: 2582-4686 SJIF 2021-3.261,SJIF 2022-2.889, 2024-
5 ResearchBib IF: 8.848 / 2024
THE MULTIDISCIPLINARY JOURNAL OF SCIENCE AND TECHNOLOGY
VOLUME-4, ISSUE-8

UDK 662.636

OPTIMIZATION OF THE COMPOSITION OF THE FOOD ENVIRONMENT IN BIOETHANOL PRODUCTION BASED ON GRAPES

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Abstract. *This article describes the effects of yeast strains, enzyme preparations and mineral components used to optimize the composition of the nutrient medium used in the production of bioethanol by processing grape waste. Methods of obtaining second-generation biofuel are one of the current topics. Biofuel production from food waste helps to solve two problems at the same time. Firstly, it causes a reduction in the negative impact on the environment by eliminating food waste, and secondly, it is used as a renewable energy source, taking into account the increasing need for energy sources resulting from economic and demographic growth. is used. In the study, the optimization of the composition of the nutrient medium and the achievement of increasing the amount of bioethanol produced in the process of alcoholic fermentation are compared with the example of experiments.*

Key words: *bioethanol, enzyme, yeast, hydrolysis, alcoholic fermentation, grapes, substrate, sugars*

Enter. It is no secret that most of the world's countries, including the USA, Brazil and most of the European countries, use bioethanol as fuel. According to the experts of the Institute of Forecasting and Macroeconomic Research, in 2024, 112.29 bln. liter of bioethanol was produced, and this indicator will reach 144.34 billion by 2029. it is mentioned that it is a liter. Obtaining bioethanol from food waste in the biofuel industry is one of the urgent tasks of today. Here it is worth noting that grape cake is one of the most common biological wastes of the grape processing industry, and it is composed of grape skins, seeds, and stalks that hold berries. In 2023, the volume of grape production in Uzbekistan was 1,800,000 tons, which in turn means the generation of a large amount of secondary waste products. During the season, one of the environmental problems associated with grape processing is the large amount of grape stalks (281x103 t), grape pomace (787x103 t), wine sediment (337x103 t) and waste water (24x106 m3) is formed. Some polluting properties of these residues, such as low pH (acidity) index and phytotoxic and antibacterial properties of phenolic substances, prevent the natural decomposition of grape residues. It is known that 60 mln. tons of grapes are grown for processing. According to FAO/WHO, 5-10 mln. tons of waste are produced, including 300-400 thousand tons of solid waste products in Uzbekistan. Failure to organize measures for processing waste products of this type causes problems from both an ecological and economic point of view. Today's industrial share of grape residues is 20-25%, usually they are not processed and are formed due to intensive accumulation of open land near the factory. This, in turn, leads to pollution of the environment and ecology. The main types of waste products generated during production are grape stalks, grape must and wine lees. In this regard, the chemical composition of grape residues is an important raw material base for the production of bioethanol for the second generation of biofuel products (BTII).

In the world, the role of bioethanol as an alternative energy in the fuel-energy complex (UEC) is increasing. One of the important indicators in the production of bioethanol is the energy balance coefficient (EBK), which is the amount of energy stored in the obtained biofuel compared to the amount of energy used for its production. According to the results of a number of studies, the first generation of bioethanol biofuel (BTI) produced from corn in the USA does not give a positive result according to the EBK indicator. A large amount of energy is spent on raw materials (including processing, planting, harvesting and further processing of raw materials) compared to the products obtained. According to the official report of the US Department of Agriculture, the EBK indicator of corn bioethanol is 1.24 units, but only 24% of the energy used for production is. The EBK indicator of cellulose bioethanol is 5-6 units, compared to gasoline, it has been determined that the amount of greenhouse gases released during the production and use of BTII is reduced by up to 85%. Bioethanol has a high octane value (99 in the engine and 105 in the research method), lower combustion temperature, no exhaust gases, because it does not contain sulfur compounds. Since bioethanol - BTII burns without producing ash, deposits do not form on engine spark plugs during the use of mixed gasoline with alcohol, and overheating is not observed during operation. For this reason, the production of bioethanol - BTII fuel based on lignin cellulose raw material is considered an urgent topic. At the same time, it corresponds to the principles of economy and fully meets the concept of today's development.

In recent years, special attention has been paid to the widespread use of biofuel products in our republic. For example, although works on obtaining biofuel were carried out by A. Azizov, N. Mominov, Sh. Imomov, B. Rakhmatov, environmentally friendly processing of fruit, vegetable and grape waste in canning and wine factories in the Republic research works based on the technology of obtaining the second generation of biofuel have not been sufficiently studied. In the decision of the President of August 22, 2019, it was determined that by 2030, the demand for the production of electricity from renewable energy sources in Uzbekistan will reach 25%. Currently, this figure is 10-12 percent. In the development strategy of New Uzbekistan for 2022-2026, special attention is paid to the continuous supply of electricity to the economy and the active introduction of "green economy" technologies to all sectors. In order to ensure the implementation of the above-mentioned tasks, one of the urgent tasks is to carry out scientific research work on the production and technology improvement of bioethanol BTII fuel based on grape juice.

Research methodology. Unimax 1010 is carried out using Incubator 1000 in a conical flask with a capacity of 2 l with a stirrer. The relative temperature of the conical flask is controlled using a TC 1/80 thermostat. Statistics 7.0 for optimizing the amount of enzyme preparations used in the development of a multienzyme complex for the enzymatic hydrolysis process. Statsoft software was used. *In this case, the rate of action of x,y,z enzyme preparations on cellulose over time and the concentration of accumulated sugars formed at the end of the reaction are determined. Unlike other experiments, here the total percentage of enzyme preparations involved in the reaction should be constant. for example 100%. 12 experiments were conducted for the enzymatic hydrolysis process. The results of the experiment are presented in the following literature [1-5]. The experimental project developed using the proposed software is shown in the following literature [1-5]. In the process of enzymatic hydrolysis, a three-factor triangular graph was created using SelloLux-A, Ultraflo Core and Brewzyme BGX enzyme preparations. The dependence of the concentration of accumulating sugars on the amount of enzyme preparations added to the reaction mass is expressed in the following equation* ***(****x, y, z****):***

$$RS=23{,}2631x + 22{,}5956y + 14{,}1481z + 7{,}5317xy + 30{,}3815xz + 29{,}1999yz;$$

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For the ideal process of enzymatic hydrolysis, the effect of the multi-enzyme complex developed in the following literature [1-5] is included. For the analysis of sugars, hydrolyzate and yeast samples formed during the fermentation process were determined in MiniSpin centrifuges (Eppendorf, Germany). The duration of mixing is 5 minutes at a speed of 10,000 revolutions per minute. The effect of Y-1693, PM-16 and Fm17 strains belonging to the class of saccharomycetes was studied for the alcoholic fermentation process. During fermentation, the volume of yeast reproduction and the concentration of bioethanol formed in the reaction mass are calculated. The relative temperature for alcoholic fermentation was 28-32 °C, pH-4.6 □ and the duration of the process was 72 hours. Six experiments were conducted to determine the optimal time for the two processes to work together. The results of the experiment are cited in the following literature [1-5].

Research results. To obtain the maximum amount of bioethanol, it is necessary to develop a nutrient medium with an optimal composition for alcoholic fermentation. During the experiment, enzymatic hydrolysis is carried out. In this case, POA (produkt, obrabotannyy azotnoy acid) acts as a substrate. First, a substrate with a dry matter content of 60 g/l is placed in the fermenter. The hydrolysis process is carried out in an 11-liter fermenter (designed by Pavlova). During the experiment, the acidity of the environment is controlled using a pH-meter in Pro 42xx. The fermenter has heat exchange elements and a device for providing the reaction mass. There is also a transmitter - M200. In the course of hydrolysis, enzyme preparations (mg/g) are introduced into the fermenter together with the substrate: "SelloLux-A" - 40, "Ultraflo Core" - 25, "BrewZyme BGX" - 50 and 42 in an environment with a pH of 4.6±0.2 hyphrolysis process is carried out at a temperature of 46±2 °C for an hour. During enzymatic hydrolysis, the total amount of RS (reducing sugar) is 48.5 g/l, including fermented sugar – 42.6 g/l, pentose – 5.9 g/l. After the end of the enzymatic hydrolysis process, the liquid part of the hydrolyzate was separated from the solid residues using vacuum filtration. In the experiment, the effect of the concentration of potassium phosphate, ammonium sulfate and yeast extract on bioethanol yield was investigated (Table 1). During the study, a three-factor experimental statistical model was developed for 10 different nutrient medium options. In addition to the above components, 0.2 g/l of potassium chloride and 1 g/l of magnesium sulfate were added to the nutrient medium for each variant. After the accumulation of a sufficient amount of hydrocarbon compounds during enzymatic hydrolysis, the nutrient medium is sterilized in an autoclave under a pressure of 5 atm for 20 minutes. The yeast cells are then placed in a fermenter to carry out alcoholic fermentation. According to the results of the research, 10 nutrient mediums with different contents were developed, and yeast cells were included in the reaction mass at a rate of 8-12% for each experiment. Yeast cell counts for all variants were initially 12 million CFU/ml. The extremity of the alcoholic fermentation process is characterized by an increase in the number of yeast cells to 100-130 million KOE/ml. The percentage of cell budding in the enzyme was 12-20%. In laboratory conditions, the hydrocarbon compounds obtained during hydrolysis are sterilized, enriched with additional active substances, and alcoholic fermentation is carried out at a temperature of 28 °C in a 2-liter flask using a TS-1/80 thermostat. An analysis of experiments in which the nutrient medium was enriched with additional components is presented in Table 1.

Table 1. The results of formation of the composition of the nutrient medium to obtain the maximum amount of bioethanol during alcoholic fermentation

	Experience numbers

Mineral additives and fermentation process activators	1	2	3	4	5	6	7	8	9	10
$(NH_4)_2SO_4$, g/l	0	4	0	4	2	0	2	2	2	2
KH_2PO_4, g/l	0	4	4	4	2	2	2	4	2	0
Yeast extract, g/l	0	20	20	0	20	10	0	10	10	10
$CaCl_2$, g/l	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2
$MgSO_4$, g/l	1	1	1	1	1	1	1	1	1	1
Indicators										
Decay rate constant $CC \cdot 10^3$, hour $^{-1}$	50,01	49,72	49,51	50,30	49,63	50,40	50,03	49,71	50,22	50,60
Residual concentration of RS, g/l	9,91	10,63	11,12	9,21	10,92	8,83	10,04	10,60	9,43	8,41
Bioethanol concentration, vol./%	2,22	1,62	1,72	2,10	2,20	2,30	2,35	2,41	2,48	2,55
Bioethanol production, %	66,92	48,84	51,85	63,31	66,32	69,33	70,84	72,65	74,76	76,87

According to the analysis of the experiment with 10 different options, the yield of bioethanol was 48.84% (for the most unsuccessful experiment), and the maximum was 76.87%. The statistical model was built on the basis of the data in the form of ternary graphical relationships presented in the following literature [5], and was created using the methodology of experimental statistical modeling. 3D distribution coordinate diagram presented in three-dimensional graphics (Fig. 1). Experimental data were processed and it was found that the maximum amount of bioethanol released during alcoholic fermentation also depends on the composition of the nutrient medium. The results of the three-factor experiment were based on the following equation (1).

$$K = 64{,}8x + 56{,}8y + 49{,}9z + 24{,}4xy + 47{,}9xz + 70{,}2yz \qquad (1)$$

where K is bioethanol concentration, %; x,y,z – (NH4)2SO4, KH2PO4 and yeast extract concentration, respectively, expressed in dimensionless form.

During the experiment, the dimensionless coefficient has a lower bound: -1 and an upper bound: +1. The minimum value of three-component mixtures is 0 g/l. The maximum amount of ammonium sulfate solution added to the feed medium was 4 g/l, 4 g/l for potassium monophosphate, and 20 g/l for yeast extract. Based on the developed statistical model, the concentration of these additives is introduced into the feed medium in 10 different proportions. The reliability of the equation was confirmed based on Fisher's test. A three-factor diagram of the response function K is shown in Figure 1. Here, it is shown that the increase in bioethanol concentration depends on the composition

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of the nutrient medium, with the optimal bioethanol yield of 77% represented as a dark red point in the triangular diagram. Then here, dark red represents the maximum bioethanol yield point and dark green represents the minimum bioethanol yield point.

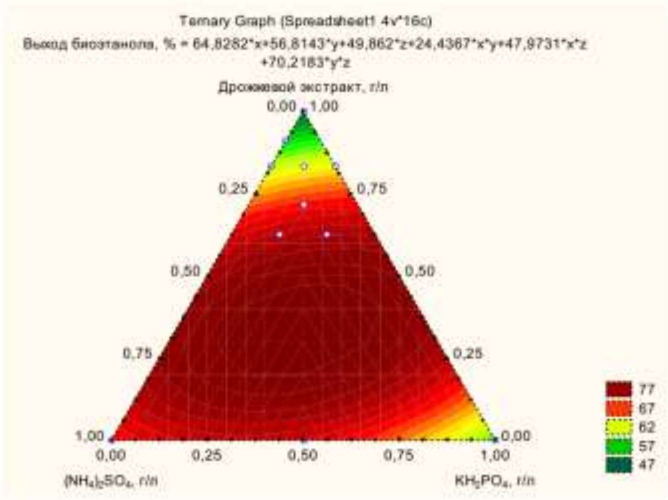

Figure 1. Dependence of bioethanol formation product (K, %) on nutrient medium content during alcoholic fermentation.

Analyzing the results based on the scheme and equation, it was found that yeast extract and ammonium sulfate solution are the most important factors influencing the formation of bioethanol during alcoholic fermentation. During fermentation, the concentration of potassium monophosphate in the amount of 0-2 g/l did not significantly affect the production of bioethanol. However, a concentration of 2-4 g/l resulted in a decrease in bioethanol concentration during alcoholic fermentation.

So, in the process of enzymatic hydrolysis, the active acidity of the nutrient medium is controlled by orthophosphoric acid. Therefore, it is assumed that a sufficient amount of phosphorus accumulates in the natural hydrolyzate and acts as a sufficient nutrient for yeast cells. Splitting of the native hydrolyzate (option #1) resulted in a bioethanol yield of 66.92%, indicating the absence of technically hazardous components or inhibitors in the medium. By adjusting the content of minerals and vitamins, a high-quality nutrient medium can be obtained, for example, 76.87% yield of bioethanol was obtained in option 10. The problem of optimizing the composition of the nutrient medium for the alcoholic fermentation process and obtaining the maximum amount of bioethanol was solved by adding additional nutrients in the following proportions: 1.90 g/l ammonium sulfate, 0.78 g/l monophosphate and 6.77 g/l By adding 1 l of yeast extract to the composition of the reaction mass, bioethanol with a percentage of 79.88% can be obtained. During the reaction, the volume ratio of bioethanol concentration was 2.65 vol./%.

Conclusion. When these nutrients were added to the reaction mixture, it was proven that 12.96% more bioethanol was produced during the experiment compared to experiment 1, which served as a control. The concentration of monophosphate does not significantly affect the mass of bioethanol formed during the reaction. If we exclude the monophosphate solution from the composition of the nutrient mass, bioethanol is produced with a share of 79.28% due to the fermentation of the reaction mass. In this case, the concentration of the produced bioethanol is 2.63 vol./% by volume. Table 1 shows the results of calculating the efficiency of the alcoholic fermentation process and the rate of consumption of hydrocarbon compounds in the reaction mass by yeast cells. The analysis of Table 1 shows that the biochemical activity of yeast cells in all 10 experiments during fermentation was 49.72-50.60·1-3 rate constant. This means that the biochemical activity of yeast is the same for all experiments. It should also be noted that the lower the residual concentration of storage sugars, the higher the substrate loss rate constant.

Gratitude. The authors would like to express their special thanks to the editor and team of the IV International Scientific and Applied Conference on Agricultural and Food Technology Research for their close assistance in the development of this article.

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