UDC663.533

OPTIMISATION OF TECHNOLOGICAL PARAMETERS OF FERMENTATION OF HIGHLY CONCENTRATED WORT FROM GRAIN RAW MATERIALS FOR BIOETHANOL PRODUCTION

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DOI: https://doi.org/10.15673/fst.v16i2.2375

Article history

Received 12.12.2021
Reviewed 18.01.2022
Revised 03.04.2022
Approved 24.05.2022

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Cite as Vancouver style citation

Kovalchuk S, DolomakinY. Optimisation of technological parameters of fermentation of highly concentrated wort from grain raw materials for bioethanol production. Food science and technology. 2022;16(2):4-14. https://doi.org/10.15673/fst.v16i2.2375

ЦитуваннязгідноДСТУ 8302:2015

Kovalchuk S., DolomakinY. Optimisation of technological parameters of fermentation of highly concentrated wort from grain raw materials for bioethanol production // Food science and technology. 2022. Vol. 16, Issue 2. P.4-14. https://doi.org/10.15673/fst.v16i2.2375

Abstract. Bioethanol is one of the most promising alternatives to fossil fuels and can be produced from various renewable sources rich in carbohydrates. A priority direction of biotechnological research is the development of new resource-saving technologies of bioethanol production. For effective bioconversion of carbohydrate-containing raw materials into bioethanol, it is advisable to optimise the technological parameters of fermenting highly concentrated wort obtained from grain raw materials. Laboratory experimental studies of the samples according to the newly developed technology have helped to substuntiate the technological parameters of fermenting highly concentrated wort and establishing the regular patterns in how the ethanol concentration in fermented washes changes depending on the initial concentration of dry matter in the wort, the industrial yeast concentration, and the fermentation temperature. Grain wort was fermented with the osmophilic thermotolerant race of distiller's yeast *Saccharomyces cerevisiae* DO–16 (IMB Y-5099) at 32–37°C. The initial concentration of dry matter was 26–30%. To optimise the technological parameters of the process of fermenting highly concentrated grain wort, an experiment was designed, which resulted in building mathematical models. To obtain the second-order regression equations in natural form, the basic level of each technological parameter of the process and its variability interval were determined. The mathematical model developed makes it possible to calculate the ethanol concentration depending on the initial concentration of the wort, the concentration of industrial yeast, and the fermentation temperature. Fermentation of highly concentrated wort for bioethanol production was optimised according to the equations of the mathematical model. It has been established that to synthesise the maximum alcohol concentration in washes using the highly concentrated wort fermentation technology, the wort concentration should be 30% of dry matter, the concentration of industrial yeast 40 million/cm³, and the temperature of the main fermentation 35°C.

Key words: bioethanol, fermentation, *Saccharomyces cerevisiae*, optimisation, highly concentrated wort.

Introduction. Formulation of the problem

Bioethanol is one of the most promising alternatives to fossil fuels and can be produced from various renewable sources rich in carbohydrates. Many countries, such as the US, Brazil, China, Canada, and several EU member states, have already announced commitment to bioethanol programmes. Biomass-derived ethanol is becoming an increasingly popular alternative to gasoline.

A priority direction of biotechnological research is the development of new resource-saving technologies that will ensure comprehensive processing of raw materials, decrease the cost of raw materials and thermal power resources, increase the quality and competitiveness of products, and reduce the man-caused load on the environment [1,2].

For effective bioconversion of carbohydrate-containing raw materials into bioethanol, it is advisable to optimise the technological parameters of fermenting highly concentrated wort from grain raw materials.

Analysis of recent research and publications

The rapid growth of energy demand and environmental problems associated with the use of fossil fuels have contributed to the development of the bioethanol industry, since this is a promising renewable and environmentally friendly fuel for transport [3].

The programme for the development of the alcohol industry for the near future provides for the establishment of large-scale production of alternative energy sources, including bioethanol. Today's most topical issue is the use of ethanol as a biofuel, both in its pure form and as a high-octane additive to petrol [4].

Bioethanol is dehydrated ethanol made from biorenewable raw materials [4]. In Ukraine, the most promising raw material for bioethanol production is sugar beet, and of cereal crops, it is maize [5-7].

Today, bioethanol manufacturing is an energy-efficient process, and research is currently underway to increase production volumes [8,9]. Bioethanol is an attractive biofuel that has potential for the country's energy security and environmental safety, as compared with fossil fuels. To date, numerous biomass resources for bioethanol production have been investigated. They can be broadly classified into sugar, starch, and lignocellulosic biomass. However, the conversion of biomass to ethanol varies greatly depending on the nature of the feedstock, mainly due to changes in the biochemical composition, and thus only a few raw materials are used commercially. In recent years, the process of biomass conversion has been significantly improved, even though most of these achievements have not yet been implemented in commercial enterprises [10].

The use of modern resource-saving biotechnologies in the alcohol industry makes it possible to develop technologies for the processing of various types of plant raw materials into ethanol. These technologies involve using broad-spectrum enzyme preparations and the fermentation of highly concentrated wort with high-yielding yeast strains resistant to elevated temperatures and increased alcohol concentrations [8,11]. For better quality indicators of both ethanol and bioethanol, it is necessary to develop a scientifically based approach to optimising the technological parameters of wort fermentation and the accumulation of fermentation by-products in it, depending on the intended purpose of the final product [12].

The application of highly concentrated wort fermentation technology to manufacture bioethanol has several advantages. It reduces the consumption of process water and power, increases an enterprise's overall productivity, and results in higher final concentrations of ethanol (with significant energy savings for distillation) [13].

The technology of highly concentrated wort fermentation is a promising universal technology that allows significant energy savings. It also makes it possible to increase the efficiency of fermentation without significant changes in the existing equipment, and to avoid losses of carbohydrates of raw materials [14]. An increase in the alcohol concentration by 1.0% vol. reduces the specific expenditure of energy carriers on wash rectification by 1150 kJ on average, and reduces the amount of post-alcohol stillage and the costs of its disposal by 9.1% [11].

The process parameters vital in ethanol production are the initial concentration of wort solids, temperature, pH, the concentration of enzyme preparations for hydrolysis of raw material components, and concentration of industrial yeast [10,15-17].

One of the most promising directions for intensification of the ethanol technology is increasing the concentration of wort obtained from grain raw materials and using new osmophilic and thermotolerant yeast strains [18]. Since the resource-saving and energy-efficient technology involves increased concentrations of dry matter in the wort, it is necessary to choose rational technological parameters of fermentation [19]. Increasing the concentration of industrial yeast is one of the ways to reduce the duration of wort fermentation.

In alcohol production, during dilution and saccharification of wort, starch is not completely hydrolysed, and this process continues during wort fermentation. The final saccharification of dextrins begins after at least 1/3 of the glucose has been fermented [19,20]. Increasing the concentration of industrial yeast will speed up the main fermentation stage and due to this, the total duration of fermentation of the wort will decrease.

This paper considers the development of technological parameters and mathematical data processing to establish the optimal parameters for fermentation of highly concentrated grain wort.

The purpose of the work is to develop and optimise the technological parameters of the fermentation of highly concentrated wort from grain raw materials to obtain bioethanol.

Objectives of the research:

1. Selection of technological parameters for highly concentrated wort fermentation.
2. Mathematical modelling of the process of fermenting highly concentrated wort.
3. Optimisation of the parameters of fermentating highly concentrated wort from grain raw materials.

Research materials and methods

Maize grain with the starch content 69.0% was used for the research. The following enzyme preparations from the manufacturer *Danisco* (Belgium) were used: Amylex 4T (activity 1158 units/cm³) as the source α-amylase, Diazyme TGA (activity 11152 units/cm³) as the source of glucoamylase, Alphalase AFP as the source of protease, Laminex 750 as the source of cellulose.

Fermentation was carried out using the osmophilic, acid-resistant, thermotolerant strain of distiller's yeast *Saccharomyces cerevisiae* DO–16 (IMB Y–5099) [21].

Methods of obtaining products and semiproducts of alcohol production in a laboratory environment

Grain mash, diluted mash, wort, alcoholic wash. To prepare the grain mash, a 50–70 g portion of maize grain (as coarsely ground as to pass fully through a sieve with meshes 1 mm in diameter) was placed into a 500 cm³ conical flask**,** and 150–130 cm³ of water was added at 20–22°C. The mixture wasstirred, and the working solution of the diluting enzyme preparation was added. The starch content of the original grain was determined by the Ewers method (ISO 712:2007). The particle size distribution of the milled grain was determined by sifting on metal and nylon-6sieves (DSTU 4525:2006). Prior to that, a solution of the enzyme preparation Amylex 4T with water (1:10) had been prepared. The enzyme preparations were added by units of activity.

Wort was prepared according to the low-temperature cooking scheme at 85–92°C using concentrated enzyme preparations, with 3 hours of exposure. The diluted mass was cooled to 50–55°C, andglucoamylase, protease, and cellulase were added, with 0.5 hours of exposure. The calculated dosage of the enzyme preparations was as follows: α-amylase **(**Amylex 4T) **–** 0.8 units AA/g of starch; glucoamylase **(**Diazym TGA) – 7.5 units GA/g; cellulolytic enzyme preparation **(**Laminex 750) – 0.35 units CA/g of raw materials, proteolytic enzyme preparation (Alphalase AFP) **–** 0.05 units PrA/g of raw materials).

Preparation of industrial yeast. Yeast *Saccharomyces cerevisiae* DO–16 (IMB Y–5099) was cultured on sterile malt wort and wort from starch-containing raw materials with the concentration 18–25% DM, in accordance with the hydromodulus. The dry matter concentration was determined with a saccharometer and a refractometer of the RPL–3 type[22]. The mash and the wort were poured into 20 ml test tubes and 0.25–0.50 l flasks in portions of 100 and 200 ml respectively.

The physiological state of yeast cells was determined by colouring the yeast cell with Lugol’s solution, the content of dead cells by colouring with methylene blue, and the number of budding yeast by counting in a counting chamber [22].

The growth medium was sterilised in an autoclave at 0.1 MPa for 30 minutes. The sterile wort was stored at room temperature.

Fermentation of wort from starch-containing raw materials. The calculated portion of industrial yeast *Saccharomyces cerevisiae* DO–16 (IMB Y–5099) introduced was 20–60 million/ml of wort.

Yeast was added to the saccharified wort in the amount 20–80 mln/cm³.In a laboratory environment, the wort was fermented by the method of“fermentation test” in conical flasks with sulphuric acid seals in an incubator where the temperature wasstably maintained at 32–37°C. The volume of a test sample was 0.2 cm³. Every 12 hours, the content of carbon dioxin released was measured by the weight method to determine the dynamics of wort fermentation [22]. Fermented wort was obtained after 3 days’ fermentation of the wort at 32–37°C.

Methods of analysing semi-products of alcohol production.

In the fermented wash, the pH and active acidity were determined electrometrically. The ethanol content was measured by the pycnometric method and the refractometric method, using an immersion refractometer [22]. The content of soluble carbohydrates, insoluble starch, alcohol-soluble carbohydrates, and dextrins was measured photoelectrocolorimetrically with the anthrone reagent [22].

All quality indicators were determined in triplicate with subsequent statistical analysis of the results obtained**.**

Methods of mathematical processing of the experimental data

To optimise the technological parameters of the fermentation process of high-concentrated grain wort, an experiment was designed, which resulted in building mathematical models. The results of the methodical studies of the technological process of fermentating high-concentrated grain wort indicate the non-linear nature of the effect that the initial concentration of wort solids, the fermentation temperature, and the industrial yeast concentration had on the ethanol concentration in the fermented washes.

The experimental findings were supposed to allow calculating the coefficients of the regression equations, that is, the dependence of the ethanol concentration c = f (DM, t, N), on the technological parameters of the process (the initial concentration of wort dry matter (DM), the fermentation temperature (t), and the industrial yeast concentration (N)). To conduct the studies, we chose an orthogonal central composite design of the second order. This design was chosen, because it was the simplest of second-order designs. In this design, the sum of scalar products of the column vectors of the matrix is equal to zero. Thus, 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:

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

where k is the number of factors; n_0 is the number of experiments in the centre of the experimental design (usually one or two experiments).

In our case, with three experiment factors $k = 3$ and two experiments in the centre of the design $n_0= 2$, 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 degree k:

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

where b_0, *bi, bij, bii* are polynomial coefficients.

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

$$y = b_0 + b_1x_1 + \ldots + b_i x_i + b_{i+1} x_1^2 + \ldots + b_{2i} x_i^2 + \ldots b_{2i+1}x_1x_2 + \ldots + b_k x_{i-1}x_i \, . \quad (3)$$

When designing the experiment, the following technological parameters of fermenting highly concentrated wort and establishing the regular patterns in the changes in ethanol concentration in fermented washes were chosen as variable factors: x_1 – industrial yeast concentration (N, mln./cm³); x_2 – fermentation temperature (t, C);x_3 – wort dry matter concentration (DM, %).

The minimum and maximum values of the technological parameters and their variability intervals were chosen based on the real process of wort fermentation used in practice. Accordingly, the range of values of the technological parameters of fermenting high concentrations of wort is as follows: the dry matter concentration is 26–30%, the wort fermentation temperature is 32–37°C, the industrial yeast concentration is 20–60 mln/cm³. The selected technological parameters of fermentation meet all the requirements for the factors of designing the experiment. The variability intervals of the experiment factors are given in Table 1. With the help of the central composite design, a quadratic polynomial (x^2), -1.68179 is obtained, these are the so-called star points. At 0, -1, and +1, linear equations are obtained, which do not allow constructing curved surfaces with maximum values (optimum of the function).

Table 1 – Matrix design for conducting experiments of the orthogonal central composite design

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	–1.6817	0	0	y_{9m}
10	1.6817	0	0	y_{10m}
11	0	–1	0	y_{11m}
12	0	1	0	y_{12m}
13	0	0	–1.6817	y_{13m}
14	0	0	1.6817	y_{14m}
15	0	0	0	y_{15m}
16	0	0	0	y_{16m}

To determine the optimal values of the technological parameters of highly concentrated wort fermentation, the partial derivatives were set to zero. According to mathematical analysis, in the place where the derivative of the function is zero, its curve reaches its maximum value, namely the optimum.

Based on the regression equations obtained, using the *ORIGIN* Pro software package, the response surfaces of the optimisation parameters were constructed from two variable technological parameters. One parameter was taken at a fixed level (e.g. t = 32°C), and by substituting this value, a dependence was obtained. The other two parameters were fixed at the basic level (two variables were taken at a fixed level). This was followed by determining the optimal values of the technological parameters of highly concentrated wort fermentation needed to achieve the maximum ethanol concentration.

Results of the research and their discussion

Selection of technological parameters of highly concentrated wort fermentation. From the data in Table 2, it can be seen that the amount of synthesised alcohol increases with the increase in the amount of industrial yeast. This is probably due to the smaller amount of carbohydrates spent on the synthesis of yeast biomass. The highest concentration of alcohol in the wash is achieved at the wort concentration 28%, and the amount of industrial yeast 40 mln/cm³. The industrial yeast concentration being 40 mln/cm³, the duration of fermentation is reduced at the initial wort DM concentration 28%. Increasing the industrial yeast concentration to 60 mln/cm³ wort did not significantly affect the increase in alcohol concentration in the wash, so further increasing the yeast concentration is impractical. It should be noted that with an increase in the amount of yeast, losses with alcohol-soluble carbohydrates decreased, which indicates more effective fermentation of carbohydrates.

Taking into account the need to prevent the development of contaminating microflora, the liquefied mass is mainly saccharified in factories directly in the course of fermentation [11]. In a production environment, boiling of grain mashes from starch-containing raw materials is carried out according to the low-temperature scheme with the use of concentrated enzyme preparations of various selective action. This scheme has a number of advantages over the high-temperature one, but does not always ensure the microbiological purity of the wort: it accelerates the development of contaminating microflora in the saccharifier, which further adversely affects the fermentation process. Saccharifying the rarefied grain mash directly in the fermenter allows avoiding these negative factors. Effective hydrolysis of dextrins in the fermenter requires developing optimal technological parameters for the fermentation process.

As can be seen from Table 2 (samples 2,3,6,7), during the fermentation of the wort at 32 and 35°C, the concentration of alcohol in the fermented wash was the highest: 14.65, 14.60% vol. and 15.42, 15.67% vol. Therefore, stage-by-stage fermentation of grain wort from maize (at 32 and 35°C) creates conditions for final saccharification of dextrins in the wort and its effective fermentation. So, this technique is particularly effective when fermenting highly concentrated worts and allows obtaining the maximum ethanol concentration in washes.

Mathematical modelling of the process of fermentation of highly concentrated wort

To substantiate the technological parameters of the fermentation of highly concentrated wort and to establish the regular patterns of changes in the ethanol concentration in fermented wash depending on the initial concentration of wort solids, the industrial yeast concentration, and the fermentation temperature, laboratory experimental studies of the samples were conducted using the above technology (Table 3).

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

Table 2 – Chemical and technological indicators of fermented wash

No.	Initial concentration of dry matter in wort, %	Temperature, °C	Concentration of yeast inoculum, mln/cm³	∑ CO_2, g/200cm³	Ethanol concentration, % vol.
1.	26±0.2	32±1	20±2	21.58±1,07	13.58±0.027
2.	26±0.2	35±1	20±2	21.60±1,08	13.59±0.017
3.	26±0.2	37±1	20±2	21.62±1,04	13.60±0.021
4.	26±0.2	32±1	40±4	21.69±1,06	13.65±0.025
5.	26±0.2	35±1	40±4	21.68±0,98	13.64±0.029
6.	26±0.2	37±1	40±4	21.68±0.85	13.64±0.017
7.	26±0.2	32±1	60±6	21.70±0.91	13.65±0.021
8.	26±0.2	35±1	60±6	21.63±1.02	13.63±0.019
9.	26±0.2	37±1	60±6	21.68±1.04	13.64±0.027
10.	28±0.2	32±1	20±2	22.27±0.95	14.52±0.022
11.	28±0.2	35±1	20±2	22.27±0.97	14.52±0.024
12.	28±0.2	37±1	20±2	22.06±0.90	14.39±0.018
13.	28±0.2	32±1	40±4	22.34±0.89	14.65±0,016
14.	28±0.2	35±1	40±4	22.25±0.87	14.60±0.014
15.	28±0.2	37±1	40±4	22.14±1.02	14.42±0.018
16.	28±0.2	32±1	60±6	22.34±1.08	14.64±0.015
17.	28±0.2	35±1	60±6	22.29±1.02	14.59±0.021
18.	28±0.2	37±1	60±6	22.14±0.93	14.42±0.022
19.	30±0.2	32±1	20±2	23.15±0.95	15.40±0.027
20.	30±0.2	35±1	20±2	23.23±0.87	15.48±0.029
21.	30±0.2	37±1	20±2	23.14±0.97	15.39±0.019
22.	30±0.2	32±1	40±4	23.30±1.02	15.50±0.021
23.	30±0.2	35±1	40±4	23.45±0,92	15.55±0.025
24.	30±0.2	37±1	40±4	23.17±0.89	15.42±0.026
25.	30±0.2	32±1	60±6	23.33±0.98	15.51±0.023
26.	30±0.2	35±1	60±6	23.26±0.94	15.44±0.025
27.	30±0.2	37±1	60±6	23.16±1.03	15.41±0.027

Table 3 – Design matrix and response function

No.	Yeast concentration *N*,mln/cm³	Temperature, °C	Dry matter *DM*, %	Ethanol concentration *C*, % vol.
1	-1	-1	-1	13.58
2	-1	-1	1	15.35
3	-1	1	-1	13.60
4	-1	1	1	14.91
5	1	-1	-1	13.65
6	1	-1	1	15.41
7	1	1	-1	13.64
8	1	1	1	14.98
9	-1.68179	0	0	14.42
10	1.68179	0	0	14.58
11	0	-1	0	14.22
12	0	1	0	14.02
13	0	0	-1.68179	12.55
14	0	0	1.68179	15.70
15	0	0	0	14.60
16	0	0	0	14.60

Estimates of the regression coefficients for the symmetrical second-order design were calculated according to the formulae:

$$b_0 = \frac{a}{n}\sum_{j=1}^{\tilde{n}} m_j\,\bar{y}_j - \frac{b}{n}\sum_{i=1}^{k}\sum_{j=1}^{\tilde{n}} m_j\, x_{ij}^2\,\bar{y}_j;$$
$$b_{ij} = -\frac{b}{n}\sum_{j=1}^{\tilde{n}} m_j\,\bar{y}_j + \frac{c}{n}\sum_{j=1}^{\tilde{n}} m_j x_{ij}^2\,\bar{y}_j - \frac{d}{n}\sum_{j=1}^{\tilde{n}} m_j x_{ij}^2\,\bar{y}_j; \quad (4)$$
$$b_i = \frac{1}{\lambda_2 n}\sum_{j=1}^{\tilde{n}} m_j\, x_{ij}\bar{y}_j \quad (i \neq 0);\; b_{i\varepsilon} = \frac{1}{\lambda_3 n}\sum_{j=1}^{\tilde{n}} m_j x_{ij} x_{\varepsilon j}\bar{y}_j \quad (i \neq \varepsilon),$$

where $\tilde{n}$ and n are, respectively, the number of design points and the total number of experiments;
mj is the number of parallel experiments (*mj* = 3) [23].

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

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

$$y = 14.58427 + 0.03728x_1 + 0.00261x_1^2 - 0.08762x_2 - 0.13174x_2^2 + 0.84043x_3 - 0.12997x_3^2 - 0.0025x_1x_2 + 0.0025x_1x_3 - 0.11x_2x_3 \quad (5)$$

After verification (using the *F*-test) whether the second-order model obtained was adequate for this technological process of fermentation, it was found that F_{calc} was less than F_{table} (0.000082 < 8.68), so the hypothesis about the adequacy of the regression equations was accepted. The obtained second-order regression equation in coded values can be used in calculations.

To obtain the regression equation in natural form, the basic level and variability interval were determined for each technological parameter of the process. In natural values, the equation (5) takes the form:

$$c_{C_2H_6O}, \text{\% vol.} = 6.525\cdot10^{-6}\cdot N^2 - 5\cdot10^{-5}\cdot N\cdot t + 4.16\cdot10^{-5}\cdot N\cdot DM + 1.8586\cdot10^{-3}\cdot N - 0.021t^2 - 0.0146\cdot t\cdot DM + 1.8467\cdot t - 0.0144\cdot DM^2 + 1.622\cdot DM - 44.323 \quad (6)$$

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

Intermediate regression equations have also been obtained. By the results of the orthogonal central composite designing of the experiment conducted, the response functions (optimisation parameter), which reflect the dependence of ethanol concentration *C* on the yeast concentration *N*, the fermentation temperature *t*, and the wort dry matter concentration *DM*, took the following form in the natural values of the variable factors:

at different temperature levels:

$$t = 32°C \rightarrow c_{C_2H_6O} = 6.5\cdot10^{-6}\cdot N^2 + 4.16\cdot10^{-5}\cdot N\cdot DM + 2.586\cdot10^{-4}\cdot N - 1.44\cdot10^{-2}\cdot DM^2 + 1.153\cdot DM - 6.81;$$
$$t = 34.5°C \rightarrow c_{C_2H_6O} = 6.5\cdot10^{-6}\cdot N^2 + 4.16\cdot10^{-5}\cdot N\cdot DM + 1.336\cdot10^{-4}\cdot N - 1.44\cdot10^{-2}\cdot DM^2 + 1.116\cdot DM - 5.7;$$
$$t = 37°C \rightarrow c_{C_2H_6O} = 6.5\cdot10^{-6}\cdot N^2 + 4.16\cdot10^{-5}\cdot N\cdot DM + 8.67\cdot10^{-6}\cdot N - 1.44\cdot10^{-2}\cdot DM^2 + 1.08\cdot DM - 4.85$$

at different levels of yeast concentration:

$$N = 20\ \text{mln/cm}^3 \rightarrow c_{C_2H_6O} = 1.8457t - 0.01467\cdot t\cdot DM - 0.021t^2 - 0.0144DM^2 + 1.6229DM - 44.28;$$
$$N = 40\ \text{mln/cm}^3 \rightarrow c_{C_2H_6O} = 1.8447t - 0.01467\cdot t\cdot DM - 0.021t^2 - 0.0144DM^2 + 1.6237DM - 44.24;$$
$$N = 60\ \text{mln/cm}^3 \rightarrow c_{C_2H_6O} = 1.8437t - 0.01467\cdot t\cdot DM - 0.021t^2 - 0.0144DM^2 + 1.6246DM - 44.19$$

at different concentrations of wort dry matter:

$$DM = 32\% \rightarrow c_{C_2H_6O} = 0.0032N + 6.5\cdot10^{-6}\cdot N^2 - 5\cdot10^{-5}\cdot N\cdot t + 1.377t - 0.021t^2 - 7.205;$$
$$DM = 29\% \rightarrow c_{C_2H_6O} = 0.003N + 6.5\cdot10^{-6}\cdot N^2 - 5\cdot10^{-5}\cdot N\cdot t + 1.421t - 0.021t^2 - 9.428;$$
$$DM = 26\% \rightarrow c_{C_2H_6O} = 0.0029N + 6.5\cdot10^{-6}\cdot N^2 - 5\cdot10^{-5}\cdot N\cdot t + 1.465t - 0.021t^2 - 11.911$$

After verification (using the *F*-test) whether the second-order models obtained were adequate for the technological process of fermentation, it was found that F_{calc} was less than F_{table}, so the hypothesis about the adequacy of the regression equations was accepted.

To study how the technological parameters of highly concentrated wort fermentation (variable factors) affected the optimisation parameters, response surfaces and their two-dimensional sections were constructed depending on two variable factors (the third factor was at a constant basic level). To this end, the *Origin* software was used (Fig. 1–3).

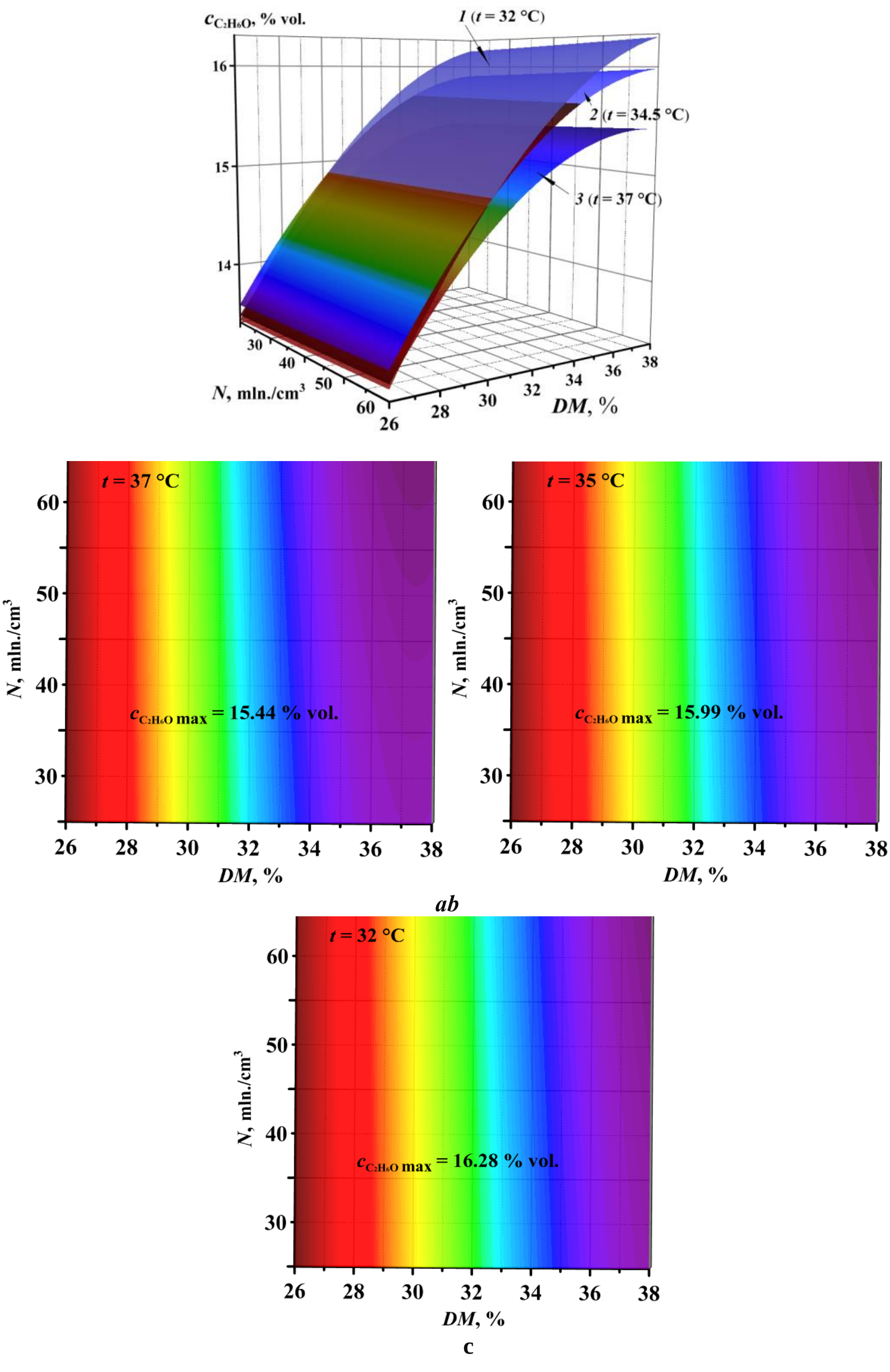

Fig. 1. Response surfaces of the dependence of ethanol synthesis on the technological parameters of fermenting highly concentrated wort: initial concentration of wort dry matter, industrial yeast concentration, fermentation temperature, and their interpretation on the plane (a – 37°C; b – 35°C, c – 32°C)

Analysis of the second-order regression equations obtained (23)–(26) and the response surfaces constructed using the *Origin* software (Fig. 1–3) has allowed finding the optimal values of the technological parameters. For the synthesis of the maximum alcohol concentration in washes with the use of the technology of highly concentrated wort fermentation, the wort concentration should be 30% of dry matter, and the industrial yeast concentration should be 40 mln/cm³ at the temperature of the main fermentation 35°C.

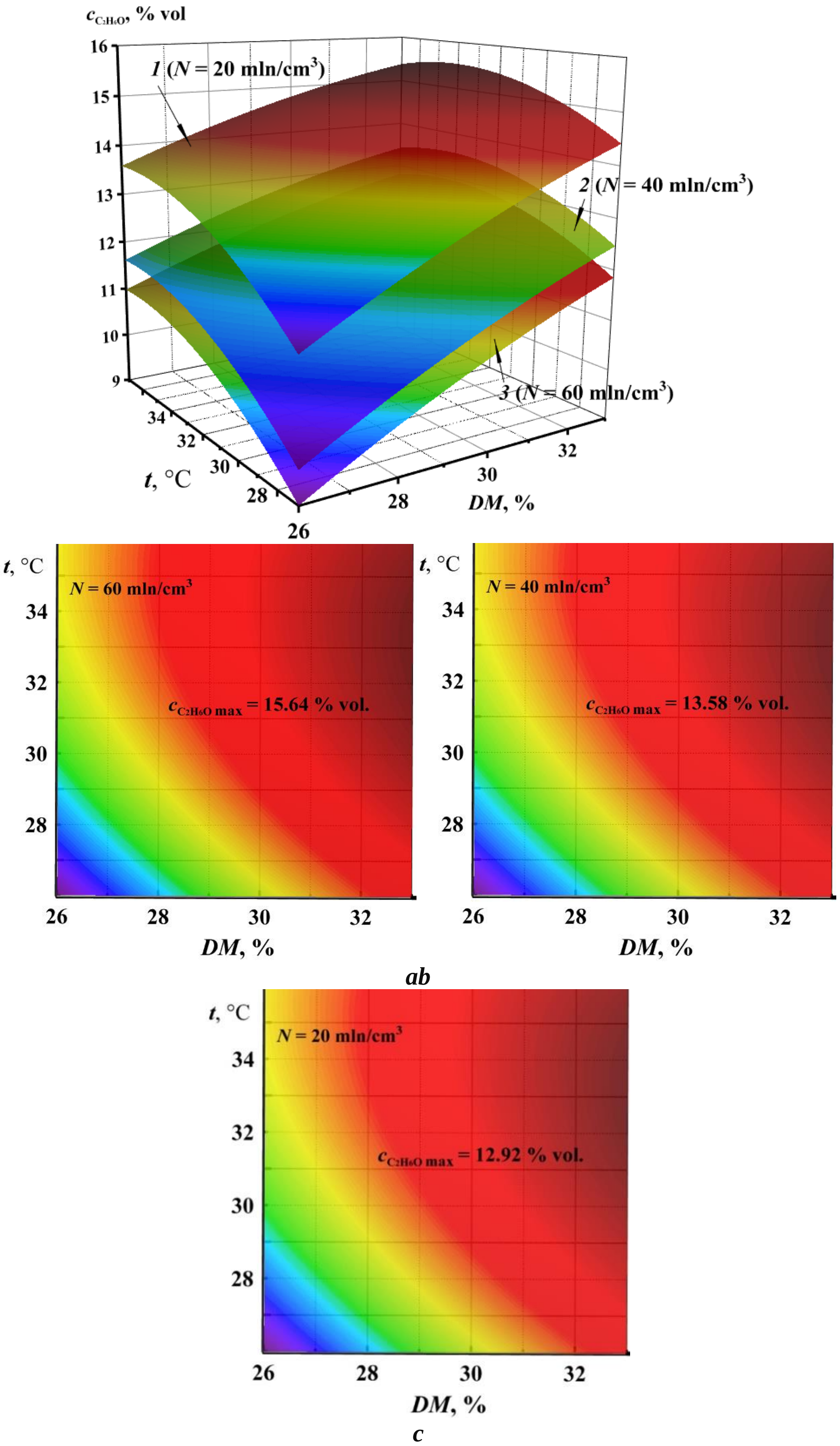

Fig. 2. Response surfaces of the dependence of ethanol synthesis on the technological parameters of fermenting highly concentrated wort: the initial concentration of wort dry matter, industrial yeast concentration, and their interpretation on the plane (a – 60 mln/cm³; b – 40 mln/cm³, c – 20 mln/cm³), fermentation temperature

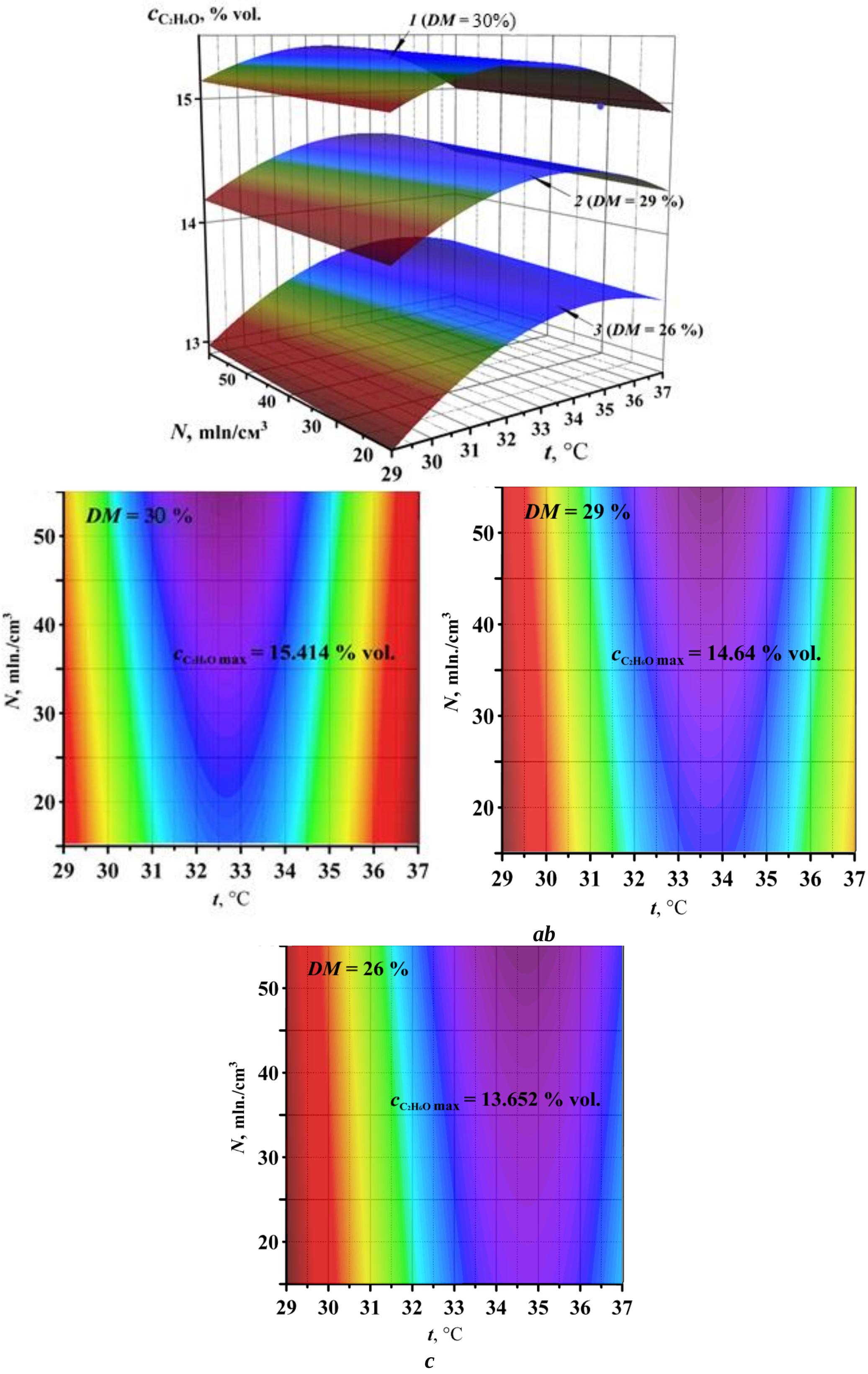

Fig. 3. Response surfaces of the dependence of ethanol synthesis on the technological parameters of fermenting highly concentrated wort: the initial concentration of wort dry matter and its interpretation on the plane (a – 30%; b – 29%; c – 26%), industrial yeast concentration, fermentation temperature

Thus, it has been proved that the process of fermenting highly concentrated wort can be directly and effectively controlled by changing its technological parameters. The research results obtained allow the practical application of the optimal values of the technological parameters of fermenting highly concentrated wort in an industrial environment to manufacture bioethanol.

Conclusion

The experimental studies have made it possible to develop the optimal technological parameters of highly concentrated grain wort fermentation with a high-yielding race of distiller's yeast *Saccharomyces cerevisiae* DO–16 (IMB Y-5099) obtained by selective breeding.

The mathematical model created makes it possible to calculate the ethanol concentration depending on the initial wort concentration (26–30%), the industrial yeast concentration (20–60 mln/cm³), and the fermentation temperature (32–37°C). The fermentation process in highly concentrated wort for bioethanol production has been optimised according to the equations of the mathematical model.

The optimal technological parameters of the fermentation process have been established. For the synthesis of the maximum alcohol concentration in washes using the highly concentrated wort fermentation technology, the wort concentration should be 30% of dry matter, the industrial yeast concentration should be 40 mln/cm³, and the temperature of the main fermentation should be 35°C.

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ОПТИМІЗАЦІЯ ТЕХНОЛОГІЧНИХ ПАРАМЕТРІВ ЗБРОДЖУВАННЯ СУСЛА ВИСОКИХ КОНЦЕНТРАЦІЙ ІЗ ЗЕРНОВОЇ СИРОВИНИ ДЛЯ ВИРОБНИЦТВА БІОЕТАНОЛУ

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Анотація.Біоетанол є однією з найбільш перспективних альтернатив викопному паливу, яке можна виробляти з різних відновлюваних джерел, багатих вуглеводами. Пріоритетним напрямом наукових досліджень в біотехнології є розробка нових ресурсозберігаючих технологій виробництва біоетанолу**.** Для забезпечення ефективного процесу біоконверсії вуглеводвмісної сировини в біоетанол доцільно оптимізувати технологічні параметри зброджування сусла високих концентрацій із зернової сировини. Для обґрунтування технологічних параметрів процесу зброджування висококонцентрованого сусла та встановлення закономірностей зміни концентрації етанолу в зрілих бражках у залежності від початкової концентрації сухих речовин сусла, концентрації виробничих дріжджів та температури зброджування, провели лабораторні експериментальні дослідження зразків за розробленою технологією. Зернове сусло зброджували осмофільною термотолерантною расою спиртових дріжджів *Saccharomyces cerevisiae* ДО–16 (IMBY–5099)за температури 32–37°С.Початкова концентрація сухих речовин становила 26–30%.Для оптимізації технологічних параметрів процесу зброджування зернового сусла високих концентрацій провели планування експерименту в результаті реалізації якого побудували математичні моделі. Для отримання рівнянь регресії другого порядку в натуральному вигляді визначили основний рівень для кожного технологічного параметру процесу та його інтервал варіювання. Створена математична модель дає можливість розрахувати концентрацію етанолу залежно від початкової концентрації сусла, концентрації виробничих дріжджів та температури бродіння. За рівняннями математичної моделі здійснено оптимізацію процесу зброджування сусла високих концентрацій для виробництва біоетанолу. Встановлено, що для синтезу максимальної концентрації спирту в бражках за технологією зброджування сусла високих концентрацій концентрація сусла повинна становити 30% сухих речовин, концентрація виробничих дріжджів 40 млн./см^3, а температура головного бродіння 35°С.

Ключові слова: біоетанол, зброджування, *Saccharomyces cerevisiae,* оптимізація, сусло високої концентрації.