic pressure.

For the assimilation of nutrients from the environment, the intracellular pressure in the yeast cell must be higher than the osmotic pressure of the environment, because when the osmotic pressure increases in solutions of salts, sugars, and other substances, plasmolysis of yeast cells occur. In solutions with a lower osmotic pressure, cells absorb water from the environment and swell. The growth rate of yeast depends on the osmotic pressure in the cell and in the wort. The larger it is, the faster the yeast reproduces [10].

Nitrogen nutrition. For normal vital activity in anaerobic conditions, yeasts need nitrogen, which they are able to assimilate in various forms. So, for example, during the reproduction of yeast, the average need for nitrogen is 20–35 mg/100 cm^3 of the medium. Under optimal cultivation conditions, every 10 billion cells absorb 20–35 mg of nitrogen [7,8].

The source of nitrogen nutrition for yeast cells is soluble nitrogen compounds (organic and inorganic). High molecular weight proteins (simple proteins and complex proteins), as well as amines, betaine, choline and purines are not assimilated by saccharomycetes, as they do not form exoproteases. The nitrogen of peptides is poorly assimilated by yeast, with an increase in the molecular weight of peptides, the ability of yeast to assimilate them decreases. But there is evidence that the presence of peptides in the environment, together with other forms of nitrogen, stimulates the consumption of amino acids.

Amides and ammonium compounds are assimilated by yeast. Nitrates are not assimilated by most yeast cultures.

Ammonia, as well as amine nitrogen, is the main source for the synthesis of cell proteins. Ammonia nitrogen is separated from ammonium salts of the environment and other nitrogen compounds and is used by yeast cells for the synthesis of amino acids.

In complex nutrient environments, the yeast cell most easily and completely assimilates nitrogen in the amine form. The nitrogen of the amide group in the

presence of amine nitrogen is practically not consumed, but in anaerobic conditions the need for amide nitrogen increases. Most yeasts can use urea as the only source of nutrition, forming the enzyme urease. There is also a non-urease pathway for the use of urea. The use of urea in alcohol production has a good effect on the development and reproduction of yeast.

Thus, yeast can synthesize almost all the necessary amino acids from carbon and ammonium. But in practice, yeast primarily uses free amino acids from the environment or vacuoles for biosynthetic processes.

The nitrogen content in the fermentable substrates largely determines the rate of synthesis and formation of yeast biomass. The most suitable for yeast, from the point of view of energy consumption, is amino acid nitrogen [8-10].

In connection with the fact that the management of the metabolism of yeast cells in the technology of cultivation of industrial yeast allows influencing the physiological and biochemical and, accordingly, the fermentation activity of yeast, it is important to research and develop ways to increase the biochemical activity of cells. The influence on yeast metabolism at the cellular and subcellular levels is carried out by adjusting the composition of the cultivation medium and technological parameters of cultivation. Nutrient media enriched with biologically active additives make it possible to improve the physiological state of yeast cultures and increase their fermentation activity [11].

The growth and reproduction of industrial strains of alcoholic yeast Saccharomyces cerevisiae is primarily determined by the balanced composition of the nutrient medium. During the fermentation of high concentrations of wort to obtain bioethanol, yeast cells are under stressful conditions. In order to prevent a decrease in the speed of the biotechnological process and to achieve an optimal yield of the target product of biosynthesis (biomass), it is necessary to add missing nutrients to the nutrient medium. Currently, there is a lot of information about the influence of various environmental factors on the growth and reproduction of microorganisms. The potential of microorganism cultures has not been sufficiently explored. When preparing nutrient media until recently, researchers mainly used methods of establishing single-factor dependence, that is, the principle of alternating changes in the experiment of each factor of the environment against the background of a constant level of others [12,13].

It is necessary to investigate the influence of various factors of the cultivated environment on the biosynthesis of yeast cells. With the help of mathematical modeling, develop response surfaces and determine the optimal technological parameters for the cultivation of industrial yeast for the fermentation of high-concentration wort.

The purpose of the presented work is to establish the optimal parameters for the cultivation of production yeast in the conditions of fermentation of high concentrations of wort for the production of bioethanol.

Research tasks:

1. Selection of technological parameters for the cultivation of production yeast for the fermentation of high-concentration wort experimentally

2. Carry out mathematical modeling of the process of cultivation of production yeast for fermentation of high-concentration wort.

3. To establish the optimal parameters for the cultivation of osmophilic yeast for the fermentation of high-concentration wort. Research materials and methods.

Research materials and methods

Methods of obtaining products and semi-products of alcohol production in laboratory conditions

Nutrient media used in the research process

For the cultivation of industrial yeast in laboratory conditions, a medium from starch-containing raw materials was used.

To prepare the grain mixture, a 500 cm³ conical flask was weighed with 50–70 g of ground corn (passage of 100% through a sieve with a diameter of 1 mm), 150–130 cm³ of water was added. It was mixed and the working solution of the diluting enzyme preparation was added. A solution of the enzyme preparation with water was previously prepared in a ratio of 1:10.

In the work, the following enzyme preparations from the manufacturer "Danisco" (Belgium) were used: Amylex 4T, as a source of α-amylase; Diazyme TGA, as a source of glucoamylase; Alphalase AFP, as a source of protease; Laminex 750 as a source of cellulose.

Enzyme preparations were added in units of activity. Corn grain with a starch content of 69.0% was used for research.

The preparation of wort was carried out according to the low-temperature boiling scheme at a temperature of 85–92°C using concentrated enzyme preparations of α-amylase, exposure for 3 h. The liquefied mass was cooled to a temperature of 50–55°C and glucoamylase, protease and cellulose were added, with an exposure time of 0.5 h. The concentration of thermostable α-amylase was 0.4; 0.60 units AA/g of starch, glucoamylase – 5.0 units. GlA/g of starch.

After saccharification, the wort was pasteurized at a temperature of 80°C for 20 minutes, cooled to a temperature of 50°C and acidified with sulfuric acid to acidity of 0.5–0.6 degrees. Yeast was cultivated at temperatures of 30, 32, 35 and 37°C.

Wort from starch-containing raw materials for cultivation was prepared with a concentration of 20, 24, 28% DM.

Preparation of industrial yeast

In laboratory conditions, pure cultures of the researched yeast from the jamb were transplanted into a test tube with sterile wort with a concentration of 9–10% *DM* and it was fermented for 24 hours at a temperature

of 30°C. After that, the contents of the test tube were sterilely transferred to a flask containing 200 ml of sterile wort and fermented for 24 hours, after which the wort was centrifuged, the sediment was washed with a physiological solution and used for wort fermentation.

Yeast was cultivated on sterile malt wort and wort from starch-containing raw materials. The dough and wort were poured into 20 ml test tubes and 0.25–0.50 L flasks of 100 and 200 ml, respectively.

The physiological state of yeast cells was determined by staining the yeast cell with Lugol's solution [14], the content of dead cells - by staining with methylene blue, the number of budding yeast and their number – in the Horyaev chamber [14].

The medium was sterilized at a pressure of 0.1 MPa for 30 min. Sterile wort was stored at room temperature.

Methods of analysis of raw materials, semi-products of alcohol production

The starch content of the original grain was determined according to the Evers method [16]. The granulometric composition of grain grinding was determined by the method of screening on metal and kapron sieves [14]. The concentration of dry substances was determined on a RPL-3 type refractometer of food laboratories [14].

In mature brew, pH and active acidity were determined by the electrometric method.

All determinations of quality indicators were determined in threefold repeatability with further statistical analysis of the obtained results.

Methods of mathematical processing of experimental data

To optimize the technological parameters of the production yeast cultivation process, an experiment was planned, as a result of which mathematical models were built. The results of methodological studies of the technological process of cultivation of production yeast indicate the non-linear nature of the influence of the initial concentration of dry substances of the cultivated wort, the temperature of cultivation and the concentration of amino nitrogen on the concentration of yeast cells.

To conduct experimental studies, the results of which allow you to calculate the coefficients of the regression equations, that is, the dependence of the concentration of yeast cells $c = f$ (*DM*, *t*, *N*), on the technological parameters of the process: the initial concentration of dry matters of the wort (*DM*), the temperature of cultivation (*t*) and the concentration of the amino nitrogen (*N*) an orthogonal central composition plan of the second order was chosen. The choice of an orthogonal central composite plan was due to the fact that this plan is the simplest among second-order plans. In this plan, the sum of the scalar products of the column vectors of the matrix is equal to zero, and accordingly, all the coefficients of the regression equation are determined independently of each other and the calculations when estimating the coefficients are simplified.

The total number of experiments *z* depends on the number of factors:

$$z = 2^k + 2k + n_0 , \qquad (1)$$

where *k* is the number of factors; n_0 is the number of experiments in the center of the experimental plan (usually one or two experiments).

In our case, for three experiment factors $k = 3$ and two experiments in the center of the plan $n_0 = 2$, the total number of experiments is $z = 2^3 + 2 \cdot 3 + 2 = 16$.

The unknown response function is most often represented by a polynomial of the kth degree:

$$y = b_0 + \sum_{i=1}^{k} b_i x_i + \sum_{\substack{i,\ j=1 \\ i<j}}^{k} b_{ij} x_i x_j + \sum_{i=1}^{k} b_{ii} x_i^2 + \cdots \qquad (2)$$

where b_0, b_i, b_{ij}, b_{ii} are polynomial coefficients.

According to the preliminary analysis of our process, the linear regression model describes it inadequately, and therefore we can achieve a satisfactory approximation of the response surface using a polynomial of the second degree. The mathematical model of the response surface in our case should look like this:

$$y = b_0 + b_1 x_1 + \ldots + b_i x_i + b_{i+1} x_1^2 + \ldots + b_{2i} x_i^2 + \ldots b_{2i+1} x_1 x_2 + \ldots + b_k x_{i-1} x_i . \qquad (3)$$

The main technological parameters of the process of cultivating production yeast and establishing the patterns of changes in the concentration of yeast cells, which were selected as variable factors when planning the experiment:

x_1 – concentration of amino nitrogen (*N*, g/dm³);

x_2 – cultivation temperature (*t*, °C);

x_3 – wort dry matter concentration (*DM*, %).

The minimum and maximum values of the technological parameters and their variation intervals were chosen based on the real process of cultivation of production yeast used in practice.

The selected technological parameters of the cultivation process meet all the requirements related to the planning factors of the experiment. The intervals of variation of the experiment factors are given in the table 1.

To determine the optimal values of the technological parameters of the production yeast cultivation process, the partial derivatives were set to zero.

On the basis of the obtained regression equations, the response surfaces of the optimization parameters were constructed from two variable technological parameters, with the other two fixed at the basic level, and also the optimal values of the technological parameters of the production yeast cultivation process were determined to ensure obtaining the maximum concentration of yeast cells for the fermentation of high-concentration wort.

Table 1 – Plan-matrix for conducting experiments of the orthogonal central composite plan

Experiment No	Variable factors			Response function (mean)
	x_1	x_2	x_3	$\bar{y}$
1	–1	–1	–1	y_{1m}
2	–1	–1	1	y_{2m}
3	–1	1	–1	y_{3m}
4	–1	1	1	y_{4m}
5	1	–1	–1	y_{5m}
6	1	–1	1	y_{6m}
7	1	1	–1	y_{7m}
8	1	1	1	y_{8m}
9	–1.68179	0	0	y_{9m}
10	1.68179	0	0	y_{10m}
11	0	–1	0	y_{11m}
12	0	1	0	y_{12m}
13	0	0	–1.68179	y_{13m}
14	0	0	1.68179	y_{14m}
15	0	0	0	y_{15m}
16	0	0	0	y_{16m}

Results of the research and their discussion

Selection of technological parameters for the cultivation of production yeast for the fermentation of wort of high concentrations by experimental means. The biochemical activity of yeast plays an important role in alcohol production. The use of osmophilic, thermotolerant strains of alcoholic yeast, optimization of their cultivation parameters allows to increase the yield of alcohol and its quality with minimal losses.

It is scientifically proven that the fermentation of high-concentration wort leads to an increase in the physiological activity of yeast and its concentration up to 30–60 million/cm³, depending on the content of dry matters in the wort [15,16].

An increase in the concentration of dry substances of fermented wort and alcohol in brews leads to a slowdown in the processes of yeast cell synthesis and fermentation [15]. Therefore, it is necessary to investigate the influence of the technological parameters of the cultivation of production yeast for the synthesis of the required concentration of yeast cells with high biochemical activity.

It is known that the metabolism in a yeast cell is directly dependent on nitrogen nutrition. With an increased concentration of grain wort, the content of non-fermentable carbohydrates increases and the yeast's need for easily assimilated nitrogen increases. Full nitrogen nutrition is an important factor in the regulation of yeast reproduction processes and their physiological activity. It is known that amino acids, which are consumed by yeast from the substrate by direct assimilation, are a more suitable source of nutrition. Therefore, providing the substrate with this source of nitrogen nutrition is one of the ways of intensification in the process of yeast cultivation [15,16].

Increasing the concentration of wort solids during fermentation should be done carefully so as not to excessively increase the osmotic pressure. Therefore, to improve bioethanol production, it is necessary to add other nutrients, such as amine nitrogen. Enrichment of the culture medium with nutrients, namely a source rich in nitrogen, was investigated for increasing the productivity and survival of yeast cells under conditions of high osmotic stress [17].

The use of environments balanced in terms of amino acids contributes to the activation of the processes of yeast cultivation, biomass synthesis and alcoholic fermentation: the concentration of cells increases by 2 times, their fermentation activity and productivity – by 20–25%, the rate of carbohydrate consumption increases. As a result, there is an intensification of the biotechnological process of obtaining ethanol [15].

An increase in the temperature of fermentation and the osmotic pressure of the environment leads to the creation of extreme conditions for the vital activity of yeast. This can lead to a decrease in the fermentation activity and reproduction rate of yeast, which, in turn, can lead to unstable work in the fermentation department. Therefore, a promising direction of scientific research is the search for ways to maintain the stability of yeast metabolic processes and increase their fermentation activity [18-20].

One of the most promising directions for the intensification of ethyl alcohol technology is to increase the concentration of wort from grain raw materials and use new osmophilic and thermotolerant yeast strains [19-21]. Given that the resource- and energy-saving technology involves an increase in the concentration of dry substances in the wort, it is necessary to choose rational technological parameters of fermentation [22-24]. Increasing the concentration of production yeast is one of the ways to reduce the duration of wort fermentation.

For this purpose, studies were conducted to determine the optimal concentration of wort dry matter,

amino nitrogen concentration, and the temperature of cultivation of production yeast for further fermentation of high-concentration wort by the yeast strain *Saccharomyces cerevisiae* DO–16 (IMB Y – 5099) selected at the Department of Biotechnology of Fermentation Products and Winemaking of the National University of Food Technologies [25].

Grinding from corn grain with a starch content of 69.0±0.1% was used for the cultivation of wort. The concentration of wort was 20, 24 and 28% of dry rheovins. Cultivation was carried out at temperatures of 30, 32, 35 and 37°C. The concentration of amino nitrogen was 0.5, 0.7, 0.9 g/dm³.

As can be seen from Table 2, during cultivation at temperatures of 30 and 32°C and a concentration of dry substances of 28% and according to the introduced amino nitrogen, the concentration of yeast cells was higher.

Mathematical modeling of the process of cultivation of production yeast for fermentation of high concentrations of wort

In order to substantiate the technological parameters of the process of cultivation of industrial yeast for the fermentation of high-concentration wort and to establish the patterns of changes in the concentration of yeast cells in the cultivation medium depending on the initial concentration of dry substances in the wort, the concentration of amino nitrogen and the temperature of fermentation, laboratory experimental studies of samples were conducted according to the developed technology (Table 3).

Since the linear response surface describes the experimental material inadequately, further experiments were conducted to refine its form using a second-order polynomial.

Table 2 – Influence of technological parameters of cultivation on the synthesis of yeast cells

№	Initial concentration of wort dry matter, %	Temperature, °C	Concentration of amine nitrogen, g/dm³	Concentration of yeast cells, million/cm³
1	20±0.2	30±1	0.5	231±23
2	20±0.2	32±1	0.5	240±24
3	20±0.2	35±1	0.5	202±20
4	20±0.2	37±1	0.5	88±8
5	20±0.2	30±1	0.7	281±28
6	20±0.2	32±1	0.7	296±29
7	20±0.2	35±1	0.7	276±27
8	20±0.2	37±1	0.7	105±10
9	20±0.2	30±1	0.9	261±26
10	20±0.2	32±1	0.9	270±27
11	20±0.2	35±1	0.9	256±25
12	20±0.2	37±1	0.9	84±8
13	24±0.2	30±1	0.5	280±28
14	24±0.2	32±1	0.5	291±29
15	24±0.2	35±1	0.5	206±20
16	24±0.2	37±1	0.5	96±9
17	24±0.2	30±1	0.7	260±26
18	24±0.2	32±1	0.7	356±35
19	24±0.2	35±1	0.7	334±33
20	24±0.2	37±1	0.7	116±11
21	24±0.2	30±1	0.9	293±29
22	24±0.2	32±1	0.9	315±31
23	24±0.2	35±1	0.9	308±30
24	24±0.2	37±1	0.9	108±10
25	28±0.2	30±1	0.5	311±31
26	28±0.2	32±1	0.5	320±32
27	28±0.2	35±1	0.5	307±30
28	28±0.2	37±1	0.5	120±12
29	28±0.2	30±1	0.7	401±40
30	28±0.2	32±1	0.7	418±41
31	28±0.2	35±1	0.7	386±38
32	28±0.2	37±1	0.7	138±13
33	28±0.2	30±1	0.9	368±36
34	28±0.2	32±1	0.9	384±38
35	28±0.2	35±1	0.9	364±36
36	28±0.2	37±1	0.9	134±13

Table 3 – Planning Matrix and Response Function

№	Dry matter *DM*, %	Temperature, °C	Concentration of amine nitrogen *N*, g/dm³	Concentration of yeast cells, *c* million/cm³
1	-1	-1	-1	231
2	-1	-1	1	261
3	-1	1	-1	88
4	-1	1	1	104
5	1	-1	-1	311
6	1	-1	1	368
7	1	1	-1	120
8	1	1	1	134
9	-1.68179	0	0	153
10	1.68179	0	0	415
11	0	-1	0	260
12	0	1	0	85
13	0	0	-1.68179	206
14	0	0	1.68179	308
15	0	0	0	276
16	0	0	0	276

Estimates of the regression coefficients for the symmetric plan of the second order were calculated according to the formulas:

$$b_0 = \frac{a}{n}\sum_{j=1}^{\tilde{n}} m_j \bar{y}_j - \frac{b}{n}\sum_{i=1}^{k}\sum_{j=1}^{\tilde{n}} m_j x_{ij}^2 \bar{y}_j;$$

$$b_{ij} = -\frac{b}{n}\sum_{j=1}^{\tilde{n}} m_j \bar{y}_j + \frac{c}{n}\sum_{j=1}^{\tilde{n}} m_j x_{ij}^2 \bar{y}_j - \frac{d}{n}\sum_{j=1}^{\tilde{n}} m_j x_{ij}^2 \bar{y}_j;$$

$$b_i = \frac{1}{\lambda_2 n}\sum_{j=1}^{\tilde{n}} m_j x_{ij} \bar{y}_j \ \ (i \neq 0); \quad b_{i\varepsilon} = \frac{1}{\lambda_3 n}\sum_{j=1}^{\tilde{n}} m_j x_{ij} x_{\varepsilon j} \bar{y}_j \ \ (i \neq \varepsilon),$$

where and n are the number of plan points and the total number of trials, respectively, m_j is the number of parallel experiments (m_j = 3).

The estimation of the significance of the coefficients of the regression equation was checked at the level of 0.05 using the Student's *t*-test. Statistically insignificant coefficients were discarded.

The response functions (optimization parameter), which reflect the dependence of the ethanol concentration on the initial concentration of wort solids, the concentration of production yeast, and the fermentation temperature, according to the results of the orthogonal central composite planning of the experiment, in the coded values of the variable factors, took the following form:

$$y = 279.222 + 50.497x_1 - 4.9503x_1^2 - 74.6375x_2 - 44.3715x_2^2 + 21.1281x_3 - 14.4962x_3^2 - 15.625x_1x_2 + 3.125x_1x_3 - 7.125x_2x_3.$$

After checking the adequacy of the obtained second-order model for this technological process of fermentation according to Fisher's *F*-criterion, it turned out that $F_{calc.}$ is less than F_{table}, so the hypothesis about the adequacy of the regression equations was accepted. The resulting second-order regression equations in coded and natural values can be used for calculation.

To obtain the regression equation in natural form, the basic level for each technological parameter of the process and its variation interval were determined. In natural quantities, the equation takes the form:

$$c_{conc.\ yeast\ cells}, million/cm^3 = 3.906DM \times c - 1.116DM \times t - 0.31DM^2 + 62.129DM - 3.622t^2 - 10.178tc + 255.27t - 362.405c^2 + 860.239c - 4874.466$$

The response values calculated by the obtained regression model are close to the experimental realizations of the observed random variable.

Intermediate regression equations were also obtained. The response functions (optimization parameter), which reflect the dependence of ethanol concentration c depending on the concentration of yeast *N*, the fermentation temperature t and the concentration of wort dry matter *DM* according to the results of the conducted orthogonal central compositional planning of the experiment in the natural values of the variable factors, took the following form:

at different temperature levels:

at t = 30°C: $c_{c.y.c.} = 28.647DM + 554.88c - 0.309DM^2 - 362.405c^2 + 3.906DM \times c - 476.29$

at t = 33,5°C: $c_{c.y.c.} = 24.74DM + 519.26c - 0.309DM^2 - 362.405c^2 + 3.906DM \times c - 387.87$

at t = 37 °C: $c_{c.y.c.} = 20.834DM + 483.63c - 0.309DM^2 - 362.405c^2 + 3.906DM \times c - 388.19$;

at different levels of amino nitrogen concentration:

at c = 0.5 g/dm³: $c_{c.y.c.} = 64.08DM + 250.18t - 0.309DM^2 - 3.622t^2 - 1.116DM \times t - 4534.95$;

at c = 0,7 g/dm³: $c_{c.y.c.}$ = 64.86DM + 248.14t – 0.309DM ^{2} – 3.622t^2 – 1.116DM×t – 4449.88;
at c = 0,9 g/dm³: $c_{c.y.c.}$ = 65.64DM + 246.11t – 0,309DM ^{2} – 3.622t^2 – 1.116DM×t – 4393.8
at different concentrations of wort dry matters:
at DM = 20%: $c_{c.y.c.}$ = 232.95t + 938.36c – 3.622t^2 – 362.405c^2 – 10.18tc – 3755.64;
at DM = 24%: $c_{c.y.c.}$ = 228.48t + 953.99c – 3.622t^2 – 362.405c^2 – 10.18tc – 3561.58;
at DM = 28%: $c_{c.y.c.}$ = 224.02t + 969.61c – 3.622t^2 – 362.405c^2 – 10.18tc – 3377.41

After checking the adequacy of the received models of the second order to the technological process according to Fisher's F-criterion, it turned out that $F_{calc.}$ is less than $F_{tabl.}$, then the hypothesis about the adequacy of the regression equations was accepted.

To study the influence of the technological parameters of the high-concentrated wort fermentation process (variable factors) on the optimization parameters, response surfaces and their two-dimensional sections were constructed depending on two variable factors (the third factor was at a constant basic level) using the *Origin* software (fig. 1–3)

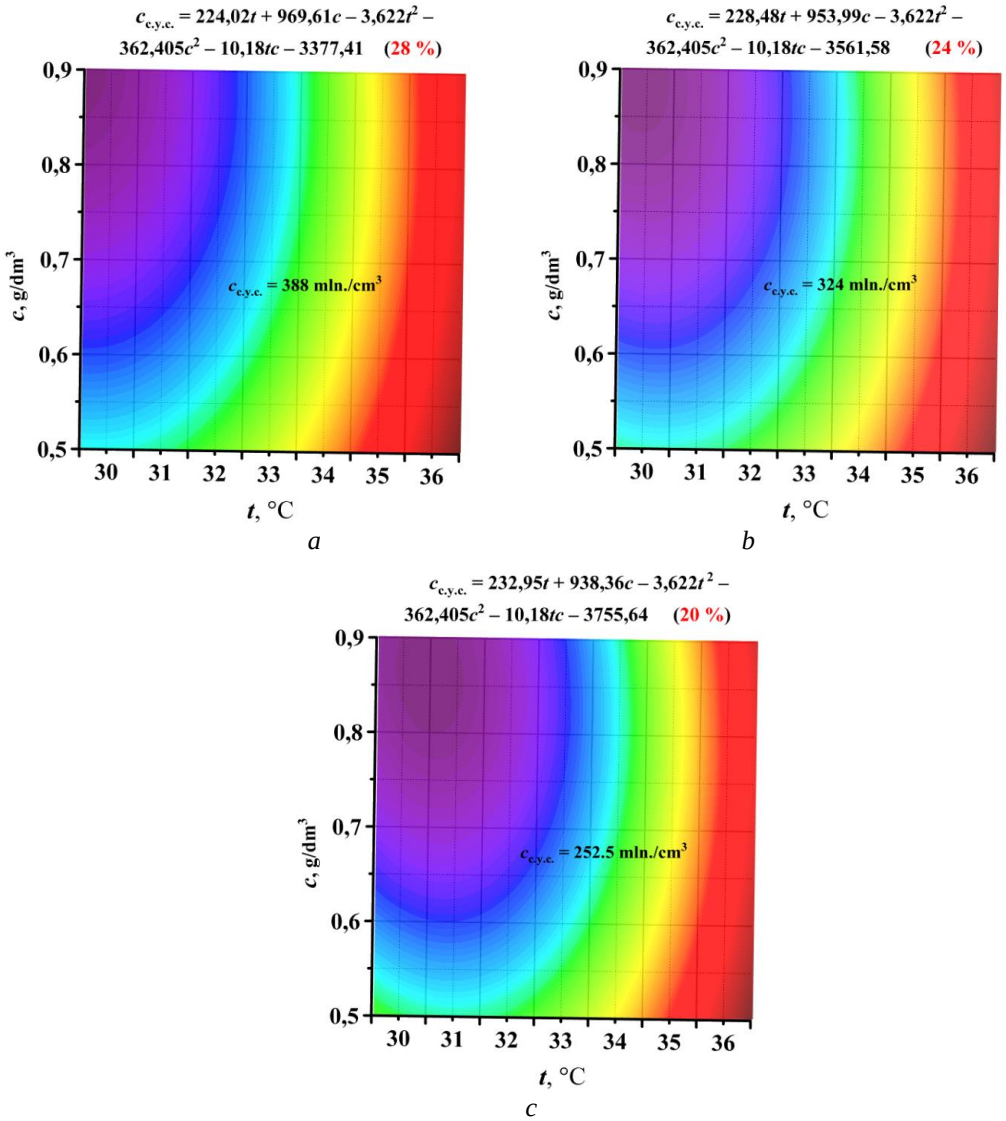

Fig. 1. Response surfaces of the dependence of ethanol synthesis on the technological parameters of the production yeast cultivation process: the initial concentration of wort dry matter (*a* – 28%; *b* – 24%, *c* – 20%), amino nitrogen concentration, fermentation temperature

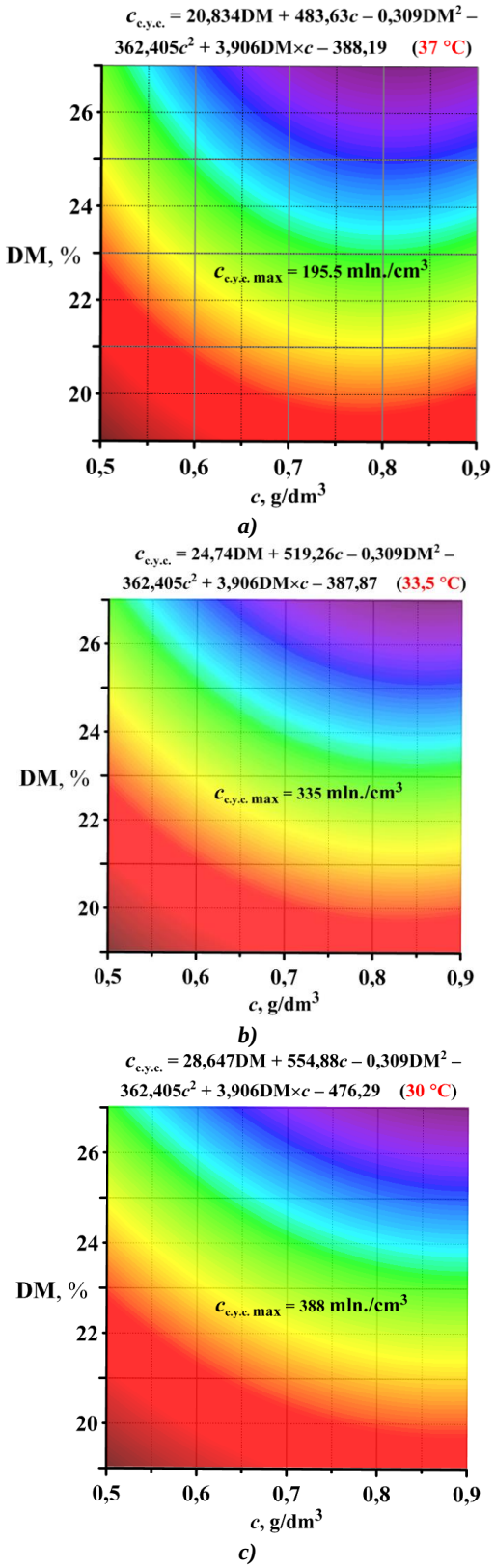

Fig. 2. Response surfaces of the dependence of ethanol synthesis on technological parameters of the production yeast cultivation process: initial concentration of wort solids, concentration of amino nitrogen, fermentation temperature (*a* –37°C; *b* – 33.5°C, *c* – 30°C)

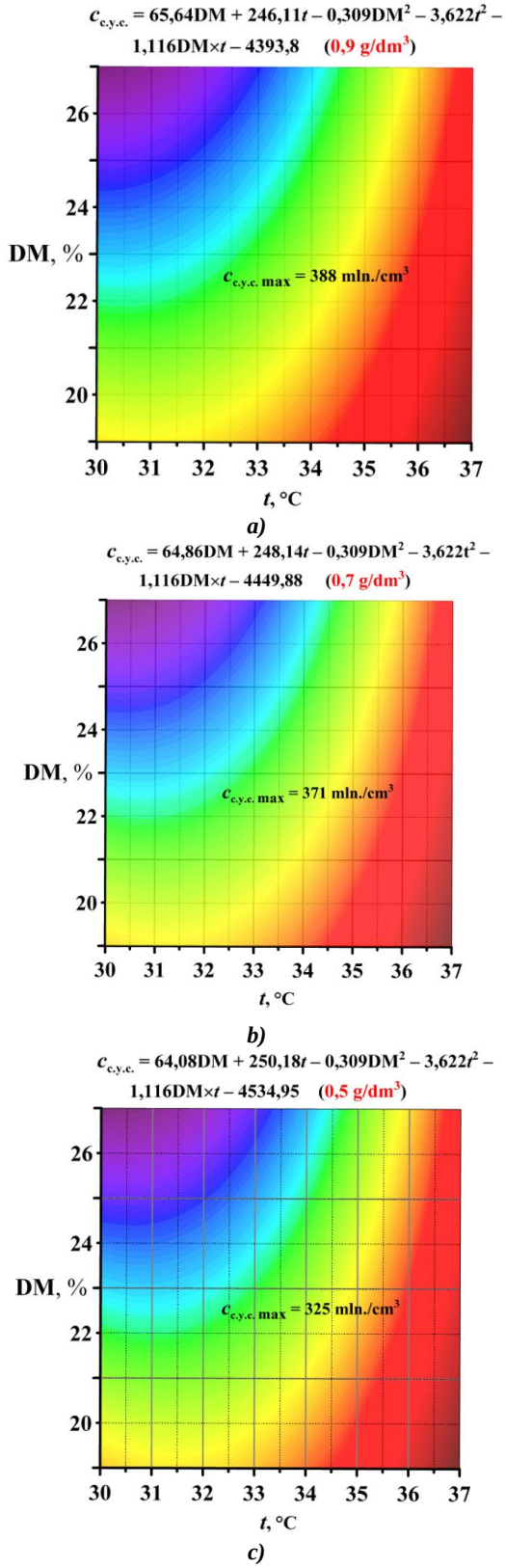

Fig. 3. Response surfaces of the dependence of ethanol synthesis on technological parameters of the production yeast cultivation process: initial concentration of wort solids, the concentration of amino nitrogen (*a* – 0.9 g/dm³; *b* – 0.7 g/dm³, *c* – 0.5 g/dm³), fermentation temperature

Thus, the possibility of effective directed management of the process of cultivation of production alcohol yeast for fermentation of high concentrations of wort by changing the technological parameters has been proven. The obtained results of the research provide the basis for the practical application of the optimal values of the technological parameters of the process of cultivation of industrial yeast for the fermentation of wort of high concentrations in industrial conditions for the production of bioethanol.

Conclusion

On the basis of experimental studies and mathematical modeling, the optimal technological parameters of industrial yeast cultivation were selected for the fermentation of high-concentration grain wort with a selected highly productive race of alcoholic yeast *Saccharomyces cerevisiae* DO-16 (IMB Y-5099).

The created mathematical model makes it possible to calculate the concentration of yeast cells depending on the initial concentration of the wort, the concentration of amino nitrogen and the cultivation temperature. Based on the equations of the mathematical model, the optimization of the process of cultivation of yeast cells for the fermentation of high-concentration wort for the production of bioethanol was carried out.

Using the Origin software, we found the optimal values of the technological parameters of the biosynthesis of yeast cells: the concentration of dry matters of grain wort 28%, the temperature of cultivation 30°C, the concentration of amino nitrogen 0.7 g/dm³.

References

1. Bušić A, Marđetko N, Kundas S. Bioethanol Production from Renewable Raw Materials and Its Separation and Purification: A Review. Food Technol Biotechnol.2018;56(3).289-311. https://doi.org/10.17113/ftb.56.03.18.5546.
2. Bhaskar T, Bhavya B, Singh R, Naik DV, Kumar A, Goyal HB. Thermochemical conversion of biomass to biofuels. In: Pandey A, Larroche C, Ricke SC, Dussap CG, Gnansounou E, editors. Biofuels – Alternative feedstocks and conversion processes. Oxford, UK: Academic Press.2011;51–77. https://doi.org/10.1016/B978-0-12-385099-7.00003-6
3. Benjaphoke S, Hasegawa D, Yokota D, Asvarak T, Auesukaree C, Sugiyama M. Highly efficient bioethanol production by a Saccharomyces cerevisiae strain with multiple stress tolerance to high temperature, acid and ethanol, New Biotechnol. 2012; 29(3); 379-386. https://doi.org/10.1016/j.nbt.2011.07.002
4. Cherubini F, Strømman AH. Principles of biorefining. Biofuels – Alternative feedstocks and conversion processes. Oxford, UK: Academic Press. 2011; 3–24. https://doi.org/10.1016/B978-0-12-385099-7.00001-2
5. Berlowska J, Kregiel D, Ambroziak W. Physiological tests for yeast brewery cells immobilized on modified chamotte carrier. Antonie Van Leeuwenhoek. 2013 Nov;104(5):703-14. https:// doi: 10.1007/s10482-013-9978-1.
6. Li Zhaofeng W, Donghai S, Yong-Cheng. Effects of nitrogen source on ethanol production in very high gravity fermentation of corn starch. Journal of the Taiwan Institute of Chemical Engineers. 2016; 70. 10.1016/j.jtice.2016.10.055.
7. Dziugan P, Balcerek M, Pielech-Przybylska K. Evaluation of the fermentation of high gravity thick sugar beet juice worts for efficient bioethanol production. Biotechnol Biofuels.2013;6,158. https://doi.org/10.1186/1754-6834-6-158.
8. Kobayashi M, Shimizu H, Shioya S. Physiological analysis of yeast cells by flow cytometry during serial-repitching of low-malt beer fermentation. J Biosci Bioeng. 2007 May;103(5):451-6. https://doi: 10.1263/jbb.103.451.
9. Maicas S. The Role of Yeasts in Fermentation Processes. Microorganisms. 2020 Jul 28;8(8):1142. https:// doi: 10.3390/microorganisms8081142.
10. Puligundla P, Smogrovicova D, Obulam VSR, Ko S. Very high gravity (VHG) ethanolic brewing and fermentation: A research update. J. Ind. Microbiol. Biotechnol. 2011;38:1133–1144. https:// doi: 10.1007/s10295-011-0999-3.
11. Shiyan P, Mudrak T, Kyrylenko R, Kovalchuk S. Effect of nitrogen and mineral composition of high-concentrated wort made from starch-containing raw materials on the cultivation of yeast. Eastern European journal of enterprise technologies. 2017; 6/11(90), 72-77. https://doi.org/10.15587/1729-4061.2017.117357
12. Sharma N. Optimization of fermentation parameters for production of ethanol from kinnow waste and banana peels by simultaneous saccharification and fermentation. Indian journal of microbiology. 2007; 47,4. 310-316. https:// doi:10.1007/s12088-007-0057-z
13. Phisalaphong M, Srirattana N, Tanthapanichakoon W. Mathematical modeling to investigate temperature effect on kinetic parameters of ethanol fermentation J. Biochem. Eng., 28. 2006; 36-43. https://doi.org/10.1016/j.bej.2005.08.039
14. Shiyan PL, Cosnytskyi VV, Oliniychuk ST. Innovative technologies of the alcohol industry. Theory and practice: monograph. Kyiv: Askania; 2009. 424 p.
15. Kovalchuk SS, Mudrak TO. Innovatsiina tekhnolohiia zbrodzhuvannia vysokokontsentro-vanoho susla iz zernovoi syrovyny. Publishing House "Baltija Publishing r. 2021; 60-100. https://doi.org/10.30525/978-9934-26-008-7.1-4
16. Mudrak T, Kovalchuk S, Kuts A, Dotsenko V. Research on the ultrathin structure of cells of different distillers yeast races and its dependence on the concentration of dry matter in wort, Food science and technology. 2020; 14 (3), p.p. 21-28. DOI: https://doi.org/10.15673/fst.v14i3.1798
17. Kovalchuk S, Mudrak T. Effectof the concentration of dry matter in wort on the characteristics of osmophilic alcoholic races of yeast, Food science and technology. 2021; 15 (1), 54–62. https://doi.org/10.15673/fst.v15i1.1967
18. Sujit KM, Manas RS. Chapter 3 - Bioethanol Production From Corn and Wheat: Food, Fuel, and Future, Bioethanol Production from Food Crops. 2019; 45-59. https://doi.org/10.1016/B978-0-12-813766-6.00003-5.
19. Yang X, Lee JH, Yoo HY. Production of bioethanol and biodiesel using instant noodle waste, Bioprocess Biosyst. Eng., 2014; 37, 1627-1635. https://doi.org/10.1007/s00449-014-1135-3
20. Kang KE, Chung D-P, Kim Y. High-titer ethanol production from simultaneous saccharification and fermentation using a continuous feeding system, Fuel, 2015; 145, 18-24. https:// doi.org/10.1016/j.fuel.2014.12.052
21. Gomes D, Cruz M, de Resende M, Ribeiro E, Teixeira J, Domingues L. Very High Gravity Bioethanol Revisited: Main Challenges and Advances. Fermentation.; 2021; 7(1):38. https://doi.org/10.3390/fermentation7010038
22. Puligundla P, Smogrovicova D, Obulam VSR, Ko S. Very high gravity (VHG) ethanolic brewing and fermentation: a research update, Journal of Industrial Microbiology and Biotechnology, 2011; 38 (9), 1133–1144, https://doi.org/10.1007/s10295-011-0999-3
23. Saprativ P Das, Debasish Das, Arun Goyal. Statistical Optimization of Fermentation Process Parameters by Taguchi Orthogonal Array Design for Improved Bioethanol Production, Journal of Fuels, 2014; 11. http://dx.doi.org/10.1155/2014/419674

24. Reisa VR, Burlamaqui Faraco Antonangelob AT, Guarnieri Bassia AP. Bioethanol strains of Saccharomyces cerevisiae characterised by microsatellite and stress resistance, Brazilian Journal of Microbiology, 2017;48 (2), p.p. 268-274. https://doi.org/10.1016/j.bjm.2016.09.017
25. Ukrainets AI, Shyian PL, Mudrak TO, Kuts AM, Kovalchuk SS, Kyrylenko RH, vynakhidnyky; Natsionalnyi universytet kharchovykh tekhnolohii MON Ukrainy, patentovlasnyk. Osmofilnyi, kyslotostiikyi shtam drizhdzhiv Saccharomyces cerevisiae IMB Y–5099 dlia mikrobiolohichnoho syntezu etylovoho spyrtu z krokhmalevmisnoi syrovyny. № 129706.2018 lyst. 12.

ОПТИМІЗАЦІЯ ПРОЦЕСУ БІОСИНТЕЗУ ДРІЖДЖОВИХ КЛІТИН ДЛЯ ЗБРОДЖУВАННЯ СУСЛА ВИСОКИХ КОНЦЕНТРАЦІЙ ПРИ ВИРОБНИЦТВІ БІОЕТАНОЛУ

С.С. Ковальчук[1], кандидат технічних наук, асистент, *E-mail:* kovalchuksvs@gmail.com
ORCID: 0000-0002-5344-1243
Ю.Ю. Доломакін[2], кандидат технічних наук, доцент, *E-mail:* dyy76@ukr.net
ORCID: 0000-0001-5279-9916
[1]Кафедра готельно-ресторанної справи
[2]Кафедра машин і апаратів харчових та фармацевтичних виробництв
Національний університет харчових технологій
вул. Володимирська 68, м. Київ, Україна, 01601

Анотація Для запобігання зниженню швидкості біотехнологічного процесу і досягнення оптимального виходу цільового продукту біосинтезу (біомаси) необхідно розробити оптимальні технологічні параметри культивування виробничих дріжджів *Saccharomyces cerevisiae* для зброджування зернового сусла високих концентрацій. Підвищення концентрації сухих речовин зброджуваного сусла і спирту в бражках призводить до сповільнення процесів синтезу дріжджових клітин та зброджування. Тому, необхно дослідити вплив технологічних параметрів культивування виробничих дріжджів для синтезу необхідної концентрації дріжджових клітин з високої біохімічною активністю. Культивування виробничих дріжджів проводили осмофільною термотолерантною расою спиртових дріжджів *Saccharomyces cerevisiae* ДО–16 (IMB Y – 5099) за температури 30, 32, 35 та 37°С. Початкова концентрація сухих речовин становила 20, 24 та 28%, концентрація амінного азоту становила 0.5, 0.7 та 0.9 г/дм³. Для підбору технологічних параметрів процесу культивування виробничих дріжджів для зброджування сусла високих концентрацій та встановлення закономірностей зміни провели лабораторні експериментальні дослідження зразків за розробленою технологією. У даній роботі досліджується ступінь впливу на синтез дріжджових клітин різних параметрів процесу в їх взаємодії, проводиться встановлення багатофакторної залежності, використовуючи методи математичного планування експерименту. Культивування виробничих спиртових дріжджів *Saccharomyces cerevisiae* ДО-16 проводили в умовах періодичної культури. Як фактори варіювання застосовували: початкову концентрацію сухих речовин сусла, концентрацію амінного азоту, температуру культивування. Створена математична модель дає можливість розрахувати концентрацію виробничих дріжджів залежно від початкової концентрації сусла, концентрації амінного азоту та температури бродіння. За рівняннями математичної моделі здійснено оптимізацію процесу зброджування сусла високих концентрацій для виробництва біоетанолу. З використанням програмного забезпечення Origin знайшли оптимальні значення технологічних параметрів біосинтезу дріжджових клітин: концентрація сухих речовин зернового сусла 28%, температура культивування 30–32°С, концентрація амінного азоту 0,7 г/дм³.

Ключові слова: біоетанол, зброджування, біосинтез дріжджових клітин, *Saccharomyces cerevisiae*, оптимізація, сусло високої концентрації