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ULTRASONIC DISINTEGRATION OF LIGNOCELLULOSE RAW MATERIALS AS A PRE-TREATMENT OF A SUBSTRATE FOR MICROBIOLOGICAL PRODUCTION OF BIOBUTANOL

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Aim. The purpose of the study was to investigate the effect of ultrasonic disintegration on the lignocellulosic raw materials (biomass of the non-cereal part of rape) with its subsequent use as a substrate for the production of biobutanol.

Methods. Butanol-producing strains and the biomass of the non-cereal part of rape *Brassica napus* were used in the present study. Ultrasonic disintegration of lignocellulosic raw materials was performed on the specially designed equipment.

Results. The effect of ultrasonic disintegration on lignocellulosic raw materials was investigated for further application in biofuel production based on microbiological conversion. The possibility of using the obtained components after the pre-treatment of lignocellulose by ultrasonic disintegration as a substrate for the microbiological synthesis of butanol was shown. The highest accumulation of butanol (2.4 g/l) was obtained with the use of 5% dry matter content in the medium, 5 min treatment and the specific power of ultrasonic disintegration of 0.72 W/ml.

Conclusions. The possibility of producer strains of the genus *Clostridium* to use cellulose in the fermentation process has been shown. When using ultrasonic disintegration for pretreatment of the non-cereal part of the biomass of rape, the accumulation of butanol increased by 3 folds.

Key words: ultrasonic disintegration, biobutanol, lignocellulosic raw materials, biofuel.

Modern biotechnology offers great opportunities for the use of lignocellulosic plant biomass, such as agricultural and woodworking plant waste. In Ukraine, more than 50 million tons of grain are harvested annually. The main grain crops in Ukraine in terms of production are corn (38.50%), wheat (28.34%), sunflower (16.43%), barley (6.74%), soybeans (4.19%) and rape (4.08%). Other crops in total make up 1.72% of the gross harvest (peas 0.78%, rye 0.23%, sorghum 0.21%, oats 0.17%, panicgrass 0.16%, rice 0.07%, triticale 0.05%, buckwheat 0.04%, etc.) [1]. Renewable energy sources of raw materials such as straw and crop residues account for about 14% [2].

Lignocellulose is the main building material of plant cell walls. The main macrocomponents of lignocellulose are cellulose, hemicellulose and lignin. The production of biofuels from biomass generally requires three successive processes, namely destruction and hydrolysis, cultivation and isolation of the final product. Numerous studies on the use of biomass have shown that the rate of hydrolysis is limited by the rigidity of cell walls, which inhibits or delays the further process of biodegradation. There are many methods of destroying cell

walls. The most common are grinding, various types of hydrolysis, ultrasonic and microwave (at 100 °C) disintegration, osmotic shock and autoclaving (at 121 °C), which have their advantages and disadvantages and, thus, different end results [3, 4].

Ultrasonic disintegration (USD) of the cell wall occurs at a relatively low temperature, compared with microwave processing and autoclaving. It does not require the pretreatment with chemical destructors, which reduces the cost of the preparation process. USD is commonly used for cell lysis and homogenization; however, it can also be an effective method of breaking hard cell membranes [5].

Biobutanol production is the next important stage in the development of various types of biofuels. The use of biobutanol should meet the growing need for environmentally friendly motor fuel. Production of second-generation biobutanol from renewable non-food sources of raw materials such as cellulose-containing waste should solve the problem of using agricultural waste [6]. One of the methods of complex preliminary preparation of lignocellulosic raw materials is USD. This method provides significant destruction of biomass and makes it possible to identify the components of lignocellulosic raw materials [7]. Subsequently, the components of lignocellulosic biomass can serve as a substrate for microbiological conversion by microorganisms of the genus *Clostridium* and the production of biobutanol [8].

The aim of the work was to study the ultrasound disintegration of lignocellulosic raw material (the biomass of non-cereal part of rape) to subsequentially use it as a substrate for biobutanol production.

Materials and Methods

The objects of the study were the strain *Clostridium* sp. IMB B-7570 from the "Collection of strains of microorganisms and plant lines for food and agricultural biotechnology" of the Institute of Food Biotechnology and Genomics of the National Academy of Sciences of Ukraine (hereinafter the Collection); green biomass of *Brassica napus* of the "Chempion Ukrayiny" variety harvested in 2021 at the Olenivske Experimental Farm of the National Research Center "Institute of Mechanization and Electrification of Agriculture" of the National Academy of Agrarian Sciences of Ukraine.

The stages of research included the preparation of plant raw materials with the preparation of a suspension based on crushed raw materials, treatment of the resulting suspension with ultrasound and its subsequent cultivation to obtain biobutanol. Preliminary preparation of plant raw materials consisted of two-stage grinding of raw materials to a given weighted average size of crushed particles (200 mesh), mixing the entire mass of crushed raw materials, preparation of suspensions with a given dry matter content and subsequent sonication of the suspension. The crusher "Elikor-5" (PJSC "Electromotor", Ukraine) was used for preliminary grinding, and the laboratory mill "LZM-1" (LLC "LIS", Ukraine) was applied for the final grinding. The weighted average size of the crushed particles of raw materials was determined by laboratory sift "RLU-3" (LLC "Status", Ukraine) with a set of laboratory sieves. Plant raw materials were ground to a weighted average particle size of 0.78 mm (passing the laboratory sieve No. 64 and retained on the sieve No. 67). The crushed raw materials were mixed for 5 minutes using a laboratory batch drum mixer [9]. For the preparation of the suspension, purified tap water was used with the corresponding mass fraction of crushed plant raw materials, taking into account its humidity. Laboratory scales TVE-1 (LLC "NVP "Technovagy"", Ukraine) were used for weighing of raw materials.

The USD of the suspension was performed with a laboratory ultrasonic bath, consisting of a stainless-steel gastronomic container of standard size "GN ¼" ("TorhOborud", Ukraine) with a depth of 65 mm, at the bottom of which were attached piezoceramic Langevin ultrasonic transducers (Fig. 1) with an operating frequency of 28 kHz and an ultrasonic power of 60 W (PE "Voron", Ukraine). The laboratory unit was powered by a 1.5 kW ultrasonic generator UCE-NT 1500 ("UCE Ultrasonic", China), which provided the set operating time and automatic tuning of the resonant frequency of the ultrasonic transducers in the range of 20–40 kHz. After ultrasound, the raw materials were immediately sent for cultivation.

The yield of butanol per unit volume of suspension and weight of dry matter (g/l) was investigated depending on the duration of ultrasonic treatment of the suspension (t, min.), the dry matter content in the suspension (s, %) and the specific power of ultrasound (μ, W/l). The specific power of the ultrasound (μ) was changed by changing the volume of the suspension at a constant power of the transducers. The duration of ultrasonic treatment of the suspension was 5 and 25 min,

the dry matter content was 50 and 100 g/l, the specific power of ultrasound was 0.18 and 0.72 W/l.

As a control, mash of rape mass of 50 and 100 g per liter of water was used and sterilized for 2 h and a pressure of 2 atm. The moisture content of the raw material was determined using a weighing moisture analyzer "RADWAG MA 50/C/1" (Poland).

Cultivation of microorganisms was performed on solid media in Petri dishes in anaerostat "AE 01" (RF) with a nitrogen atmosphere to obtain single colonies. To obtain the inoculum, single colonies were selected and placed in a liquid medium. Glycerol medium was used as the inoculum medium with the following composition (g/l): glycerol p. a. — 20, yeast extract — 1.0; $(NH_4)_2SO_4$ — 0.6; $(NH_4)_2HPO_4$ — 1.6; pH 6.5. The medium was sterilized for 30 min at a pressure of 1 atm. and was used to accumulate and add to the fermentation medium the same concentration of bacteria in the active phase. The inoculum was fermented for 24 h and the accumulation of bacteria was evaluated according to the turbidity standard [9]. The anaerostat was placed in a thermostat at a temperature of 35 ± 1 °C. Cultivation was performed in 500 ml flasks using 250 ml of medium, the flasks were covered with hydroacid seals with concentrated sulfuric acid. The flasks were weighed and thermostated at 35 ± 1 °C. After 72 h of culture, the cells were pelleted using a "Labofuge 400R" ultracentrifuge (Germany) at 13,000 rpm for 10 min. After cultivation, fermentation products were distilled off from the culture fluid. The presence of ethanol and butanol in the culture fluid was determined using a gas chromatograph with a flame ionization detector. A 3 m long packed column was used with Carbowax 1500 on chromaton N-A-W-DMSC (0.20–0.25 mm). The temperature of the column was 60 ± 2 °C, that of the evaporator was 160 ± 5 °C. The ratio of nitrogen-hydrogen-air flows was 1: 1: 10.

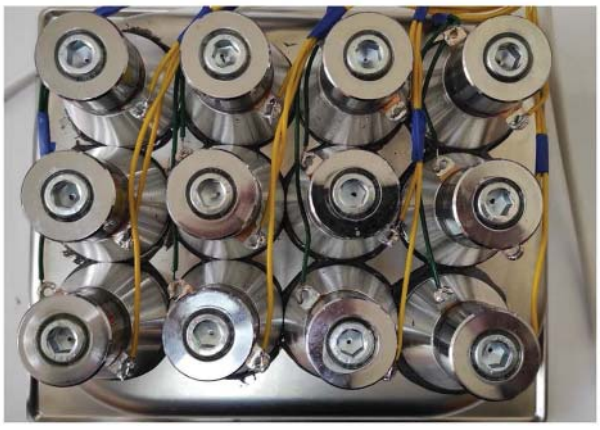

Fig. 1. **Laboratory ultrasonic bath with transducers (bottom view)**

All experiments were performed in triplicates. Statistical processing of experimental data was done using Microsoft Excel. The difference between the two means was considered significant at $P < 0.05$.

Research of ultrasound decomposition of plant raw materials to obtain biobutanol was conducted at the Department of Labor Protection and Biotechnical Systems in Animal Husbandry in the National University of Life and Environmental Sciences of Ukraine, and the State Institution "Institute of Food Biotechnology and Genomics of the National Academy of Sciences of Ukraine" (laboratory of industrial and food biotechnology).

Results and Discussion

The main idea of the research was to establish the parameters of pre-treatment of plant raw materials, in particular, grinding and USD, at which the accumulation of biobutanol in the cultivation process would be maximal. The results of the study showed the possibility of using USD of rape biomass and its use as a substrate for biobutanol (Table). The technological parameters of USD and solvent accumulation are given in Table. Experiments 1–8 were performed with biomass after USD, and experiments 9 and 10 were conducted without USD and served as a control. The accumulation of biobutanol after USD of rapeseed biomass was greater than that of untreated rapeseed biomass and untreated rape biomass of other plants [10]. The duration of ultrasonic treatment of the suspension t did not have a significant effect on the final result, which indicates the need to adjust the limits of change of this parameter and set the upper limit at 5 minutes.

The USD parameters indirectly affected the accumulation of the target product. In some samples there was an increased accumulation of ethanol instead of butanol. In our opinion, this change is due to the change in the carbon-nitrogen ratio of the medium after USD. The largest accumulation of biobutanol (2.44 g/l) was for the following values of parameters: s = 50 g/l, μ = 0.72 W/ml, which corresponds to the upper limit of variation of these parameters. The lowest accumulation of biobutanol (1.16 g/l) was observed in the case of s = 100 g/l and μ = 0.18 W/ml, which corresponds to the lower limit of variation.

Technological parameters of USD and solvent accumulation

Experiment	t, min	s, g/l	μ, W/l	Solvent accumulation, g/l			Plant biomass residue, g/l
				ethanol	acetone	butanol	
1	25	100	0.72	0.34 ± 0.07	0.09 ± 0.01	1.57 ± 0.09	73.55 ± 0.02
2	25	100	0.18	0.30 ± 0.07	0.03 ± 0.01	1.16 ± 0.07	34.26 ± 0.07
3	**25**	**50**	**0.72**	**0.32 ± 0.07**	**0.20 ± 0.03**	**2.44 ± 0.09**	**18.46 ± 0.06**
4	25	50	0.18	0.31 ± 0.07	0.07 ± 0.01	1.63 ± 0.06	34.55 ± 0.03
5	5	100	0.72	0.33 ± 0.07	0.06 ± 0.01	1.22 ± 0.04	85.56 ± 0.04
6	5	100	0.18	0.24 ± 0.04	0.06 ± 0.01	1.24 ± 0.03	83.29 ± 0.05
7	**5**	**50**	**0.72**	**0.33 ± 0.07**	**0.20 ± 0.04**	**2.37 ± 0.09**	**18.55 ± 0.01**
8	**5**	**50**	**0.18**	**0.52 ± 0.07**	**0.10 ± 0.02**	**2.16 ± 0.09**	**19.76 ± 0.02**
9	–	50	–	0.13 ± 0.04	0.02 ± 0.01	0.24 ± 0.02	42.55 ± 0.03
10	–	100	–	0.05 ± 0.01	0.02 ± 0.01	0.73 ± 0.04	96.14 ± 0.07

Note: the largest accumulation of butanol is highlighted in fat.
Hereinafter: $P < 0.05$ compared to control, native medium used as control.

In control samples that did not undergo USD, the accumulation of biobutanol was 0.24 and 0.73 g/l, depending on the biomass concentration of 50 and 100 g/l, respectively.

The dependences of biobutanol accumulation on the parameters s and μ are obtained in the form of power functions for the specific accumulation per unit volume of suspension Bv, g/l (1)

$$Bv = 7.84563 \cdot s^{-0.721369} \cdot \mu^{0.142132}, \quad (1)$$

where s is the content of dry matter in the suspension, %; μ is the specific power of ultrasound, W/ml;
and for the specific accumulation per unit mass of dry matter Bm, g/kg (2)

$$Bm = 784.563 \cdot s^{-1.72137} \cdot \mu^{0.142132}. \quad (2)$$

For dependence (1), which is adequate for 95% confidence interval, the coefficient of multiple determination D = 0.882368, the coefficient of multiple correlation R = 0.939344. For Fisher's test, F = 18.7527; the probability F of the criterion P = 0.996669. All model coefficients are significant at a confidence interval of at least 94%. For dependence (2), which is also adequate at 95% confidence interval, the coefficient of multiple determination D = 0.974346, the coefficient of multiple correlation R = 0.98709. Fisher's test F = 94.9496; the probability F of the criterion P = 0.999808. All model coefficients are significant at a confidence interval of at least 94%. Graphical view of dependences (1) and (2) is shown in Fig. 2 and Fig. 3, respectively.

As can be seen from Fig. 2 and Fig. 3, the increase in dry matter content in the suspension leads to a decrease in the accumulation of biobutanol. This can be explained by the increase in acoustic resistance of the treated suspension and the corresponding decrease in the effective action of ultrasound. The effect of the specific power of ultrasound on the accumulation of biobutanol within the experiment is less intense, but leads to an increase in the yield of butanol. The effectiveness of this factor increases in the case of reducing the dry matter content in the suspension.

The accumulation of butanol increased due to the higher bioavailability of raw materials. The increase in the bioavailability of the substrate may be due to the fact that during USD, the number of crystalline zones of cellulose decreases and the number of amorphous zones, which are easily broken down by enzymes, rises. Ultrasonic pretreatment of the substrate can change the surface morphology of lignocellulosic materials, partially disrupt the cell wall, which leads to increased availability of cellulose fibers to enzymes (cellulase) and enhances the yield of sugars during hydrolysis [11–13]. In bacteria of the genus *Clostridium*, such enzymes that break down cellulose are part of the extracellular multiprotein complex, the cellulosome.

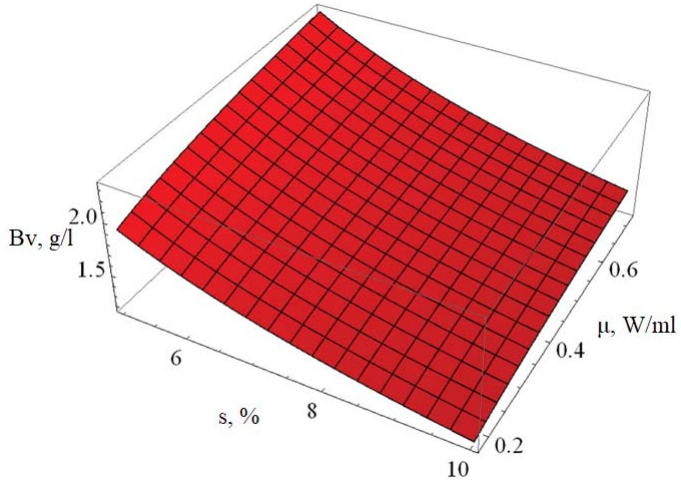

Fig. 2. **The dependence of the specific accumulation of biobutanol per unit volume (Bv) of the suspension on the dry matter content of the suspension (s) and the specific power of ultrasound (μ)**

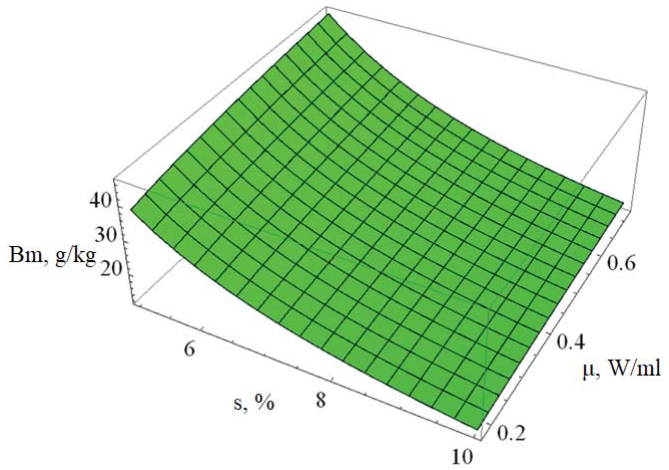

Fig. 3. **The dependence of the specific accumulation of biobutanol per unit mass of dry matter (Bm) on the dry matter content of the suspension (s) and the specific power of ultrasound (μ)**

The increase in the content of available carbohydrates in suspensions depends on the degree of destruction of lignocellulose, so it is important to find the optimal percentage of its destruction in suspension, for further use in biotechnology of liquid biofuels.

Ultrasound treatment of wheat straw [12] and sugar cane stalks [13] with ultrasound frequency of 20 and 24 kHz for 35 and 47 minutes in media of potassium hydroxide and sodium hydroxide, respectively, led to the destruction of 50 to 75 % of lignocellulose. This duration of USD, obviously, is beyond the economic feasibility of use in biofuel technologies.

USD of lignocellulosic biomass has both advantages and disadvantages compared to other pretreatment methods [14, 15]. It should be emphasized that it is not always possible to transfer a certain method of pretreatment of the substrate from one type of plant biomass to another. The choice of the method of pretreatment of lignocellulosic biomass depends on its composition and by-products formed as a result of processing. These factors significantly affect the material and financial costs in biofuel technology, which are associated with the method of pretreatment of lignocellulosic biomass.

Conclusions

Producer strains of the genus *Clostridium* can use plant lignocellulosic raw materials as a substrate in the cultivation process. The use

of ultrasound disintegration for pretreatment of the non-grain part of rape biomass increases the accumulation of butanol. The optimal values of technological parameters of USD as a pretreatment of plant biomass are established. These results indicate the effectiveness of ultrasound as an agent of pretreatment of plant raw materials in liquid biofuel technologies.

This study was conducted within the framework of the Ministry of Education and Science of Ukraine "Development of technology for ultrasonic disintegration of plant biomass of the non-grain part of the crop", State registration No. 0120U102083.

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УЛЬТРАЗВУКОВА ДЕЗІНТЕГРАЦІЯ ЛІГНОЦЕЛЮЛОЗНОЇ СИРОВИНИ ЯК ПОПЕРЕДНЯ ПІДГОТОВКА СУБСТРАТУ ДЛЯ МІКРОБІОЛОГІЧНОГО ОТРИМАННЯ БІОБУТАНОЛУ

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Мета. Дослідження впливу ультразвукової дезінтеграції на лігноцелюлозну сировину (біомасу незернової частини ріпаку) з подальшим використанням її як субстрату для отримання біобутанолу.

Методи. Для досліджень використовували штами-продуценти біобутанолу; біомасу незернової частини ріпаку *Brassica napus*. Ультразвукову дезінтеграцію лігноцелюлозної сировини виконували на спеціально створеному обладнанні.

Результати. Досліджено вплив ультразвукової дезінтеграції на лігноцелюлозну сировину з подальшим її використанням для отримання біопалива за допомогою мікробіологічної конверсії. Показано можливість застосування отриманих компонентів лігноцелюлози як субстрату після ультразвукової дезінтеграції для мікробіологічного синтезу бутанолу. Встановлено, що найбільше накопичення бутанолу (2,4 г/л) отримано за використання вмісту 50 г/л сухої речовини у середовищі та 5 хв оброблення. Зміна питомої потужності ультразвукової дезінтеграції практично не впливала на накопичення спиртів.

Висновки. Показано, що штами-продуценти роду *Clostridium* можуть використовувати рослинну лігноцелюлозну сировину як субстрат у процесі культивування. Встановлено, що за використання ультразвукової дезінтеграції для попередньої обробки незернової частини біомаси ріпаку накопичення бутанолу збільшилось утричі.

Ключові слова: ультразвукова дезінтеграція, біобутанол, лігноцелюлозна сировина, біопаливо.

УЛЬТРАЗВУКВАЯ ДЕЗИНТЕГРАЦИЯ ЛИГНОЦЕЛЛЮЛОЗНОГО СЫРЬЯ КАК ПРЕДВАРИТЕЛЬНАЯ ПОДГОТОВКА СУБСТРАТА ДЛЯ МИКРОБИОЛОГИЧЕСКОГО ПОЛУЧЕНИЯ БИОБУТАНОЛА

Е. А. Тигунова[1], *М. О. Уманский*[2], *В. В. Братишко*[2], *А. В. Балабак*[3], *С. М. Шульга*[1]

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Цель. Исследование влияния ультразвуковой дезинтеграции на лигноцеллюлозное сырье (биомасса незерновой части рапса) с последующим использованием её как субстрата для получения биобутанола.

Методы. Для исследования использовали штаммы-продуценты бутанола; биомассу незерновой части рапса *Brassica napus*. Ультразвуковую дезинтеграцию лигноцеллюлозного сырья выполняли на специально созданном оборудовании.

Результаты. Исследовано влияние ультразвуковой дезинтеграции на лигноцеллюлозное сырье с дальнейшим использованием для получения биотоплива с помощью микробиологической конверсии. Показана возможность использования после обработки лигноцеллюлозы ультразвуковой дезинтеграцией полученных компонентов как субстрата для микробиологического синтеза бутанола. Установлено, что наибольшее накопление бутанола (2,4 г/л) получено с использованием 5% содержания сухого вещества в среде, 5 мин обработке и удельной мощности ультразвуковой дезинтеграции 0,72 Вт/мл.

Выводы. Показана возможность штаммов-продуцентов рода *Clostridium* применять целлюлозу в процессе ферментации. Установлено, что при использовании звуковой дезинтеграции для предварительной обработки незерновой части биомассы рапса накопление бутанола увеличилось в 3 раза.

Ключевые слова: ультразвуковая дезинтеграция, биобутанол, лигноцеллюлозное сырье, биотопливо.

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