UDC 664.15 https://doi.org/ 10.15407/biotech9.05.038

TWO- PRODUCT ALCOHOL OBTAINING TECHNOLOGY BASED ON CONTINUOUS GRADIENT YEAST GENERATING

L. V. Levandovskyi
V. S. Mykhailyk

Kyiv National University of Trade and Economics, Ukraine

E-mail: levan39@yandex.ua

Received 30.07.2016

The objective is to use a serial connection of yeast generators (according to the battery principle) and by means of this to increase the rate of medium dilution therein from 0.18–0.20 to 0.88–0.96 h^{-1}, to reduce the solids concentration in the yeast mash from 17 to 10–12% and to enter the whole volume of it into the first streamwise device. The amount of molasses that has not been added to the mash in order to reduce the concentration of solids in it, was introduced into the last two yeast generators. Consequently, the experiments demonstrated the intensification of biomass accumulation, the increase of the amount of yielded ethanol by 29%, and the increase in productivity of yeast culturing by 18%.

Key words: yeast, yeast generator, fermentation.

Alcohol branch of food industry of Ukraine is a powerful industry that provides comprehensive processing of raw materials (grain or sugar beet molasses) to produce ethanol and several other useful products [1]. Despite some reduction of alcohol production volume in the last decade, the problems of energy saving, greater use of raw materials, reduction of contaminated waste, etc. are still relevant for businesses [2].

One of the advanced areas of integrated molasses processing in the alcohol production is two products (ethanol and baking yeast) obtaining from it. This technology is referred to as two-product technology; it is operated at 15 alcohol enterprises of Ukraine and provides the desired output of each of these products and quality standards [3].

However, quite often the market situation of these products requires the increasing of baking yeast's production while the alcohol production power is unchanged or even reduced. This can be achieved by increasing the outcome of yeast, compared to the volume of produced ethanol.

The solution of this problem requires the intensification of yeast biosynthesis in the medium [4–12].

For many years the two-product production was based on the classic single-threaded molasses mash fermentation technology. Its main feature was the use of mash with the same solids concentration (S) — 21–22%, that at first comes on a full scale to the yeast generators for growing the industrial yeast, and then as industrial yeast enters the main fermenter for anaerobic fermentation. Using this technology, 8–9 vol. % of alcohol and 18–22 g/l of yeast biomass are accumulated in spirit mash [2]. The pressed yeast yield, which in alcohol technology is accepted to count as the ratio of the produced biomass amount with moisture content of about 75% (kg) to the amount of the obtained anhydrous alcohol (decalitres — dal), for this technology is 1.8–2.0 kg/dal.

Scientists of the Ukrainian Research Institute of Alcohol and Biotechnology of Food Products have improved this technology towards the biosynthesis of yeast intensification. The essence of technology is to reduce solids concentration in yeast mash from 21–22 to 16–17% and to enter the underissue part of molasses into the first and the second fermenters [2]. Due to the reduction of medium osmotic pressure in the course of yeast cultivation, their accumulation increases, and the yield increases by 10%.

The next step improving the process was a developing of a new approach in conducting the yeast generation process. It was serial devices connection (battery principle) with the differential aeration of the medium: increased — in the first yeast generators with a gradual decrease of this parameter toward the last apparatus [3]. The proposed method improves the biomass throughput process, the rate of sugars fermentation and economic factor for their usage.

The aim of the study was the further improvement of cultivation stage of biomass producer in two-product alcohol production technology towards increasing the crop of baking yeast.

Materials and Methods

As a producer of alcohol and yeast biomass the yeast *Saccharomyces cerevisiae* strain M-5 were used, which were received from the collections of industrial microorganisms of the Ukrainian Research Institute of Alcohol and Biotechnology of Food Products [1]. The sterile malt wort was filled in a test tube with yeast on wort agar. Then a gradual dilution of pure culture of alcohol producers was performed, firstly under laboratory conditions and then in industrial pure culture devices followed by the insertion into the yeast generator for growing the industrial yeast under the natural pure culture conditions in accordance with applicable regulations [3].

The objects of research were also: raw materials (sugar beet molasses), molasses mash, spirit mash and its distillates.

Sugar beet molasses containing 48.8% of fermentable carbohydrates and 77.6% of solids was the raw material for molasses mash making. Molasses was enriched with nitrogen (urea) and phosphate (H_3PO_4) nutrients in accordance with applicable rules, and H_2SO_4 was added in an amount that ensures active mash acidity at pH 5.0–5.2.

In molasses mash and in the fermented medium, the content of solids was determined by refractometric method, and pH was determined by electrometric method.

Spirit mash (medium after fermentation) was analyzed in terms of: medium pH, yeast biomass — by weighting on conversion to 75% humidity, alcohol concentration — in wort distillate by picnometer method [4]. The amount of unfermented carbohydrates in the mash was determined by resorcin colorimetric method [3]. Besides, the content of glycerol in mash was determined by distillation colorimetric method, and in mash distillates — the content of higher alcohols (by photo colorimetric method with reagent p-dimethylbenzaldehyde) by the accepted in science and practice of alcohol production methods [4].

The study was performed under industrial conditions at Barskyy distillery (Bar, Ukraine). Yeast fermenter (Figure) consists of six yeast generators *1* (each one of 50 m^3), recirculation pump *2* and eight series-connected fermenters *3* (each one of 100 m^3).

Statistical analysis of the obtained data was performed using Microsoft Excel. The defining of abovementioned indicators was conducted in three — four repetitions. The difference between the results of measurement was considered significant at $P < 0.05$.

Results and Discussion

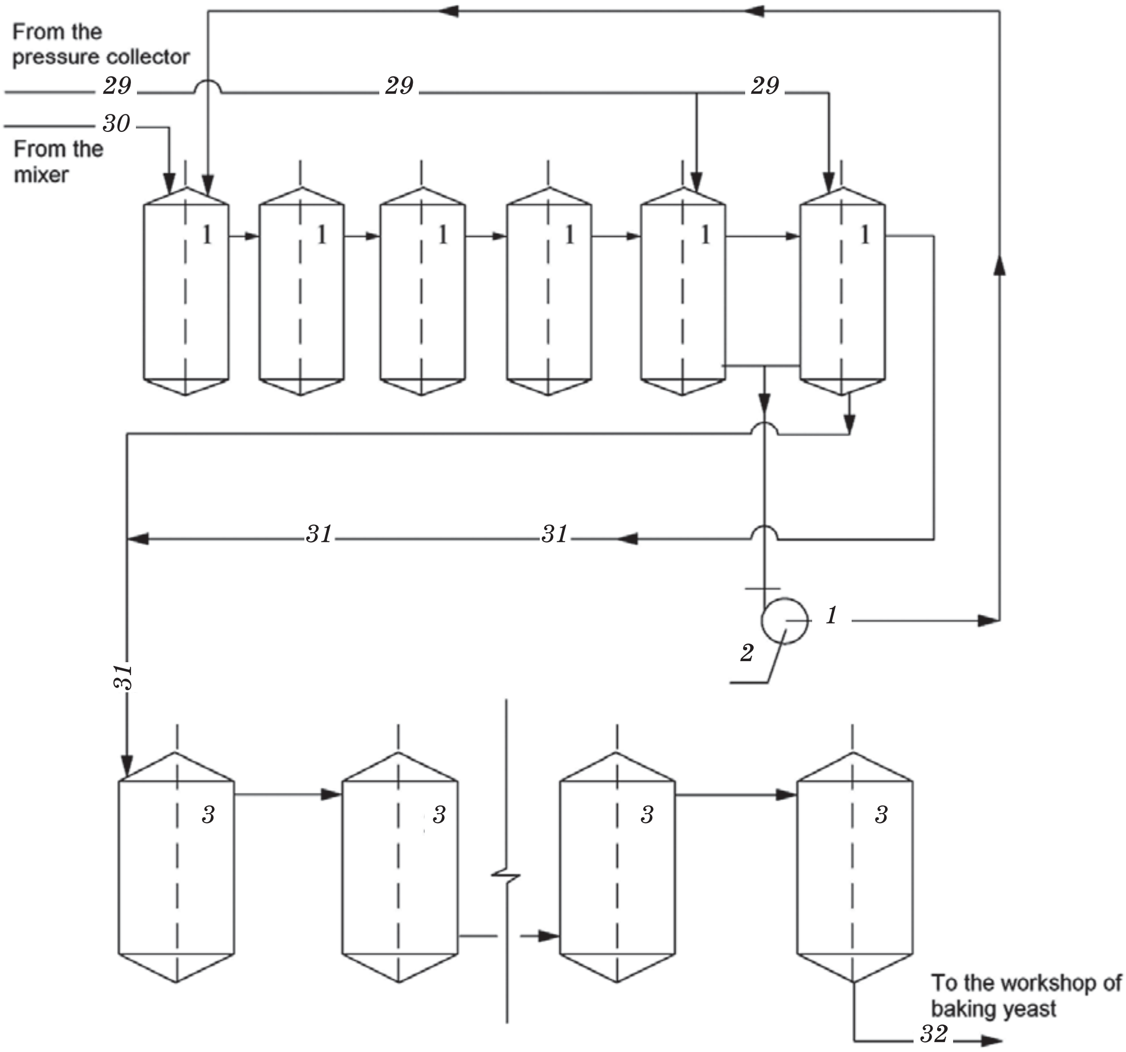
Considering the known theoretical and practical biotechnology principles of positive impact of carbohydrate substrate stepped adding during microbial cultivation on the biomass synthesis intensity [5–7, 12], and also the results of our previous laboratory investigations [8, 9], it was recommended in research option to improve the process of battery yeast generation in such a manner. Solids concentration in yeast mash was reduced from 17 to 10–12% and it was added fully into the first streamwise device. The amount of molasses that has not been added to the mash in order to solids concentration reduction in it was introduced into the last two yeast generators. Thus, the initial concentration of mash solids (C_i) was increased up to a value close to 17 g/100 cm^3. Aeration intensity in the first and the second devices were maintained at 10–15 and were gradually reduced to 3–5 m^3/m^3 of medium · h in the last two devices. In order to prevent yeast washing out during yeast generation the part of medium (30–40% of the value of the wort inflow) from the last (the sixth) apparatus was recirculated by pump into the first one. The industrial yeast was administered from the sixth apparatus into the first fermenter. Required initial concentration of fermented wort solids in anaerobic stage (22 g/100 cm^3) has been reached by input of the calculated amount of molasses into the first two fermenters.

An advanced single-threaded fermentation method widely used in ethanol plants that produce alcohol and baking yeast was used as a control [2, 3].

Technological parameters of both processes are presented in Table 1 and 2.

The obtained results suggest that the proposed yeast generation method helps intensify the biosynthesis of yeast, resulting in increase in biomass accumulation as in finished yeast (up to 31.1 vs. 22.5 g/dm^3 in control, $P < 0.05$) and in spirit mash (up to 39.3 vs. 30.5 g/dm^3, $P < 0.05$). In pilot trial the productivity of yeast generation at that has increased from 3.7 to 4.9 $kg/m^3 \cdot h$ ($P < 0.05$). Characteristically, the growth of yeast in the anaerobic stage of the process (i.e., from the first to the last fermenter) was about the same in both variants — 8.2 (experiment) and 8.0 g/dm^3 (control). This indicates that yeast generation stage is foreground rather than fermentation stage in the accumulation of biomass in the whole process.

It has been established that in biotechnology at alcohol obtaining from carbohydrate containing raw materials [10–12], the improvement of yeast cultivation conditions and their physiological state leads to the weakens of glycerol accumulation,



Apparatus-technological diagram of fermentation process with gradient continuous yeast generating:
1 — yeast generators; *2* — pump; *3* — fermenters
Conventional notation: *29* — molasses; *30* — molasses wort; *31* — industrial yeast; *32* — spirit mash

Table 1. **Indicators of yeast generating technological mode for serial connection of devices and molasses wort fermentation**

Indicators	The number of yeast generator					The number of fermenter				
	1	2	3	4	5	1	2	3	5	7
The apparent solids concentration, %	9.6±0.3	8.6±0.3	7.1±0.2	8.3±0.3	8.4±0.2	9.0±0.3	8.2±0.2	7.8±0.2	7.0±0.2	6.8±0.1
pH of the medium	5.1±0.1	5.1±0.1	5.0±0.1	5.1±0.1	5.1±0.1	5.1±0.1	5.1±0.1	5.2±0.1	5.2±0.1	5.2±0.1
Yeast biomass, g/dm^3	14.0±1.1	19.4±1.2	26.9±1.6	28.5±1.4	31.1±1.5	29.7±1.5	–	36.5±1.8	38.0±1.8	39.3±2.0
Alcohol concentration, vol.%	1.3±0.1	2.2±0.1	2.9±0.2	3.8±0.2	4.7±0.2	6.7±0.2	7.2±0.3	7.5±0.2	8.0±0.2	8.2±0.2
C_i, $g/100\ cm^3$	12.1	12.4	12.2	14.9	16.6	–	–	21.9	21.5	21.6
Unfermented carbohydrates, $g/100\ cm^3$	–	–	–	–	2.9±0.14	4.30±0.3	–	1.87±0.08	–	0.28±0.02
The content of: higher alcohols, vol.% glycerol, $g/100\ cm^3$	0.043±0.002 0.20±0.01	– –	– –	– –	0.053±0.003 0.25±0.02	– –	– –	– –	– –	0.062±0.003 0.65±0.03

Note: here and in the Table 2 «–» means "not defined".

which occurs in the glycolytic monosaccharides cleavage and requires the consumption of the latter. This contradicts the basic aim of alcohol fermentation that is the maximal conversion of sugars into alcohol, so glycerol formation minimizing is an important task of this technology.

It has been shown in our investigations (Table 1 and 2) that for equal values of alcohol concentration in spirit mash in the control and experimental versions (8.2 vol. %) the glycerol formation in the test process slightly decreases (0.65 vs. 0.72 $g/100\ cm^3$). We can assume that this is the result of improving the physiological state of yeast cells due to solids concentration reduction in yeast wort in the first stage of the cultivation with the gradual inflow of the substrate in the final stage of the process. This fact became also a prerequisite for better yeast cells reproduction and their biomass increase.

According to the known biochemical mechanisms of alcohol fermentation [2, 9] the increase of the amount of synthesized yeast biomass is regularly accompanied by increased biosynthesis of higher alcohols: in industrial yeast — up to 0.053 (Table 1) vs. 0.042 vol. % in the control (Table 2), and in spirit mash — up to 0,062 (Table 1) vs. 0,056 vol. %, $P < 0.05$ (Table 2).

Thus, the investigations give enough reasons to claim, that the yield of baking yeast at application conditions of the proposed method of producer cultivation is significantly increased within two-product alcohol production technology.

Table 3 shows a comparative assessment of traditional technology and the one, proposed by us, according to the results of industrial and departmental tests.

Table 2. **Indicators of yeast generating technological mode and wort fermenting by single-threaded way (control)**

Indicators	**Yeast generators**	**The number of fermenter**			
		1	3	5	8
The apparent solids concentration, %	8.4 ± 0.2	9.7 ± 0.3	8.0 ± 0.2	6.7 ± 0.2	7.5 ± 0.2
pH of the medium	5.0 ± 0.1	5.0 ± 0.1	5.0 ± 0.1	5.1 ± 0.1	5.1 ± 0.1
Yeast biomass, g/dm^3	22.5 ± 1.4	24.8 ± 1.6	27.9 ± 1.8	29.2 ± 1.7	30.5 ± 2.0
Alcohol concentration, vol.%	5.0 ± 0.2	5.2 ± 0.15	6.8 ± 0.2	8.0 ± 0.15	8.2 ± 0.15
C_i, g/100 cm^3	17.5	19.2	2.9	21.3	21.3
Unfermented carbohydrates, g/100 cm^3	2.55 ± 0.10	3.60 ± 0.22	1.15 ± 0.09	0.42 ± 0.03	0.26 ± 0.01
The content of: higher alcohols, vol.% glycerol, g/100 cm^3	 0.042 ± 0.002 0.30 ± 0.02	 0.052 ± 0.003 –	 0.052 ± 0.002 –	 0.054 ± 0.003 –	 0.056 ± 0.002 0.72 ± 0.03
Biomass productivity of cultivation process, kg/m^3·h	3.7	–	–	–	–

Table 3. **Comparative evaluation of traditional (control) and advanced (experiment) technologies by the results of industrial tests**

Test options	**Duration of yeast cultivation, h**	**Rate of medium dilution in particular yeast generator, h^{-1}**	**Biomass of finished yeast, g/dm^3**	**Biomass productivity of cultivation process, kg/m^3·h**	**The flux rate of finished yeast, m^3/h**	**The amount of molasses which was entered into the main fermenters, m^3/h**	**The amount of spirit mash, m^3/h**	**Yeast outcome, kg/dal of alcohol**
Industrial tests								
Control	5.5	0.18	25	4.55	46.3	2.65	49.0	3.29
Experiment	6.3	0.96	34	5.40	41.4	2.60	43.0	4.14
Departmental tests								
Control	5.3	0.20	21.5	4.01	51.0	2.90	53.7	3.00
Experiment	6.8	0.88	31.9	4.70	39.5	2.70	42.2	4.46

Increasing the medium dilution rate in yeast generators with serial connection up to 0.88–0.96 vs. 0.18–0.20 h^{-1} in the control (P < 0.05) had a positive effect on the microbiological state of the medium and physiological properties of yeast. The number of cells of infecting microflora was not increased from the first to the sixth yeast generator even after 4 days of apparatus operation, and the yeast cells were large and had a significant percentage of budding yeast. As a result, the productivity of yeast generation process according to the proposed gradient continuous cultivation technology has increased from 4.55 to 5.40 $kg/m^3 \cdot h$ (industrial tests) and from 4.01 to 4.7 $kg/m^3 \cdot h$ (departmental tests) with no changes in the quality of baking yeast which meets the requirements of State Standards of Ukraine 4812: 2007.

Thus, the investigations in laboratory and industrial conditions have allowed to intensify the two-product alcohol and baking yeast production technology by increasing their output and yeast biomass cultivation productivity. The developed technology is referred to the Departmental Commission and is implemented in several industrial enterprises.

REFERENCES

1. *Shiyan P. L., Sosnytskiy V. V., Oliynichuk S. T.* Innovative technologies of alcohol industry. Theory and practice. *Kyiv*: *Askaniia*. 2009, 424 p. (In Ukrainian).
2. *Marynchenko V. A., Domaretskyy V. A., Shiyan P. L.* Technology of alcohol. *Vinnytsia: Podolie-2000*. 2003, 496 p. (In Ukrainian).
3. Typical technological regulation of production molasses-alcoholic mash and pressed baking yeast: TR Ukr 18.8049-2004. *Kyiv: UkrNDIspyrtbioprod*: *Ministry of Agrarian Policy of Ukraine*. 2004, 62 p. (In Ukrainian).
4. *Polygalina G. V.* Technochemical control of alcohol and distillery production. *Moskwa*: *Kolos*. 1999, 336 p. (In Russian).
5. *Ghorbani F., Younesi H.* The kinetics of ethanol production from cane molasses by saccharomyces cerevisiae in a batch bioreactor. *Energy Sources, Part A: Recovery, Util. Environm. Effects*. 2013, 35 (11), 1073–1083.
6. *Bouallagui H., Touhami Y., Hanafi N., Gharani A., Hamdi M.* Performances comparison between three technologies for continuous ethanol production from molasses. *Biomass Bioen*. 2013, V. 8, P. 25–32.
7. *De Andrade R., C ndida Rabelo S., Maugeri Filho F., Maciel Filho R., Carvalho da Costa A.* Evaluation of the alcoholic fermentation kinetics of enzymatic hydrolysates from sugarcane bagasse (*Saccharum officinarum* L.). *J. Chem. Technol. Biotechnol*. 2013, 88 (6), 1049–1057.
8. *Levandovskyi L. V., Nichik O. V., Yakovenko A. A.* Influence of cultivation duration of alcohol yeast on the results of fermentation of molasses wort. *Production of alcohol and alcoholic beverages*. 2012, N 2, P. 13–15. (In Russian).
9. *Levandovskyi L. V., Nichik O. V., Semenyuk Yu. V.* Influence of the composition of molasses wort on the effectiveness of two-product of alcohol production and baker's yeast. *Production of alcohol and alcoholic beverages*. 2012, N 1, P. 11–13. (In Russian).
10. *Fakruddin M., Quayum M., Ahmed M., Choudhury N.* Analysis of key factors affecting ethanol production by Saccharomyces cerevisiae IFST-072011. *Biotechnology*. 2012, 11 (4), 248–252.
11. *Pe a C., Arango R.* Evaluation of ethanol production using recombinant strains of Saccharomyces cerevisiae from sugar cane molasses. *DYNA*. 2009, V. 159, P. 153–161.
12. *Herrera W. E., Filho R. M.* Development of a monitoring hybrid system for bioethanol production. *Chem. Engin. Transact*. 2013, V. 32, P. 943–948.

ТЕХНОЛОГІЯ ДВОПРОДУКТОВОГО ОТРИМАННЯ СПИРТУ НА ОСНОВІ ГРАДІЄНТНО-НЕПЕРЕРВНОГО ДРІЖДЖЕГЕНЕРУВАННЯ

Л. В. Левандовський
В. С. Михайлик

Київський національний торговельно-економічний університет, Україна

E-mail: levan39@yandex.ua

Метою роботи було застосувати послідовне з'єднання апаратів-дріжджегенераторів (батарейний принцип) та збільшити за рахунок цього швидкість розбавлення середовища в них з 0,18–0,20 до 0,88–0,96 год$^{-1}$, зменшити концентрацію сухих речовин дріжджового сусла з 17 до 10–12% і вводити його в повному обсязі в перший за потоком апарат. Кількість меляси, що її недодали в сусло з метою зменшення в ньому концентрації сухих речовин, вводили в два останніх дріжджегенератори. У результаті було доведено факт інтенсифікації накопичення біомаси, підвищення виходу дріжджів відносно кількості спирту кількості етанолу на 29% і збільшення продуктивності процесу культивування дріжджів на 18%.

Ключові слова: дріжджі, дріжджегенератор, зброджування.

ТЕХНОЛОГИЯ ДВУХПРОДУКТНОГО ПОЛУЧЕНИЯ СПИРТА НА ОСНОВЕ ГРАДИЕНТНО-НЕПРЕРЫВНОГО ДРОЖЖЕГЕНЕРИРОВАНИЯ

Л. В. Левандовский
В. С. Михайлик

Киевский национальный торгово-экономический университет, Украина

E-mail: levan39@yandex.ua

Целью работы было использовать последовательное соединение аппаратов-дрожжегенераторов (батарейный принцип) и увеличить за счет этого скорость разбавления среды в них с 0,18–0,20 до 0,88–0,96 ч$^{-1}$, уменьшить концентрацию сухих веществ дрожжевого сусла с 17 до 10–12% и вводить его в полном объеме в первый по потоку аппарат. Количество мелассы, не добавленное в сусло с целью уменьшения в нем концентрации сухих веществ, вводили в два последних дрожжегенератора. В результате был доказан факт интенсификации накопления биомассы, повышения ее выхода относительно количества этанола на 29% и увеличения производительности процесса культивирования дрожжей на 18%.

Ключевые слова: дрожжи, дрожжегенератор, сбраживание.