Теоретично обґрунтовано та експериментально підтверджено доцільність збагачення високонцентрованого сусла із крохмалевмісної сировини азотним та мінеральним живленням для дріжджових клітин. Досліджено вплив азотного живлення в якості амінного та аміачного азоту, а також мінерального живлення в якості наночастинок та іонів металів. Експериментально встановлено, що додавання азотного і мінерального живлення дозволяє отримати високу концентрацію дріжджових клітин, з високою фізіолого-біохімічною активністю, а також скоротити тривалість дріжджегенерування

Ключові слова: дріжджова клітина, високонцентроване сусло, сухі речовини, дріжджегенерування, наночастинки металів, мінеральне та азотне живлення

Теоретически обоснована и экспериментально подтверждена целесообразность обогащения высоконцентрованого сусла с крохмалсодержащего сырья азотным и минеральным питанием для дрожжевых клеток. Исследовано влияние азотного питания в качестве аминного и аммиачного азота, а также минерального питания в качестве наночастиц и ионов металлов. Экспериментально установлено, что добавление азотного и минерального питания позволяет получить высокую концентрацию дрожжевых клеток, с высокой физиолого-биохимической активностью, а также сократить продолжительность дрожжегенерирования

Ключевые слова: дрожжевая клетка, высоконцентрованое сусло, сухие вещества, дрожжегенерирование, наночастицы металлов, минеральное и азотное питание

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EFFECT OF NITROGEN AND MINERAL COMPOSITION OF THE HIGH-CONCENTRATED WORT MADE FROM STARCH-CONTAINING RAW MATERIALS ON THE CULTIVATION OF YEAST

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1. Introduction

Physiological-biochemical activity of yeast is of great importance for the fermentation of wort with high concentration. Using new breeds of yeast and adjusting biochemical composition of nutrient medium make it possible to significantly improve indicators of fermentation. It was scientifically substantiated that the necessary condition for the fermentation of wort with high concentration is the enhancement of physiological activity of yeast depending on the content of dry substances (DS) in wort and the temperature of fermentation [1].

Increasing the temperature of fermentation and the osmotic pressure of the medium leads to the creation of extreme conditions for the life activity of yeast. This may contribute to the lowering of regenerative and fermentative activity of yeast, which in turn results in the unstable work of fermentation separation. Therefore, it is an important direction of scientific research to search for ways to maintain the stability of yeast-generating processes, yeast metabolism, and enhance fermentative activity.

2. Literature review and problem statement

Higher concentrations of dry substances in wort predetermines the growth in the influence of nitrogen and mineral nutrition on the reproduction of yeast cells and wort fermentation [1].

The presence of free amino acids in the medium allows the intensification of yeast reproduction process. This increases not only the density of the yeast population, but the fermentative activity and cell productivity as well. Ammonia, as well as amine nitrogen, is the main source for the synthesis of protein substances in the cell. Ammonia nitrogen, cleaved from the ammonium salts of the medium and other nitrogen compounds, is used by yeast cells in order to synthesize amino acids. Nitrogen content in fermented substrates largely determines the rate of synthesis and the formation of yeast biomass. The most suitable for yeast, in terms of energy costs, is the nitrogen of amino acids [2].

The use of media balanced by amino acids contributes to the activation of processes of yeast-generation, to the synthesis of biomass and alcohol fermentation.

Yeast cell most easily and completely assimilates nitrogen in the amine form. Nitrogen of amidic form in the presence of amine nitrogen is almost never consumed, but, under anaerobic conditions, the need for this component grows.

It is known that the role of micro elements in life activity is extremely important [3]. A feature of micro elements is the ability to enter compounds with organic substances, such as proteins, peptides, amino acids, organic acids, sugars, and vitamins. Special role belongs to the bonds of metals with enzymes and vitamins. The presence of certain micro elements in the composition of these complexes enhances biological activity. Virtually all micro elements are the activators of enzymes and simultaneously part of the molecules [4].

Those activators that contribute to the increased activity of enzymes include metal ions and certain anions. As data from the scientific literature indicate, the activators of enzyme preparations are most often the ions of magnesium, calcium, potassium, mangan, cobalt and zinc [5]. Ions of mineral substances are required to maintain pH, osmotic stability, transportation of nutrients and as cofactors during fermentative-catalytic reactions [6].

It is known that minerals are capable of forming complexes with nucleic acids, affecting physical-chemical properties, the structure and biological function of nucleic acids.

Micro elements have an important role in protein biosynthesis in ribosomes, they actively participate in the stabilization of ribonucleic acids. Manganese impacts realization of the Krebs cycle reactions.

The main functions of zinc are related to the metabolism of carbohydrates, proteins, phosphates, as well as the formation of DNA and ribosomes. Zn, Mg influences the activity of key enzymes of carbohydrate exchange. The inclusion of zinc in the cell under any conditions bears a character of active transport, it affects the penetration of membranes and stabilization of cell components [7].

Nanotechnologies are a promising area of interdisciplinary research, which opens up a wide range of possibilities in different sectors [8].

Nanotechnologies are one of the most important tools in production, and it is assumed that agrifood nanotechnology will become in the near future the driving economic force. Nanoparticles, due to their unique characteristics, including small size, shape, high surface area, charge, chemical properties, solubility and degree of agglomeration [9] are the object of research in various areas [10]. Nanotechnologies may potentially find application in the food industry and processing as new instruments for transporting biologically active compounds to target areas [11]. Therefore, we should consider it promising to study the application of metal nanoparticles for yeast-generation.

3. The aim and objectives of the study

The aim of present work was to study the influence of nitrogen and mineral nutrition on the accumulation of yeast cells in the process of yeast-generation. This would provide obtaining yeast cells with high physiological-biochemical activity.

To accomplish the aim, the following tasks must be solved:

– to determine the amount of amine oxide in corn grain wort of various concentration;

– to establish experimentally the optimal concentration of amine and ammoniac nitrogen, as well as the concentration of macro- and microelements in the nano- and ionic forms during cultivation of yeast cells;

– to conduct research in order to determine the required number of cycles in using mineral nutrition.

4. Materials and research methods

The study was conducted under laboratory (Fig. 1) and industrial conditions. We used a culture of the yeast breed Saccharomyces cerevisiae DO-16 as the object of research.

To prepare industrial yeast and fermentation, we used wort with a concentration of 20 % DS and 28 % DS. In the study we used milled corn grains with a dispersion of 90 %, a 100 % pass through a sieve with a hole diameter of 1 mm and a 100 % pass through a sieve with a hole diameter of 0.5 mm. Physiological state of yeast cells was determined by coloring the yeast cells with a solution of Lugol. The content of dead cells – by coloring with methylene blue. The total number of yeast cells was determined by the method of direct counting in the Goryaev's chamber.

Fig. 1. Preparation of samples for culturing the yeast under laboratory conditions

Nanoparticles from aqueous dispersions were borrowed at the Scientific-Research Laboratory of the National University of Food Technologies (Ukraine), maintained by the method of volumetric electric-arc dispersion of current-conducting metals in a fluid.

Fig. 2. Laser spectrometer Zeta Sizer Nano (Malvern, United Kingdom)

Research into electrophoretic mobility and distribution of particles by size in the colloid system was conducted by the method of photo-correlation spectroscopy LCS using the laser spectrometer Zeta Sizer Nano (Malvern, United King-

dom) (Fig. 2) with an electrode system for measuring ζ-potential Universal Dipcell (ZEN1002) and a cell – Disposable polystyrene (DTS0012).

5. Results and discussion of the study of influence of amine and ammoniac nitrogen on the accumulation of yeast cells

At present, distilleries that produce alcohol mostly use corn grain. The yield of this culture is higher compared to other grain crops by 2–3, while the content of starch is 61–70 %.

We conducted a study to determine the amount of amine nitrogen in the wort from corn with different concentration. Table 1 shows that corn grain wort regardless of its concentration has a relatively low content of nitrogen nutrition.

Table 1

Content of amine nitrogen in wort made from corn

No. of entry	Concentration of DS, %	Content of amine nitrogen, mg/100 cm³	Content of amine nitrogen, g/dm³
1	20	8.4	0.08
2	25	14.0	0.14
3	27	16.24	0.16
4	28	18.06	0.18
5	30	19.88	0.19
6	31.5	21.8	0.21

Thus, one of the tasks of present study is to estimate effect of the concentration of amine nitrogen on the process of accumulation of yeast cells, which was adjusted by introducing an amino acid into wort, specifically glycine. Glycine was introduced to the yeast wort to a concentration of amine nitrogen of 0.3; 0.5; 0.7; 0.9, and 1.2 g/dm³, as well as seeding yeast – 15 mln/cm³. We used the yeast breed S.Cerevisiae DO-16 for the cultivation. The study was carried out using grain corn wort with a concentration of 20 % and 28 % of DS. The wort was acidified with sulfuric acid to pH 5.0. Orthophosphoric acid was introduced as phosphorus nutrition in line with standard consumption. A sample of yeast, cultivated with the addition of nitrogen nutrition as urea in line with standard consumption, served as control. Duration of the yeast cultivation was 24 hours [12].

Based on the data obtained (Fig. 3), it was established that in the process of culturing yeast an increased concentration of amine nitrogen in wort leads not only to the growing rate of yeast cells reproduction, by 1.4–1.5 times, but also to an increase in the total population, by 40–50 %, compared with control, depending on the concentration of DS in wort. However, increasing the concentration of amine nitrogen by larger than 0.7 g/dm³ does not cause a significant increase in the growth of cells.

It is known [12] that in order to provide yeast with nitrogen nutrition, the process of yeast-generation involves urea calculated to 400–600 g/m³.

By analyzing the data obtained, it was decided to conduct a study to determine the optimal concentration of urea (as ammoniac nitrogen), introduced to the wort in the amount of 400, 600, 800 and 900 g/m³, at wort concentration of 20 and 28 % of DS.

It was established (Fig. 4) that an increase in the concentration of nitrogen nutrition in the substrate led to the growing accumulation of yeast cells, by 40–60 %, which amounted to 260–430 mln/cm³ depending on the content of dry substances in wort. The addition of urea to the substrate, larger than 800 g/m³, contributed to a slight increase in the accumulation of yeast cells.

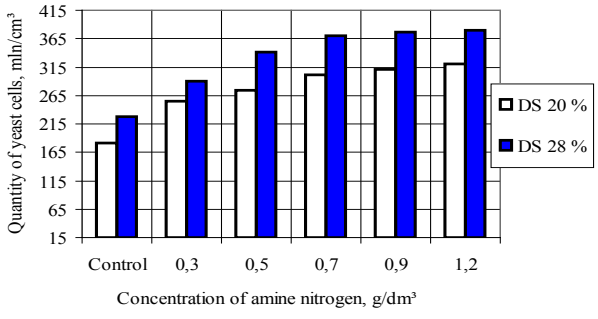

Fig. 3. Effect of the concentration of amine nitrogen on the synthesis of yeast cells in the process of yeast-generation

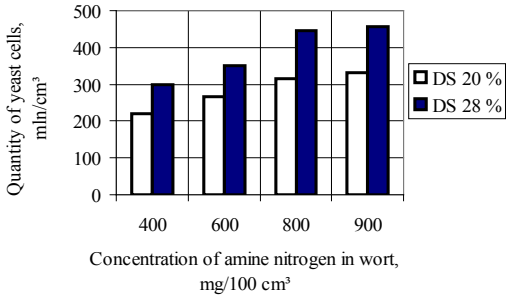

Fig. 4. Effect of the concentration of ammoniac nitrogen in wort on the synthesis of yeast cells in the process of yeast-generation

Based on the results of data obtained, it was established that the introduction of nitrogen nutrition of up to 600 and 800 g/m³ to serve as urea, regardless of the concentration of wort, is more appropriate. At optimal concentration of nitrogen nutrition, regardless of the form of its introduction, yeast has a high content of glycogen, the average size of cells is 5×2.0 μm and 8×2.3 μm. At optimal concentration of ammonia and amine nitrogen, duration of yeast-generation was 15–17 hours.

6. Results of research into effect of nanoparticles and ions of metals, serving as mineral nutrition, on the accumulation of yeast

One of the techniques to intensify the process of yeast-generation is the use of preparations based on nanoparticles – aqueous dispersions of biogenic preparations [12]. In the study we used nanoparticles of aqueous dispersions with the following dimensions: Cu – 200 nm, Mn – 180 nm, Zn – larger than 30 nm, Fe – 200 nm, Mg in the form of micro fractions.

To perform the research, we used corn grain wort with a concentration of 28 % DS. Seeding yeast were introduced calculated to 15 mln/cm³. Concentration of metals in the nanoform was 1.2 μg/m³, 10 μg/m³, 30 μg/m³. Wort for culturing the yeast was prepared according to the requirements of technical regulations [13].

The need for micro elements can increase by several times, under condition that the cell is under stressful conditions, such as high temperature and osmotic pressure.

An analysis of the data has revealed (Fig. 5) that the application of nanoparticles of Zn, Mg, Fe, Mn and Cu at a concentration of 1.2 μg/m³ resulted in the accumulation of the largest quantity of yeast cells and amounted to 190–420 mln/cm³. Increasing the concentration of these components to 10 and 30 mg/cm³ contributed to a reduction in the concentration of cells when cultured.

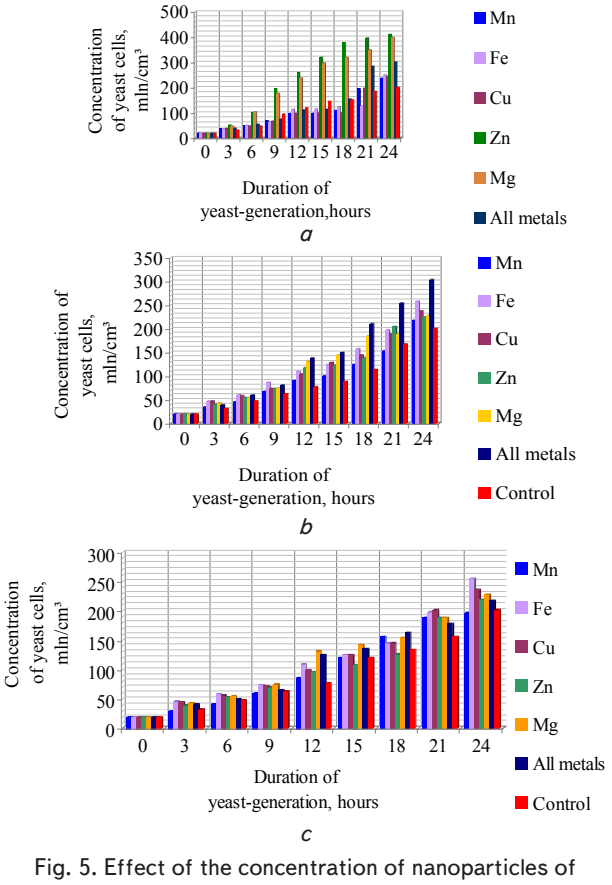

Fig. 5. Effect of the concentration of nanoparticles of different metals on the accumulation of yeast cells in the process of yeast-generation: *a* – concentration of metals nanoparticles 1.2 μg/cm³; *b* – concentration of metals nanoparticles 10 μg/cm³; *c* – concentration of metals nanoparticles 30 μg/cm³

The largest quantity of yeast cells was observed when using nanoparticles of metals Zn and Mg and which amounted to 420 and 380 mln/cm³, respectively. Based on the data obtained, in the course of present study we investigated combined use of metals. Cultivation of yeast was conducted on wort with a concentration of 20 and 28 % of DS (Fig. 6).

Combined utilization of the above-specified metals in the nanoform allowed us not only to obtain a high concentration of yeast cells, which ranged from 370 to 480 mln/cm³, but also to reduce duration of yeast-generation to 15–17 hours.

To obtain a comparative characteristic, we investigated the use, at the stage of yeast-generation, of metal ions of Zn^{2+}, Mg^{2+} as the solutions of $ZnSO_4$ and $MgSO_4$ salts in the amount of 40, 50, and 60 g/m³ of wort (Fig. 7), at a wort concentration of 28 % DS.

It was established that the introduction of 50 g/m³ of ions of metals Zn^{2+}, Mg^{2+} at the stage of yeast-generation contributed to an increase in the concentration of yeast to 320–370 mln/cm³, reduced duration of yeast-generation to 17–20 hours and improved physiological characteristics. Increasing the concentration of metal ions up to 60 g/m³ did not significantly affect the accumulation of yeast biomass.

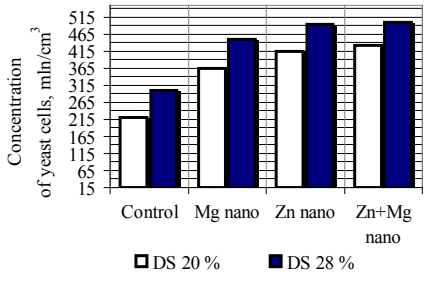

Fig. 6. Effect of Zn and Mg metal nanoparticles on the accumulation of yeast cells in the process of yeast-generation

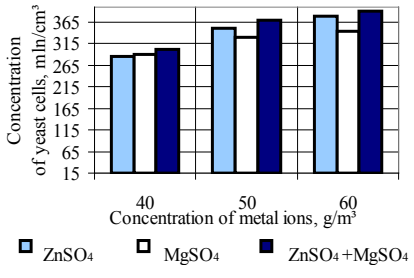

Fig. 7. Effect of metal ions on the synthesis of yeast cells in the process of yeast-generation

An analysis of the data obtained demonstrated the feasibility of using metals Zn and Mg and their mixture in both nano- and ionic forms. That is why we carried out research of the application of these metals at the stage of yeast-generation at repeated use, i. e. in cycles (Table 2). One cycle is the duration of the process of yeast-generation when obtaining industrial yeast; according to regulation, it lasts for 24 hours [12].

Table 2

Effect of cyclicity on the synthesis of yeast cells using different forms of mineral nutrition

Form of nutrition	Number of cycles											
	1	2	3	4	5	6	7	8	9	10	11	12
	Concentration of yeast cells, mln/cm3											
Nanoparticles of Zn	301	338	340	303	236	198	298	301	295	289	280	225
Nanoparticles of Mg	270	340	365	310	219	201	239	248	238	251	301	298
Mixture of nanoparticles of Zn and Mg	327	437	443	391	280	171	245	278	344	304	321	307
$ZnSO_4$	273	338	295	287	161	181	126	158	166	181	173	170
$MgSO_4$	238	256	271	253	162	205	324	298	301	341	293	274
Mixture of components of $ZnSO_4$ and $MgSO_4$	219	268	398	371	298	263	242	274	231	189	202	141
Control	220	224	228	228	224	223	224	220	225	221	221	205

Based on data in Table 2, it was established that the addition of mineral nutrition contributed to an increase in the synthesis of yeast cells to the second and third cycles depending on the form of the examined metals. An increase in the number of cycles of yeast-generation led to a decrease in the concentration of yeast cells from 5 to 28 %. We observed inhibition of the growth rate of yeast cells. In addition, dimensions of the cells decreased, which could possibly indicate the oversaturation of cells with the examined components.

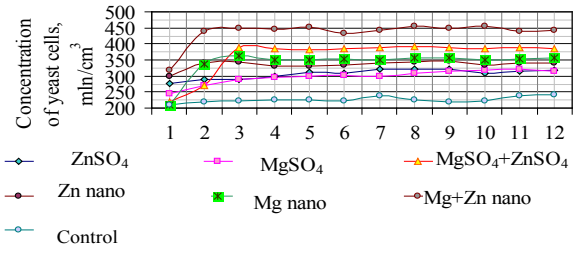

Fig. 8. Study of cyclic use of nutrition for yeast

Based on the data received, we conducted a study to determine the optimal number of cycles using mineral nutrition. Given the results of research, it was established (Fig. 8) that the addition of metals in nano- and ionic forms at the stage of yeast-generation should be performed cyclically at the following ratio: 3 cycles with metals and the same without metals; it will provide stabilization in the accumulation of yeast cells and high fermenting activity.

Through the use of an additional source of nitrogen and mineral nutrition for yeast we obtain a high concentration of physiologically-active yeast cells, which will make it possible to ferment the wort with high concentrations.

At present, the distilleries of Ukraine accumulate alcohol in mash to 14 %. That is why our task was to select optimal concentrations of nitrogen and mineral nutrition for yeast in the cultivation. A cyclic application of nutrition is proposed.

The benefits of the fermentation of wort with high concentrations include the economic factor. After all, bringing down the costs is especially relevant today. Application of energy-saving technology makes it possible to increase specific yield of alcohol, to reduce energy consumption, reduce the amount of distillery dregs.

The shortcoming of present research is the complexity of application of nanoparticles from aqueous dispersions under industrial conditions, due to the fact that these systems are not stable over a long period of time.

Further development of present work might include design of the technology for industrial yeast in order to ferment wort with a concentration of DS higher than 28 %, as well as the application of other types of nutrition.

7. Conclusions

1. It was determined that corn grain wort has a low content of nitrogen nutrition regardless of its concentration. This necessitates introduction of additional sources of nitrogen nutrition, as urea, or the amino acid glycine. Given the fact that the distilleries utilize carbamide as nitrogen nutrition, since the nitrogen of amino acids is the best source for yeast cells, we selected the amino acid glycine for comparison.

2. It was established experimentally that in the course of cultivating the yeast on wort with a concentration of 20, 28 % DS, a necessary precondition is the adjustment of nitrogen nutrition with additional introduction of glycine to serve as amine nitrogen, the concentration of 0.7 g/dm^3 of wort, and ammoniac nitrogen (to serve as carbamide) in the amount of 800 g/m^3, which made it possible to increase the concentration of yeast cells to 280–430 mln/cm^3 in the process of culturing the yeast depending on the concentration of wort and the type of nitrogen nutrition, respectively.

3. We have proven the feasibility of cyclic use of mineral nutrition, both in ionic and in nano forms, during yeast cultivation, in order to obtaining cells with high physiological-biochemical activity.

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