Современная наука и инновации.
2024. № 2 (46). С. 59-68.
Modern Science and Innovations.
2024;2(46):59-68.

*ТЕХНОЛОГИЯ ПРОДОВОЛЬСТВЕННЫХ ПРОДУКТОВ /
TECHNOLOGY OF FOOD PRODUCTS*

Научная статья / Original article

УДК 336.76
https://doi.org/10.37493/2307-910X.2024.2.6

ТЕХНОЛОГИЯ ПРОДОВОЛЬСТВЕННЫХ ПРОДУКТОВ | TECHNOLOGY OF FOOD PRODUCTS

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Получение инвертного сахара из целлюлозы для последующей его ферментации кормовыми дрожжами

Production of invert sugar from cellulose for subsequent fermentation with feeder yeast

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Аннотация. *Получение микробиального белка для животных может быть реализовано с помощью различных технологий, отличающихся по эффективности, экологичности и долгосрочности времени жизни на рынке, в том числе в связи с ограниченностью ресурсов. В настоящем исследовании изучена возможность получения инверсного сахара из древесных опилок методом кавитации, используя ультразвуковую ячейку, при этом были получены образцы гидролизатов с массовой долей инвертных сахаров более 30 % и в лабораторных условиях показана эффективность использования полученных сахаров для получения биомассы кормовых дрожжей, обогащенных биоэлементами, пробиотиком, пребиотиком и другими биологически активными веществами. В качестве основы питательной среды при производстве кормового протеина исследовалась сухая стандартная питательная среда для дрожжей, молочная сыворотка, сухая молочная сыворотка, пермеат, сухой пермеат. Лабораторные данные свидетельствуют, что дальнейшие испытания должны быть переведены на производственный уровень, однако узким местом для реализации такой технологии являлось отсутствие производительного, экологического и эффективного метода получения инвертного сахара из древесных опилок. В качестве научной новизны работы – представленная экологичная технология кавитационного процесса при помощи усовершенствованной проточной ячейки Ч-245 ООО «Новотех-ЭКО (г. Вологда, Россия), при этом производительность ультразвукового оборудования в таком формате может быть подобрана под конкретную производительность линии переработки молочной сыворотки/пермеата путём параллельного использования нескольких проточных ячеек. Таким образом, с помощью предложенной технологии, одновременно могут быть решены экологические проблемы, связанные с недоиспользованием* *молочного вторичного сырья, получена эконмическая выгода от применения древесно-стружечных отходов с помощью экологической биотехнологии. При этом гибкость биотехнологий позволяет получать функциональный кормовой продукт для сельскохозяйственных животных с конкретным набором биологически-активных веществ, в частности биоэлементов.*

Ключевые слова: кормовой протеин, ультразвуковая кавитация, молочная сыворотка, биотехнология, экология

Для цитирования: *Полянская И. С., Воропай Л. М., Кузнецова О. Б., Абакумова Е. А. Получение инвертного сахара из целлюлозы для последующей его ферментации кормовыми дрожжами // Современная наука и инновации. 2024. № 2 (46). С. 59-68. https://doi.org/10.37493/2307-910X.2024.2.6*

Благодарности: *авторы выражают благодарность ООО «Новотех-ЭКО (г. Вологда) за предоставленное УЗ-оборудование*

Abstract. *Microbial protein for animals can be produced by using various technologies that differ in efficiency, environmental friendliness and life on the market, including due to limited resources. The work presents the study dedicated to the possibility of producing inverse sugar from sawdust by the cavitation method using an ultrasonic cell. In the course of the research samples of hydrolysates with a mass fraction of inverse sugars of more than 30% have been produced. The work also shows the effectiveness of using the resulting sugars for developing feed yeast biomass enriched with bioelements, probiotic, prebiotic and other biologically active substances. Dry standard nutrient medium for yeast, whey, dry whey, permeate, dry permeate have been studied as the basis of the nutrient medium for the feed protein production. Laboratory data suggests that further testing should be carried out at the production scale, but the problem point for implementation of such technology has been a lack of a productive, environmentally friendly and efficient method for producing inverse sugar from sawdust. The scientific novelty of the work is presented by environmentally friendly technology of the cavitation process using an improved Ch-245 flow cell (Novotekh-EKO LLC (Vologda, Russia)), while the productive capacity of ultrasonic equipment in this format can be adjusted for the specific performance of the whey processing line /permeate by parallel use of several flow cells. Thus, the proposed technology helps in simultaneous solving environmental problems associated with underutilization of secondary dairy raw materials as well as in gaining economic benefits from using wood chip waste. At the same time, the flexibility of biotechnologies makes it possible to develop a functional feed product for farm animals with a specific set of biologically active substances, bioelements, in particular.*

Keywords: feed protein, ultrasonic cavitation, whey, biotechnology, ecology

For citation: *Polyanskaya IS, Voropay LM, Kuznetsova OB, Abakumova EA. Production of invert sugar from cellulose for subsequent fermentation with feeder yeast. Modern Science and Innovations. 2024;2(46):59-68..* https://doi.org/10.37493/2307-910X.2024.2.6

Acknowledgments: *the authors express their gratitude to Novotech-EKO LLC (Vologda) for the ultrasound equipment provided*

Introduction. Qualitatively and quantitatively, protein for farm animals is one of the most expensive items of expenditure, while at the same time being the most important lever for animal productivity. Trends and technologies in the production and use of plant, fungal and microbial proteins for animal feeding are the focus of domestic and world science [1, 2]. This has led to the emergence of a fairly large number of innovations in this area, which are also associated with a critical understanding of the use of natural resources in obtaining proteins using traditional and alternative methods. The shortage of feed protein in the world is estimated at approximately 30 million tons/year, and in Russia – at 2.3 million tons/year. At the same time, there is a close connection between the food industry, biomedicine and feed production, since the effective conversion of feed proteins in the body of productive farm animals ultimately ensures the necessary level of metabolism of humans as the main consumer of the final products obtained from these animals [3].

Operating timber industry enterprises provide only 65% of wood processing. Of these, unclaimed waste makes up from 30 to 50% of the total volume of raw materials used and approaches 10 million m3 ^{per} year. The subject area under study is a technology for obtaining carbohydrate raw materials (inverse sugar) from waste from the wood processing industry (sawdust, shavings) for its subsequent use in the industrial biotechnology of microbial synthesis of feed protein peptides, or individual essential amino acids, to solve several problems in a complex, taking into account those specifically posed in the region tasks.

For example, simultaneous microbial conversion of secondary dairy raw materials and carbohydrates from wood waste makes it possible to use consortia of the most important microorganisms for specific animal species - probiotics - to increase biomass.

It is known that wood waste is a valuable natural raw material containing fractions of polysaccharides, monosaccharides, lignin, ethers and mineral components. Therefore, the introduction of new technologies for deep wood processing into production cycles is an urgent problem.

One of the methods for deep processing of wood raw materials is hydrolytic technologies, which result in the formation of wood hydrolysates containing soluble fractions of hexosans, pentosans, lignin, essential oils and mineral impurities. The formation of alcohols, glycerol, acetic acid, etc. also occurs. The by-products formed in this case cause a delay in the growth of some strains of feed yeast and belongs to the group of chemical inhibitors [4, 5].

In this regard, the study of the influence of the chemical composition of ultrasonic extracts of wood waste on the adaptation processes of feed yeast strains is relevant.

An analysis of sources on the topic, including a patent search, shows that in industry, to obtain hydrolysates from wood waste, acid hydrolysis using solutions of sulfuric, hydrochloric and nitric acids, and alkaline hydrolysis using solutions of sodium hydrosulfite and sodium hydroxide are used. For example, acid hydrolysis of crushed fractions of wood waste in a solution of sulfuric and hydrochloric acids at a pressure of 1.5-2.0 bar is widely used [6]. Shredded wood waste is loaded into the reactor, a hot acid solution is added and boiled for 3 hours at a pressure of 1.5-2.0 bar. Next, the liquid mixture containing solid fractions is fractionated to separate extracts and solids. The extract is distilled and neutralizers are introduced until a pH value of 6.0 is obtained. Feeding yeast is grown on it. The disadvantage of the proposed method is the high consumption of acids and the complexity of the technological cycle.

To eliminate these shortcomings, an improved acid method for the hydrolysis of wood waste using a hydrochloric acid solution has been developed [7]. In order to activate hydrolysis and extraction, the wood is crushed to a flour fraction, steamed in bunkers with steam to soften the cellulose fibers, and a solution of hydrochloric acid with a concentration of 2.5 mol/l is added. The mixture is stirred for 40 - 45 minutes at temperatures below 100 ^{0} C and hydrated cellulose is obtained, which is washed and fungal micelles are cultivated on it. The efficiency of sugar extraction is 21-22%, however, hydrochloric acid causes corrosion fatigue of the equipment, the process itself is lengthy, which is a significant drawback of this technology.

There is a known method for producing hydrolysates in alkaline media using a solution of sodium hydrosulfite with a concentration of 0.5 – 5.0% when processing crushed birch waste [8]. Hydrolysis is carried out with constant stirring, maintaining a hydromodulus of 1:100 for 24 hours at a temperature of 25-28 ^{0}C with the addition of microorganisms. Under these conditions, enzymatic hydrolysis of cellulose occurs to form dextrins, which are extracted and the same type of consortium of microorganisms is added to the extract. The result is solid protein supplements and extracts that are added to animal feed. Lignin content is allowed up to 2.4%. However, this technology is of limited use, as it requires expensive equipment and compliance with temperature conditions and air humidity during storage and transportation of additives.

Currently, the integrated processing of plant waste - the technology of biotransformation of cellulose-containing waste - has become widely used. The technology includes the stages of treating sawdust with unipolar water, followed by the addition of acidic or alkaline solutions, fractionating the mixture and isolating the solid fraction, which is cultivated with yeast strains and fungal micelles. The disadvantage of this method is the high cost and high consumption of unipolar water and reagents [9].

In recent years, ultrasonic technologies have been used to activate hydrolysis and extraction processes in technological cycles [10,11]. According to work [10], the use of ultrasound for pre-treatment of lignocellulosic substrates in a heterogeneous environment leads to an increase in their reactivity during bioconversion into sugars with the help of cellulolytic enzymes. The disadvantage of the technology is the duration of ultrasonic activation with the simultaneous use of enzymatic hydrolysis.

Ultrasonic technology for producing protein feed is known, which includes treating wood with an alkali solution of 1-2% concentration while sonicating for 3 hours [12]. The activated mass of sawdust is crushed in a mill to a size of 50-100 microns and microorganisms are introduced. A significant disadvantage of this technology is the high consumption of sodium hydroxide and the problem of recycling alkaline waste. Remaining alkali impurities in treated wood slow down the growth of feed yeast strains.

Unlike known methods, the work proposes to use a sodium acetate solution with a concentration of 0.005 mol/l, which provides a pH value of 8.0-8.5.

To achieve this goal, the following research questions are being solved: experimental selection of environmentally friendly technological conditions for ultrasonic sonication of different types of wood waste to obtain nutrient media for the production of feed protein; study of the influence of the chemical composition of extracts on the adaptation processes of feed yeast strains. The research empirical part reflects the search for solutions to these problems.

Materials and research methods. To solve the above problems and test the hypothesis of the possibility of using our proposed production technology, the experiment was carried out according to the following scheme: obtaining ultrasonic extracts from wood waste, cultivating fodder yeast strains on a factory nutrient medium, transplanting them into the resulting extracts, studying the influence of the chemical composition of nutrient media on yeast adaptation processes by changing their growth rate.

When performing the experiment, gravimetric, titrimetric, spectrophotometric, ionometric and biotechnological methods of analysis were used. An extract of sugary fractions of wood waste is obtained from spruce and birch sawdust using a low-frequency ultrasonic contact-type reactor, which ensures deep penetration of the solvent into the structure of wood particles and, due to cavitation effects, causes an increase in temperature by 60-65^{0}C, acidification of the environment, and an increase in the rate of hydrolysis of wood components with their subsequent extraction. As a solvent, in contrast to existing technologies, a weakly alkaline solution of sodium acetate was used, during the hydrolysis of which the medium is alkalized to pH values of 8.0 - 8.5. The preparation of wood waste (spruce and birch sawdust) was carried out according to the following method: samples of sawdust were taken, placed in a container and a solution of sodium acetate with a molar concentration of 0.005 mol/l was added, maintaining a hydromodulus of 1:10. Subjected to ultrasonic sounding for 45 minutes and left after stopping sounding for 3 hours to stabilize the system. After adaptation, the mixture was filtered and the extract was selected, which is a nutrient medium for growing feed yeast. The solid residue of sawdust was further used as a filler in the production of wood concrete.

To assess the content of reducing substances in the extracts, a method that complies with the requirements of GOST 19222-84 was used. Toxicity of grown yeast strains and extract - biotechnologically using the BIOLAT device. Ciliates were used as a standard when studying the toxicity of media: if the toxicity index is $T<0.4$, the media is acceptable toxic; if $0.4<T<0.7$ – the environment is moderately toxic; $T>0.7$ – toxic environment.

The work design can be classified as a case study, which consists of an in-depth study of a specific research problem.

Research results and their discussion. At the first stage of the work, ultrasonic extracts were obtained for growing strains of feed yeast. The best results were obtained when using sawdust stabilization in a sodium acetate solution. In this case, after fractionating the sawdust, they were placed in an ultrasonic reactor, a solution of sodium acetate was added at a hydromodulus of 1:10, and sonication was carried out for 45 minutes at a cavitation number of 800-1000. To increase the degree of extraction and hydrolysis of sugar fractions, stabilization was carried out in the same solution of sodium acetate at a temperature of 30-350C. The conditions for obtaining a nutrient medium from various wood wastes are presented in Table 1.

Table 1 – Conditions for obtaining hydrolysates

Column header	**Number sample**	**Column header**				**Percentage of sugary substances**
		Temperature, ^{0} C	**Number cavitation**	**pH**	**Stabilization time, hour.**	
Birch sawdust	1	85	800-900	8.2	1	19.8
	2	98	900-1000	7.9	2	24.3
	3	98	800-900	7.9	3	35.8
	4	90	800-900	7.9	4	35.9
Spruce sawdust	5	85	800-900	8.4	1	15.5
	6	95	800-950	8.2	2	21.8
	7	95	800-950	8.2	3	29.8
	8	95	850-950	8.2	4	29.9

Source: compiled by authors

The results indicate that the efficiency of extraction of sugar fractions depends on the type of sawdust, stabilization time, and cavitation number. When using birch sawdust, the sugar content in hydrolysates is always higher than the content of sugar fractions in hydrolysates obtained from spruce sawdust. Probably, the resinous substances present in spruce sawdust interfere with the processes of extraction and hydrolysis of polysaccharides due to the formation of internal plugs. A relationship has also been established between sugar content and stabilization time after ultrasonic sonication. The optimal time is three hours at a wood extract temperature of 35-40 ^{0}C, which ensures a sugar content of up to 35.8% in birch sawdust hydrolysates and 29.8% in spruce sawdust hydrolysates. A further increase in stabilization time does not cause a change in the sugar content in the extracts. To confirm the completeness of extraction of sugar fractions from wood waste, sawdust is re-treated in an ultrasonic reactor in a sodium acetate solution for 45 minutes. After processing, sugars are not detected, which indicates complete extraction of sugar fractions from wood after stabilization.

From the extracts obtained, samples No. 3 and 7 with a high content of sugar fractions and pH values of 7.9 and 8.2 were selected, which are used as a nutrient medium for growing different strains of feed yeast.

At the second stage of the experiment, 8 strains of feed yeast U248, U697, U815, U361, U251, U192, U428, U731 are cultivated on a standard nutrient medium for 7 days in a thermostat at a temperature of 300C. It was found that after 36-48 hours of cultivation, strains U815, U251, U192, U428 die. In the first two days there is no increase in biomass. Therefore, the standard culture medium is not adapted to these strains. To prove the impossibility of using ultrasonic extracts for cultivating these strains of feed yeast, they were cultivated on the obtained wood extracts. The experiment shows that the death of the four strains studied is observed within a day and this excludes the possibility of their use.

The standard nutrient medium is most adapted for strains U248, U697, U361, U731, for which an increase in their biomass is observed (Figure 1).

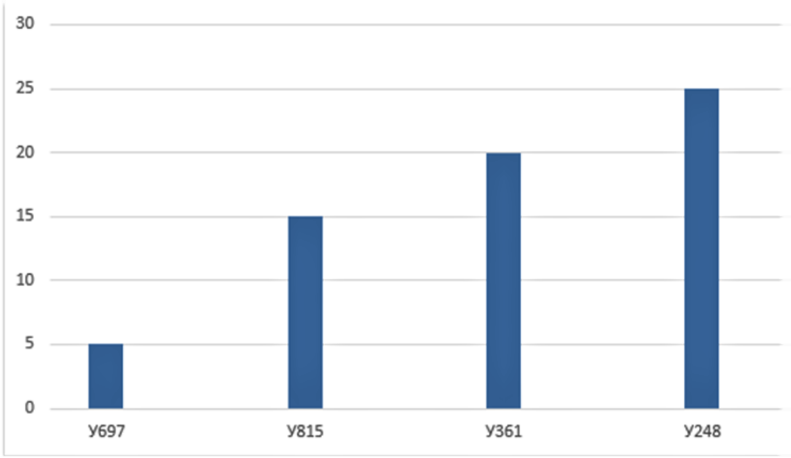

Figure 1 – Change in the mass of yeast strains in the factory nutrient medium, % Horizontal - names of feed yeast strains
Source: compiled by authors

At the next stage of the experiment, observations were made over the course of nine days on the adaptation of different strains of feed yeast to the ultrasonic extract. Adaptation was determined by changes in the concentration of sugars in the extracts and was calculated as % relative to the initial concentration. The results are presented in Table 2.

Table 2. Determination of adaptation of feed yeast to ultrasonic extract of birch sawdust by changes in sugar concentration

% change in the concentration of sugar fractions within 1-9 days							
2	3	4	5	6	7	8	9
9.3	10.3	11.2	11.2	12.0	12.2	13.1	13.2
9.7	10.9	11.4	11.1	12.8	13.7	14.1	14.9
9.3	13.9	14.2	11.4	14.5	14.5	15.3	15.8

Source: compiled by the authors

The change in sugar concentration during cultivation of strain U361 after seven days is 12.2%. For strain U697, the concentration changes by 13.7%, and for strain U815 by 15%. A sample of strain U248, which was adapted to a standard nutrient medium, dies after three days when placed in an ultrasonic extract. Thus, the most adapted strain to the ultrasonic extract obtained by sonicating birch sawdust is strain U815.

Research also shows that when cultivating a nutrient medium obtained from pine extracts, strains U361, U697, U815, U248 die after 2-3 days.

Thus, the ultrasonic extract obtained from birch sawdust based on sodium acetate can be used as a nutrient medium for growing strains of feed yeast U361, U687, U815.

Next, the toxicity of fodder yeast strains grown on an ultrasonic extract of birch sawdust was determined. A sample of strain U697 has a toxicity index of 5.30 and belongs to the second toxicity class - moderately toxic. For samples of strains U815, the toxicity index is 0.12; for strain U361, the toxicity index is 0.30. Therefore, these two yeast strains are classified as toxicity class 1 and are acceptably toxic. They can be used as dietary supplements for animal feed.

Conclusion. Compared with predecessor studies, including with the participation of a number of authors of this study [13], the presented study obtained new results to substantiate the choice of environmentally friendly technological conditions for ultrasonic sonication of various types of wood waste to obtain nutrient media for the production of feed protein. These new data relate to the use of new adapted equipment (cavitation cell) to obtain sugary substances from sawdust, as well as to the design of the preparation of wood waste, including the use of a solution of sodium acetate with a concentration of 0.005 mol/l, which allows obtaining a higher yield of sugary substances, according to compared with prototype options using an ecological method, while strains of feed yeast were found, with the help of which the protein obtained using ultrasonic hydrolyzate of wood waste, as shown by the biological research method, has a low toxicity index.

In this case, the solid residue of sawdust is used as a filler in the production of wood concrete. Thus, the investigated method of using ultrasonic extracts of wood waste to grow fodder yeast is not only environmentally friendly and low-waste.

According to the results of the presented study on the selection of environmentally friendly technological conditions for ultrasonic sonication of different types of wood waste to obtain nutrient media for the production of feed protein:

1. The technological conditions for obtaining ultrasonic extracts from unclaimed birch and spruce waste were selected experimentally.

2. To determine the adaptation of different strains of feed yeast U248, U697, U815, U361, U251, U192, U428, U731, they were cultivated on a standard nutrient medium. Based on changes in the concentrations of sugar fractions and pH values of the nutrient medium, features and adaptations to the nutrient medium were studied. It was established that strains U251, U192, U428, U731 are not adapted to the nutrient medium and die during cultivation.

3. When studying the processes of adaptation of feed yeast strains (U248, U697, U815, U361) to ultrasonic extracts obtained from birch and spruce sawdust, it was found that strains U697, U815, U361 are adapted to birch extracts and not adapted to extracts from pine sawdust.

4. It has been established by biological methods that when cultivating strains U815, U361, the resulting protein belongs to the class of permissibly toxic and can be used for feed purposes for farm animals.

5. The developed technology for cultivating strains of feed yeast on waste birch sawdust can further reduce the environmental load on the environment if whey or permeate is used in the nutrient medium.

The practical significance of the study lies in the development of laboratory tests with a view to industrial scale production of microbial feed protein using sugary substances of sawdust. In this study, the possibility of obtaining inverse sugar from sawdust by the cavitation method using an ultrasonic cell was studied, samples of hydrolysates with a mass fraction of inverse sugars of more than 30% were obtained, and the effectiveness of using the resulting sugars to obtain biomass of fodder yeast enriched with bioelements was shown under laboratory conditions. probiotic, prebiotic and other biologically active substances.

A patent was received for a method for producing a functional feed product for farm animals, which uses cavitation treatment of sawdust [14].

Laboratory data indicates that testing must be transferred to the production level, which becomes possible using a line of ultrasonic flow cells.

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Вклад авторов: все авторы внесли равный вклад в подготовку публикации.
Конфликт интересов: авторы заявляют об отсутствии конфликта интересов.

Contribution of the authors: the authors contributed equally to this article.
Conflict of interest: the authors declare no conflicts of interests.

Статья поступила в редакцию: 14.03.2024;
одобрена после рецензирования: 17.04.2024;
принята к публикации: 10.06.2024.

The article was submitted: 14.03.2024;
approved after reviewing: 17.04.2024;
accepted for publication: 10.06.2024.