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THE EFFECT OF THE INTENSITY OF AERATING THE MEDIUM ON THE METABOLIC ACTIVITY OF ALCOHOL YEAST

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Abstract. The article presents the results of investigating how the intensity of aerating the medium effects on the cultivation process and the metabolic activity of alcoholic yeast *Saccharomyces cerevisiae*, strain U-563, in the modern technology of alcohol and baking yeast from molasses. The chemical and technological parameters of media at the aerobic and anaerobic stages of the process, the level of accumulation of the major and secondary products of yeast metabolism, and their enzymatic activity have been determined by methods commonly employed in science and in the practice of alcohol biotechnology. The objects of research were the yeast *Saccharomyces cerevisiae*, molasses wort, the medium in the process of yeast cultivation, and fermented wash. It has been established that two factors are the most important in the accumulation of alcoholic yeast biomass: the intensity of aerating the medium, and the staged introduction of the substrate during biomass cultivation. The more aerated the medium, the more intensively secondary metabolites of yeast *Saccharomyces cerevisiae* are formed (glycerol, aldehydes, higher alcohols, volatile acids, and esters) – both at the yeast generation stage and during anaerobic fermentation. When yeast *Saccharomyces cerevisiae* is grown in a gradient-continuous manner in a battery of series-connected apparatuses, with undiluted substrate (molasses) added by degrees, yeast biosynthesis is significantly enhanced compared to the traditional homogeneous-continuous method. The results obtained indicate the active metabolism of carbohydrates in the Krebs cycle, when the medium is intensively aerated. Besides, the results reveal the high reactivity of aldehydes and esters that results in their transformation into other compounds, and in a great decrease in their amount at the anaerobic stage of the process. However, a progressive increase is observed in glycerol, higher alcohols, and volatile acids, starting from the first yeast generator and up to the last fermentation apparatus, irrespective of the level of aerating the medium during yeast cultivation. These findings can be effectively used to manufacture food, technical, and fuel ethanol industrially from sugar-based raw materials in the course of co-production of alcohol and baking yeast.

Key words: gradient-continuous yeast generation, fermentation, yeasts *Saccharomyces cerevisiae*, aeration intensity, alcohol, secondary metabolic products.

ВПЛИВ ІНТЕНСИВНОСТІ АЕРУВАННЯ СЕРЕДОВИЩА НА МЕТАБОЛІЧНУ АКТИВНІСТЬ СПИРТОВИХ ДРІЖДЖІВ

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Анотація. У статті представлено результати досліджень впливу інтенсивності аерування середовища на процес культивування і метаболічну активність спиртових дріжджів *Saccharomyces cerevisiae* штаму У-563 у сучасній технології спирту і хлібопекарських дріжджів із меляси. Хіміко-технологічні показники середовищ в аеробній та анаеробній стадіях процесу, рівень накопичення основних і вторинних продуктів метаболізму дріжджів та їхню ферментативну активність визначали за прийнятими в науці і практиці біотехнології спирту методиками. Об'єктом досліджень були дріжджі *Saccharomyces cerevisiae*, мелясне сусло, середовище в процесі культивування дріжджів і дозріла бражка. Встановлено, що найсуттєвішу роль у накопиченні біомаси спиртових дріжджів відіграють два фактори: інтенсивність аерування середовища і ступінчатий ввід субстрату протягом процесу культивування біомаси. Із підвищенням ступеня аерування середовища посилюється утворення вторинних продуктів метаболізму дріжджів *Saccharomyces cerevisiae*: гліцерину, альдегідів, вищих спиртів, летких кислот і складних ефірів як на стадії дріжджегенерування, так і при анаеробному бродінні. Вирощування дріжджів *Saccharomyces cerevisiae* градієнтно-безперервним способом у послідовно з'єднаних апаратах з поступовим додаванням нерозбавленого субстрату (меляси) забезпечує суттєве посилення біосинтезу дріжджів в порівнянні з традиційним гомогенно-неперервним способом. Отримані результати свідчать про активний метаболізм вуглеводів у циклі Кребса в умовах інтенсивного аерування середовища, а також про високу реакційну здатність альдегідів і складних ефірів, внаслідок чого вони перетворюються на інші сполуки і кількість їх в анаеробній стадії процесу значно зменшується. Разом з тим встановлено поступальне збільшення гліцерину, вищих спиртів і летких кислот, починаючи від першого дріжджегенератора і до останнього бродильного апарата, незважаючи на рівень аерування середовища при культивування дріжджів. Наведені результати можна ефективно використати у промисловому виробництві харчового, технічного і паливного етанолу із цукровмісної сировини в умовах спільного виробництва спирту та хлібопекарських дріжджів.

Ключові слова: градієнтно-безперервне дріжджегенерування, зброджування, дріжджі *Saccharomyces cerevisiae*, інтенсивність аерування, спирт, вторинні продукти метаболізму.

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Introduction. Formulation of the problem

The research is in the field of biotechnology of alcohol and baking molasses yeast. For this technology, a topical task is intensifyin of yeast biomass synthesis and studying the changes in yeast metabolism during this process. The problem of increasing the yeast accumula-

tion in the wort has been investigated by intensifying the aeration of the medium and by the staged introduction of the substrate in the process of cultivating the producer.

Analysis of recent research and publications

In the biotechnology of microbial synthesis, commonly used is the method when, during the process, the substrate is loaded in a staged, sequential way in order to reduce the inhibitory effect of its increased concentration at the beginning of the producer cultivation or of the target product synthesis [1-5]. Ukrainian scientists have performed a series of studies to optimize yeast cultivation in the technology of co-production of alcohol and baking yeast [2,6]. As a result, the optimal parameters for the batch cultivation of the producer have been determined.

An example of how the method of staged introduction of the substrate in the biotechnology of alcohol from sugar-containing raw materials in continuous producer cultivation can be scientifically and technologically developed is the yeast generation technology using the battery principle of the equipment configuration (serially connected yeast generators) [1,2]. The authors, basing on the results of laboratory experiments [6], bench testing, and experimental-industrial studies [7,8], have shown that dispersed input of carbohydrate feed during the yeast production process increases the process productivity for the yeast biomass and results in its higher activity.

However, an analysis of scientific and practical achievements in this field shows that in gradient-continuous yeast generation for inoculating yeast growing, there are still plenty of potential reserves left. In particular, a question of high scientific interest is how the technological parameters that intensify biomass synthesis effect on the formation of its secondary metabolic products, and on the physiological activity of yeast as an alcohol producer [9-14]. The reason why the problem is important is the need to minimize the accumulation of certain substances (primarily glycerol, volatile acids, esters, and aldehydes) by yeast. As these metabolic products are formed from fermented sugars, an increase in their accumulation worsens the economical efficiency of alcohol and yeast biomass synthesis, which contradicts the goal of the technology of co-production of these two products [3,15,16]. However, it is also known that increasing the physiological activity of alcoholic yeast reduces unproductive losses of sugars and increases the yield of target products [5,11].

One of the key parameters of inoculating yeast growing in the co-production technology is the intensity of the aeration of the medium. In the science and practice of traditional alcohol technology, it is a well-known fact that yeast biomass synthesis in the wort intensifies with more intensive aeration of the medium [2,13]. According to some researchers' data, secondary products are also formed under these conditions [17,18]. However, in the professional literature on gradient-continuous cultivation of alcoholic yeast in the co-production of alcohol and bakery yeast, no such studies have been found.

The purpose of this work was to study how the intensity of aerating the medium influences the cultivation process and the metabolic activity of yeast *Saccharomyces cerevisiae* in the modern technology of alcohol and molasses bakery yeast, with gradient-continuous yeast generation used.

Research tasks:

1. To investigate the influence of the intensity of aerating the medium on the process of cultivation and wort fermentation.
2. To determine the dependence of yeast biomass accumulation and its physiological activity on the manner of inputting the substrate into the medium.
3. To determine the effect of the intensity of aerating the medium on the formation of yeast's secondary metabolic products.

Research materials and methods

The objects of research were: raw materials (sugar-beet molasses), molasses wort, yeast *S. cerevisiae*, fermented wash and its distillates.

Sugar-beet molasses from Kamyanets-Podilsky sugar plant, containing 78.5% of dry substances (DS) and 48.4% of fermented carbohydrates, served as the raw material for molasses wort.

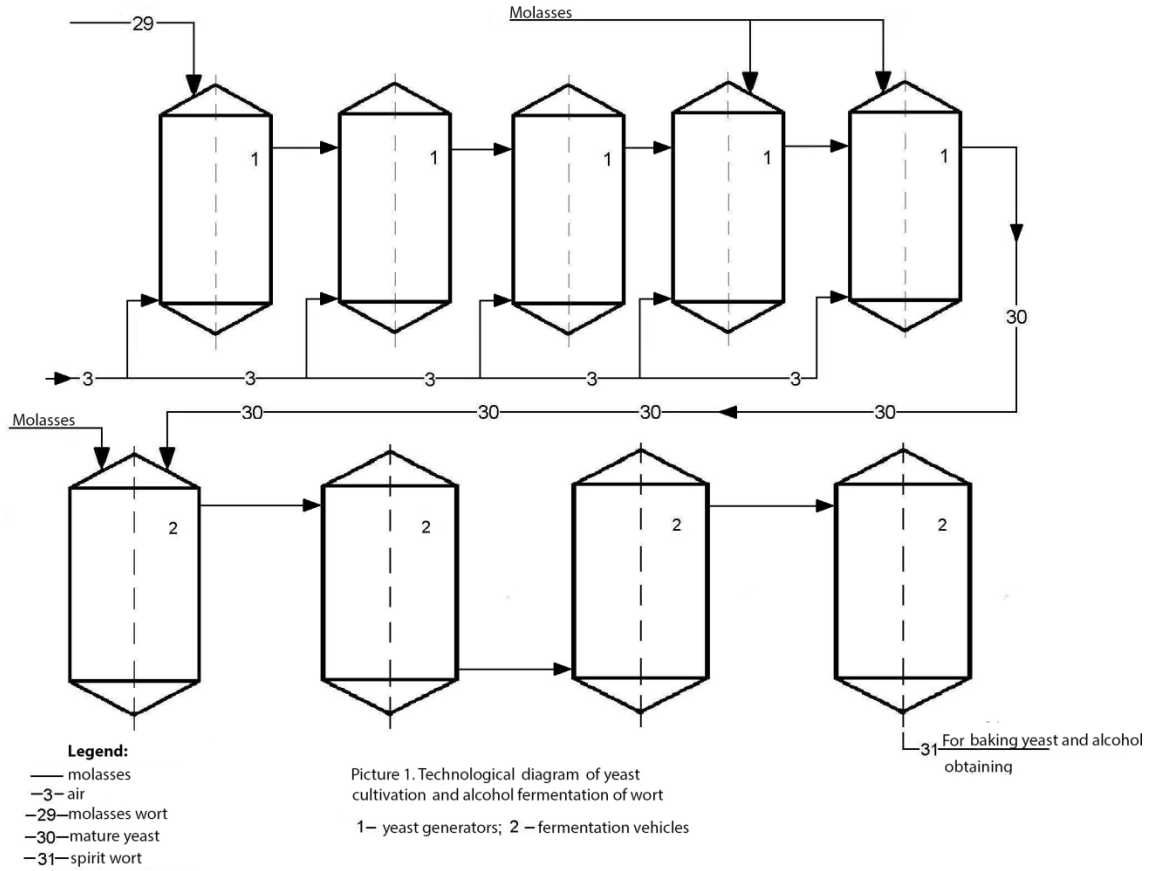
As the producer of alcohol, a yeast *S. cerevisiae* strain U-563 was used. It was taken from the collection of industrial microorganisms of the Ukrainian Research Institute of Alcohol and Biotechnology of Food Products [2]. The inoculating yeast was prepared according to the current regulations [2]: from a test-tube with its pure culture, on molasses wort enriched with nitric and phosphorous, in several stages. The first three stages were carried out in the laboratory – in flasks and bottles (sterile wort), and the following ones – in pure culture apparatuses and yeast generators (in conditions of natural pure culture).

The influence of aeration intensity on the process of wort cultivation and fermentation was studied at Dovzhok Alcohol Plant (Khmelnytsky region). The fermentation installation consisted of five yeast generators (useful capacity of each 27 m^3), connected in the upper part with overhead communication lines and four serially connected fermentation apparatuses (35 m^3 each). All yeast generators in their lower part were equipped with aeration devices to input and disperse air in the medium. The aeration intensity was set according to the conditions of a particular experiment. All apparatuses of the fermentation installation were equipped with heat-exchangers of the coil-pipe type to maintain automatically the optimum temperature of the medium (+28–30°C). In the fermentation apparatuses, conditions for fermentation of anaerobic sugars (without the aeration of the medium) were created.

The experimental variant was the process of yeast cultivation in a gradient-continuous manner (Fig. 1).

Molasses wort was injected into the first apparatus (1), and the medium was flowing, one after another, from the first to the fifth yeast generator. In the fourth and fifth apparatuses, undiluted molasses was added to increase the initial concentration of DS of the wort to the

planned level. From the fifth apparatus, mature yeast came into the main fermentation apparatus 2, where undiluted molasses was also added continuously to increase the initial concentration of wort DS to the level determined by the experimental conditions. From the last (the fourth) fermentation apparatus, the fermented wash was sent for separation followed by obtaining commercial bakery yeast. The wash without yeast was sent to the brew-rectification department to produce rectified alcohol [2].



Picture 1. Technological diagram of yeast cultivation and alcohol fermentation of wort

1 – yeast generators; 2 – fermentation vehicles

As a control, the traditional technology of two-product manufacturing was used. According to it, yeast is cultivated in a homogeneous-continuous manner in yeast generators ("chemostats") working in parallel [1]. In this case, the wort was separately introduced into each of the yeast generators, and the mature yeast from each apparatus was joined in one stream and injected into the main apparatus of the fermentation battery. Further, the process of anaerobic fermentation of the medium occurred in the same way as in the experimental variants.

In molasses and molasses wort, the dry substances (DS) content was determined by the refractometric method, pH – by the electrometric method, and acidity – by electrometric titration. The amount of fermentable sugars in molasses was calculated according to the values of direct, inverse polarization and to the invert sugar contents [19].

In semi-manufactured products and fermented wash, the observed and actual concentrations of DS were determined by the areometric method, pH – by the electrometric method, yeast concentration – by the weight method (with humidity taken as 75%), alcohol concentration – in the distillates of the wash by the areometric method.

In existing technologies, the concentration of DS in molasses solutions (molasses wort) is determined directly in the wort with hydrometers (saccharometers) traditionally used in alcohol biotechnology [2]. However, for culture media, when the amount of DS continuously decreases and ethanol accumulates as a result of alcoholic fermentation of sugars, this method is not objective. For this purpose, a parameter called "initial concentration of dry substances" has been developed and introduced into the practice of controlling the technological process of fermentation; the notation is IC [19]. Its value in the fermented media is determined as follows. In the sample selected, two parameters are determined: the alcohol content (a) and the actual concentration of dry substances (ACDS), by the above mentioned methods. IC is calculated by the formula (1):

$$IC = ACDS \cdot g + 1{,}541 \cdot a, \quad (1)$$

where a is the concentration of alcohol in the medium under study, vol. %;

1,541 is the coefficient of conversion of formed alcohol into dry substances (sucrose);

g is the density of the solution at a given DS concentration (g/ cm^3), which is calculated as follows:

$$g=200/(200-n), \quad (2)$$

where n is the reading of the hydrometer (saccharometer) for the given ACDS, % of DS.

The amount of unfermented carbohydrates in the molasses wash was determined by the resorcinol-colorimetric method [2]. The glycerol content was determined by the distillation-colorimetric method [19]. In wash distillates, the amount of volatile organic acids was determined by titration with an alkali [19], the amount of esters – by their saponification [19], the amount of aldehydes – by the photocolorimetric method using Schiff's (fuchsin-sulfide) reagent, and the amount of higher alcohols – by the photocolorimetric method with the paradimethylbenzaldehyde reagent according to the methods [19] used in the science and practice of alcohol production.

The enzymatic activity of yeast was analyzed by the zymase activity, which was determined by the gasometric method in the Yeletsky device, and by the raising power measured by the emersion rate of a dough ball [19]. The results were statistically processed with the Microsoft Office standard program by the methods of variation and correlation statistics. The mean values and standard errors (M±m) were determined. The values obtained for $P<0.05$ were considered reliable.

Results of the research and their discussion

The results of comparing the yeast biomass accumulation in conditions of different aeration intensities and different methods of yeast cultivation technology (Table 1) indicate the following.

Table 1 - The main results of wort fermentation under different conditions of yeast cultivation

Parameters	Yeast cultivation method		
	Homogeneous-continuous (chemostat) – control	Gradient-continuous (battery) – experiment	
		Variant 1	Variant 2
DS Concentration in the wort for yeast, %	5–6	5–6	5–6
Intensity of aerating the medium during cultivation, $m^3/m^3 \cdot h$: – in the first two apparatuses – in the next three	 15 15	 10 7	 10 7
IC of industrial yeast, which was increased in the experimental variants due to inputting molasses into the fourth and the fifth apparatuses, g/100 cm^3	5–6	9–10	12–13
Concentration of biomass in mature yeast, g/dm^3	29±2	36±2	42±2
pH of the medium	4.8	4.8	4.9
IC in the second fermentation apparatus, g/100 cm^3	12–13	12–13	12–13
Concentration of dissolved oxygen in the industrial yeast medium: – mg/dm^3 – % of saturation	 3.5 94	 3.1 92	 3.0 91
pH of the medium	4.8	4.8	4.9
Concentration of alcohol in industrial yeast, vol.%	2.5	2.9	3.0
Concentration of biomass in fermented wash, g/dm^3	34±2	41±2	46±2

In the control variant, the yeast generators functioned as chemostats (the wort inflow and selection of the yeast medium were individual for each apparatus). At the 5–6 % concentration of wort DS and aeration intensity of about 15 $m^3/m^3 \cdot h$, the biomass accumulation in mature yeast was 29 g/dm^3. In the experimental variants (the battery system of connecting yeast generators), with the same DS concentration of the initial wort fed into the first yeast generator, the medium was successively flowing from the first to the last apparatus. The undiluted molasses was continuously fed in equal portions into the last two (the 4th and the 5th) apparatuses to increase the IC to 9–10 g/100 cm^3 (experimental variant 1) and to 12–13 g/100 cm^3 (experimental variant 2). The concentration of dissolved oxygen at the end of the cultivation process, due to the efficient air distribution system in all apparatuses, was 91–94% of full saturation. In this case, the accumulation of biomass in mature yeast was 36 and 42 g/dm^3 respectively compared to 29 g/dm^3 in the control, though the intensity of aerating the medium in the last three yeast generators of both experimental variants lowered decreased to 7 $m^3/m^3 \cdot h$ (this parameter in the control was maintained, throughout the process, at 15 $m^3/m^3 \cdot h$). This result proves the effectiveness of gradual injection of additional amount of substrate at the stage of yeast cultivation in a battery apparatus system. At the same time, it is obvious that there is a direct relationship between the amount of substrate introduced (as the IC parameter indicates) and the biomass of the producer in mature yeast. The latter was fed into the main fermentation apparatus in a continuous stream, where an appropriate amount of undiluted molasses was added, too, to increase the value of IC in the wash up to 12–13 g/100 cm^3 in the control and the first experimental variants.

As it can be seen from the results (Table 1), the stage of yeast cultivation played a key role in the biomass accumulation at the end of the process (in fermented wash), as the increase of biomass at the anaerobic stage of the control and the experimental variants was approximately the same. The largest amount of biomass in the fermented wash was found in experimental variant 2 (45–47 g/dm^3), in which all the carbohydrate substrate was introduced at the yeast generation stage.

Table 2 shows the results of gradient-continuous yeast cultivation with a differentiated level of aerating the medium, namely: in variant 1, the amount of the air injected into the first three yeast generators of the battery was 15 m^{3}/m^{3} h, and in variant 2–7 m^{3}/m^{3} h. In the fourth and the fifth apparatuses of both variants, this parameter was the same – 3–5 m^{3}/m^{3} h.

Table 2 – Technological parameters of yeast cultivation and wort fermentation with different intensity of aerating the medium

Parameters	The value of the parameter	
Molasses wort		
Concentration of DS, %	6.0	6.0
Undiluted molasses was fed into the 4nd and the 5th yeast generators to increase the IC to the level, g/100 cm^3	11–12	11–12
Mature yeast		
Intensity of medium aeration, $m^{3}/m^{3}\cdot h$:		
– in the first three yeast generators	15–20	6–8
в д – in the last two	3–5	3–5
Visible concentration of DS, %	4.5–5.5	3.6–4.2
Biomass, g/dm^3	45±2	36±2
Alcohol, vol. %	3.0–3.5	3.5–4.1
Fermented wash		
Visible concentration of DS, %	4.0–4.5	3.0–4.0
Biomass, g/dm^3	48±2	41±2
Alcohol, vol. %	3.3–3.6	3.8–4.0
Unfermented carbohydrates, g/100 cm^3	0.20	0.20

Molasses wort with 6% of DS was fed into the first yeast generator, and undiluted molasses – in the fourth and the fifth ones to increase the IC value to about 12 g/100 cm^3 in both variants. It has been established that under these conditions biomass accumulation in the mature yeast in the first variant was 44, and in the second 36 g/dm^3. The same tendency was observed in anaerobic fermentation of the medium: the amounts of yeast at the end of the process, that is, in the fermented wash, were 48 and 41 g/dm^3, respectively.

The results summarized in Table 1 and Table 2 show that, with all the rest of the conditions of alcohol yeast cultivation remaining the same, the most important role in its accumulation is played by two factors: the intensity of aerating the medium, and the staged input of the substrate in the course of biomass cultivation.

The specific character of this method of yeast cultivation, with differentiated aeration and molasses introduction distributed over time, aroused scientists' interest in the peculiar features of yeast metabolism at various stages of cultivation and of anaerobic fermentation of wort and their enzymatic activity. Scientists, judging by the specialized literature, have never studied these aspects of the problem.

The experiments were carried out in two modes. In both of them, the wort was injected into the first yeast generator, and undiluted molasses in the fourth and the fifth ones to raise the IC of the medium to 8–9 g/100 cm^3. Mature yeast from the fifth yeast generator came to the first fermentation apparatus; and here, the molasses was injected to make the IC of the medium about 18 g/100 cm^3. These two modes were distinguished by the intensity of aerating the medium in the course of gradient-continuous cultivation: in one, this parameter gradually decreased from 25 (in the first yeast generator) to 10 m^{3}/m^{3} h (in the fifth) (Table 3), and in the other, respectively, from 15 to 5 m^{3}/m^{3} h (Table 4).

In both modes, the dynamics of secondary fermentation products and the enzymatic activity of yeast during their cultivation and anaerobic fermentation of wort were studied.

The comparative estimation of the data of these tables confirms the well-known regularities concerning the influence of the intensity of aerating the medium on yeast accumulation: a higher value of this parameter results in an increase in the amount of biomass in industrial yeast from 45 (Table 3) to 57 g/dm^3 (Table 4), and, respectively, in fermented wash from 55 to 66 g/dm^3. It has been determined that zymase activity of yeast, which reflects the rate of monosaccharides conversion to ethanol and CO_2, remained at almost the same level throughout the process from the beginning of the yeast generation to the end of the anaerobic stage, regardless of aeration intensity at the stage of yeast cultivation, and was within narrow limits of 32–36 min. The same tendency was also observed in the raising power, which characterizes the ability of yeast to perform alcoholic fermentation in wheat dough. The value of this parameter in the whole yeast-fermentation system also varied but little both throughout the whole process in general and, in particular, depending on the aeration intensity during yeast generation: fluctuations were 31 to 35 min.

Analysing the changes in the secondary products content in the medium during biomass cultivation and subsequent anaerobic fermentation of the wort, one can make the following generalizations.

Intensification of the aerobic pathway of carbohydrates catabolism during yeast cultivation, as a result of an increase in the intensity of aerating the medium (from 5–15 to 10–25 $m^{3}/m^{3}\cdot h$), was accompanied by an in-

crease in the mass, or volume, concentration of all the secondary fermentation products considered that were present in the medium: glycerol, aldehydes, higher alcohols, volatile acids, and esters.

Table 3 – Dynamics of technological parameters and biosynthesis of secondary fermentation products during yeast cultivation with high aeration intensity

Parameters	Number of a yeast generator in the battery					Fermentation apparatus		
	1	2	3	4	5	1	2	4 (fermented wash)
Aeration intensity, $m^3/m^3 \cdot h$	25	25	20	15	10	0	0	0
Actual concentration of DS, %	3.0	2.7	3.3	4.0	5.2	9.5	9.0	7.9
Acidity, deg	0.3	0.3	0.5	0.6	0.7	0.7	0.8	0.8
Biomass, g/dm^3	34	38	42	48	57	58	64	66
Alcohol, vol. %	1.1	0.9	1.1	1.7	2.4	4.8	5.9	6.5
IC, $g/100\ cm^3$	4.6	4.0	5.0	6.6	8.8	16.9	17.8	17.8
Unfermented carbohydrates, $g/100\ cm^3$	0.98	1.10	1.05	1.10	1.20	2.4	1.3	0.29
Zymaze activity of yeast, min	34	33	36	34	35	35	32	33
Raising power, min	33	31	33	35	35	32	34	33
Glycerol: $g/100cm^3$ g/dm^3 of anh. al.	 0.120 109.1	 0.125 134.9	 0.135 113.6	 0.152 89.4	 0.160 66.7	 0.375 78.1	 0.398 75.8	 0.468 72.0
Aldehydes: vol. %, cm^3/dm^3 of anh. al.	 0.093 84	 0.184 204	 0.192 175	 0.176 104	 0.141 58	 0.082 17	 0.075 13	 0.057 9
Higher alcohols, vol. %	0.016	0.019	0.022	0.028	0.035	0.046	0.047	0.044
Volatile acids: mg/dm^3 of the wash g/dm^3 of anh.al.	 – –	 36 4.2	 96 8.7	 – –	 132 5.5	 216 4.5	 – –	 264 4.1
Esters: mg/dm^3 of the wash g/dm^3 of anh. al.	 – –	 105 11.7	 70 6.4	 – –	 53 2.2	 47 0.75	 – –	 48 0.75

Note: The sign dash "–" in Tables 3 and 4 means "not defined"

However, by the nature of changes in their concentration during the process, the yeast metabolic products under study can be divided into two groups.

Glycerol, higher alcohols, and volatile acids, the amount of which is steadily increasing throughout the two-stage process, represent one of these groups. It can be assumed that these substances are the end products of certain biochemical transformations and are not involved in other reactions.

As can be seen from Tables 3 and 4, in both modes, there was a steady increase in the accumulation of these metabolic products, from the first yeast generator and up to the last fermentation apparatus. Moreover, it is evident that there is a direct relationship between the increase in the aeration intensity and the amount of secondary products formed, both in the mature yeast and in the fermented wash.

The other group of metabolic products of yeast can include esters and aldehydes, the amount of which during aerobic cultivation of yeast significantly exceeds their content in the medium of the next (anaerobic) stage. The greater aeration intensity during yeast cultivation (Table 3) contributes to the increase in accumulation of these metabolites compared to the lower aeration intensity mode (Table 4).

We have also calculated the indexes of secondary fermentation products accumulation in relation to the alcohol concentration in each apparatus of the yeast fermentation installation. These indexes illustrate each product's synthesis rate in comparison with the rate of alcohol formation.

The results indicate that the synthesis rate of these substances, when oxygen is actively introduced into the medium at the stage of yeast cultivation, significantly exceeded the rate of alcohol formation. The amount of aldehydes, with higher aeration of the medium during cultivation, increased to 175–204 cm^3/dm^3 of the alcohol formed, and decreased to 9 cm^3/dm^3 of anhydrous alcohol (anh. al.) in the fermented wash (Table 3), and with a lower level of aeration, the values were, respectively, 85–93 and 6 cm^3/dm^3 of anh. al. (Table 4). For esters, these values were, respectively, 6.4–11.7 and 0.75 g/dm^3 of anh. al. (Table 3), and 2.2–4.8 and 0.5 g/dm^3 of anh. al. (Table 4).

These results indicate the active metabolism of carbohydrates in the Krebs cycle, in a medium intensively aerated, as well as high reactivity of aldehydes and esters, which results in their turning into other compounds and in a great decrease in their number at the anaerobic stage of the process.

Table 4 – Dynamics of technological parameters and biosynthesis of secondary fermentation products, with the aeration intensity reduced

Parameters	Number of a yeast generator in the battery			Fermentation apparatus		
	1	3	5	1	2	4 (fermented wash)
Aeration intensity, $m^3/m^3 \cdot h$	15	10	5	0	0	0
Actual concentration of DS, %	3.4	4.2	5.5	8.7	6.9	5.9
Acidity, deg	0.2	0.3	0.4	0.7	0.8	0.8
Biomass, g/dm^3	32±2	36±2	45±2	48±2	53±2	55±2
Alcohol, vol. %	1.1	1.4	1.6	3.2	6.6	6.8
IC, $g/100\ cm^3$	5.1	6.3	8.0	16.0	18.0	18.2
Unfermented carbohydrates, $g/100\ cm^3$	1.12	1.40	1.33	2.10	1.14	0.27
Zymaze activity of yeast, min	34	33	35	36	34	34
Raising power, min	35	33	32	34	35	35
Glycerol: $g/100cm^3$ g/dm^3 of anh. al.	 0.115 104.5	 0.120 85.7	 0.125 78.1	 0.312 96.8	 0.344 52.1	 0.375 55.1
Aldehydes: vol. %, cm^3/dm^3 of anh. al.	 0.088 78	 0.130 93	 0.136 85	 0.080 25	 0.045 7	 0.039 6
Higher alcohols, vol. %	0.017	0.020	0.022	0.024	0.032	0.032
Volatile acids: mg/dm^3 of the wash g/dm^3 of anh. al.	 24 2.1	 – –	 24 1.5	 36 1.1	 – –	 72 1.1
Esters: mg/dm^3 of the wash g/dm^3 of anh. al.	 53 4.8	 42 3.0	 35 2.2	 35 1.1	 – –	 35 0.5

Conclusions

Thus, the results of the work have helped establish the following:

1. Cultivation of a yeast *Saccharomyces cerevisiae* strain U-563 by gradient-continuous method in a battery of series-connected apparatuses with gradual addition of undiluted substrate (molasses) significantly enhances yeast biosynthesis compared to the conventional homogeneous-continuous method.
2. Increasing the intensity of the aeration of the medium in the battery system of yeast generation increases biomass accumulation in a mature yeast medium.
3. With an increase in medium aeration, the formation of secondary yeast metabolic products (glycerol, aldehydes, higher alcohols, volatile acids, and esters) is intensified both at the stage of yeast generation and during anaerobic fermentation. The results obtained indicate active metabolism of carbohydrates in the Krebs cycle in conditions of intensive aeration of the medium, as well as high reactivity of aldehydes and esters, which results in their transformation into other compounds and a great decrease in their amount at the anaerobic stage of the process. However, there is a progressive increase in glycerol, higher alcohols, and volatile acids, starting in the first yeast generator and up to the last fermentation apparatus, irrespective of the aeration level of the medium during yeast cultivation.

The results can be effectively used in the industrial production of food, technical and fuel ethanol from sugar-based raw materials in the course of co-production of alcohol and bakery yeast.

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