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LIGNOCELLULOSIC BIOMASS AFTER EXPLOSIVE AUTOHYDROLYSIS AS SUBSTRATE FOR BUTANOL OBTAINING

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The aim of the investigation of the effect of the explosive autohydrolysis on lignocellulosic biomass (saving, switchgrass biomass) for consequent use as a substrate to produce biofuels such as butanol. Butanol-producing strains, switchgrass *Panicum virgatum* L. biomass and its components after autohydrolysis were used in the study. The thermobaric pressure pretreatment of lignocellulosic biomass was carried out using specially designed equipment. The effect of explosive autohydrolysis on lignocellulosic biomass for further use in producing biofuels using microbial conversion was studied. Components of lignocellulosic biomass were fractionated after undergoing thermobaric treatment. The possibility of using different raw material components after using explosive autohydrolysis processing to produce biobutanol was found. Products of switchgrass biomass autohydrolysis were shown to need further before fermentation purification from furfural formed by thermobaric pretreatment and inhibiting the growth of microorganisms. The ability of strains of the genus *Clostridium* to use cellulose as a substrate for fermentation was proved. It was found that using explosive autohydrolysis pretreatment to savings allowed to boost the butanol accumulation by 2 times.

Key words: explosive autohydrolysis, butanol, lignocellulosic biomass, biofuels.

Lignocellulose is one of the virtually unlimited sources by the number of substrates for microbial conversion. A lot of lignocellulosic waste is produced in forestry and agriculture, pulp and paper and wood industries, which poses a serious environmental problem, the increasing pollution. Unfortunately, most of the waste is not recycled although it can be used, for example as biofuels. An effective biological conversion requires pretreatment of lignocellulosic biomass [1]. One of the methods of its complex pretreatment is explosive autohydrolysis. This method provides almost total destruction of the biomass and allows to separate components of lignocellulosic material [2]. Later lignocellulosic biomass components can serve as a substrate for microbial conversion, including microorganisms of the genus *Clostridium*, and as a source for obtaining biofuels such as butanol [3].

The aim of the study was to explore the effect of explosive autohydrolysis on lignocellulosic biomass (saving, switchgrass biomass) for consequent use as a substrate to produce biofuels such as butanol.

Materials and Methods

For the study we used butanol producing strains *Clostridium acetobutylicum* IMB B-7407 (IFBG C6H), *Clostridium sp.* IMB B-7570 (IFBG C6H 5M) from "Collection microorganism's stains and plants line for food and agriculture biotechnology" of the Institute of Food Biotechnology and Genomics of the National Academy of Sciences of Ukraine; biomass of switchgrass *Panicum*

virgatum L., and components of switchgrass after autohydrolysis (cellulose, lignin and arabinogalactan); savings of Obukhiv pulp and paper mill.

Moisture mass fraction in initial samples and in products of reaction was determined according to [4], crude ash mass fraction according to [5], lignin's by [6], soluble carbohydrates' by [7], resins' and fats' by [8]. Mass fraction of cellulose was conducted by nitrogen-alcoholic method [9].

Thermobaric preprocessing of lignocellulosic materials was performed by [2] on specially constructed equipment at a temperature of 200 °C for 10 min. Mush-like mixture was received and directly used as substrate or fractionated (Fig. 1).

The process of fractionation can be described by following stages. Mixture of products (620 g) after autohydrolysis was transferred to a Buchner funnel and filtered out liquid fraction (310 g). Air-dry residue was placed in a conical flask of volume 500 ml, and hot water was added (300 ml), liquor ration 50 (solid phase to liquid ratio). Extraction of soluble carbohydrates was done for three hours on water bath at 100 °C, then the second filtration in a Buchner funnel. The resulting solid residue was dried. To prevent the destruction of soluble sugars, the filtrate was steamed at water bath at 50 ± 5 °C, with simultaneous extraction of furfural with water. Arabinogalactan was precipitated by ethanol with liquor ration 5. The residue was filtrated, washed with ethanol and dried. The ethanol was removed from the filtrate solution on rotary evaporator (Rotadest, Hungary). The result was a concentrated solution of soluble sugars.

The presence of furfural after explosive autohydrolysis (almost non-waste technology to separate plant material into three main components: lignin, cellulose and hemicellulose by applying high temperature (100–200 °C) water vapor to wooden complex; reaction time is 1–10 min) was determined by gas chromatography. We used gas chromatograph Agilent-7890A (USA) with flame ionization detector; silica intermediate polar capillary column HP-5 (length 30 m, inner diameter 0.320 mm, film thickness 0.25 μm); carrier gas: helium (300 mL/min); programmed temperature from 40 °C to 280 °C in increments of 5 °C / min; temperature of evaporator 250 ± 5 °C; temperature of detector 280 ± 5 °C. The volume of the sample was 1.0 μl.

Results of the analysis were processed using the method of absolute calibration by chromatographic peak areas.

The savings and switchgrass biomass were dried at 30 ± 1 °C for 168 hours. The dried biomass was ground using mill Cyclone MSh 1 (Ukraine) to particles sized 200 mesh. The moisture was determined by moisture analyzer RADWAG MA 50/C/1 (Poland).

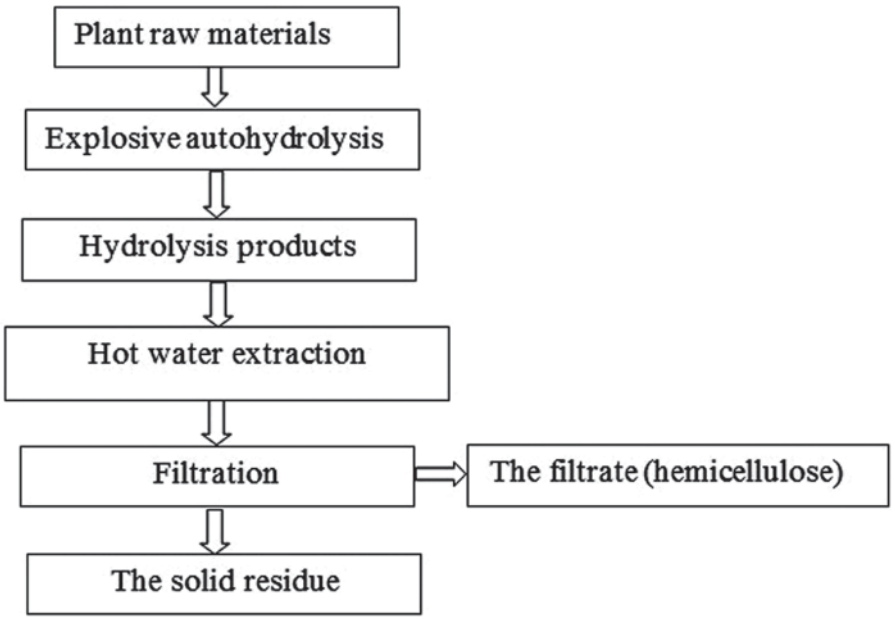

Fig. 1. **Scheme of fractionation of lignocellulosic biomass after explosive autohydrolysis**

Winogradsky medium and slices of potatoes smeared with chalk were used as activation medium for butanol producing strains. To ascertain the purity of culture, MAV medium was used [10]. To prepare mash's leavens (out of switchgrass, savings), samples of 50.0 g of the substance were taken per 1 l of water, and sterilized for 2 hr at a pressure of 0.2 MPa. To confirm the properties of cellulolytic strains, solid medium of the following composition was used: $(NH_4)_2SO_4$ — 0.6 g/dm^3; $(NH_4)_2HPO_4$ — 1.6 g/dm^3; agar — 30 g/dm^3; pH 6.2. It was sterilized during 30 min at 0.1 MPa. Discs of filter paper (1.0 g), previously sterilized for 30 min at 0.2 MPa, were inserted in medium. On the discs, relevant strains were put in droplets of liquid medium.

Culture of microorganisms at solid medium was performed following [11] in anaerobic culture apparatus AE 01 (RF) under a nitrogen atmosphere. The apparatus was kept in thermostat at 35 ± 1 °C. In five days, the fermentation was stopped and the cells were precipitated using ultracentrifuge Labofuge 400R (Germany), then the supernatant was distilled and fermentation products defined.

Presence of ethanol, acetone and butanol in culture liquid was determined using gas chromatograph "Kristall-5000 lux" (RF) with flame-ionization detector and packed column (3 m in length), phase Carbowax 1500 on chromaton NAW-DMSC (0.20–0.25 mm). The column temperature was 60 ± 2 °C, the evaporator's 160 ± 5 °C, Nitrogen: Hydrogen: air ratio was 1:1:10.

Statistical data analysis was performed using Microsoft Excel program. All experiments were done in triplicate. The difference between the two averages was considered probable by $P < 0.05$ (significant results marked with *).

Results and Discussion

One of the promising renewable raw materials are lignocellulosic materials, including switchgrass *Panicum virgatum* L. biomass. The possibility of conversion of switchgrass biomass to produce butanol was shown previously [11]. To enhance butanol production by strains IFBG C6H and IFBG C6H 5M, switchgrass was pretreated by explosive autohydrolysis. The components of switchgrasss biomass before and after autohydrolysis are given in Table 1.

As follows from the data, after the explosive autohydrolysis, the content of basic components changed, due to the restructuring of biomass components [12, 13]. The growing portion of water-soluble substances was primarily due to destruction of hemicelluloses [12]. A slightly increased lignin content, according to [13], was due to the lowered pH of the reaction medium. At the same time, during fractionation after autohydrolysis (delignification of cellulose) there was a chemical effect on lignin, which induced its polymerization, and therefore the amount of insoluble in acid lignin decreased. Therefore, for the fermentation of substrate after autohydrolysis it was necessary to use active producers of cellulases. Bacteria of the genus *Clostridium* can serve as such [1]. Chosen strains IFBG C6H and IFBG C6H 5M were precipitated to filter paper to confirm the possibility of cellulose fermentation (Fig. 2).

After 72 hours of culturing on filter paper, clear areas appeared, and after 120 hours destruction of paper around the colonies was observed. Results of the study demonstrated the presence of cellulases in both cultures and the ability of these strains to use cellulose as a carbon source. To test whether lignocellulose was used as a substrate, culturing of microorganisms on individual components of switchgrass after explosive autohydrolysis was performed (Fig. 3).

As a result of studies it was found that the content of furfural increased in plant biomass after autohydrolysis, inhibiting the bacterial growth. To avoid the inhibitory effect, furfural was removed and biomass components were separated. In this study it was found that cultured strains-producers using the biomass components produced butanol with different levels of accumulation. The highest concentration of butanol (2.8 g/dm^3) was in the case of the strain IFBG C6H 5M cultured on switchgrass biomass after autohydrolysis and extraction of furfural. These data suggest that the explosive autohydrolysis is a promising way of preparing lignocellulosic biomass before fermentation.

Another possible substrate are savings, paper industry waste product. To establish the impact of autohydrolysis on savings, its component composition was studied before and after autohydrolysis (Table 2).

The data show that nearly half of savings consists of inorganic components that cannot serve as a culturing medium for microorganisms. As savings are waste of

Table 1. **Switchgrass biomass content after autohydrolysis**

Components	The initial biomass, %	Biomass after hydrolysis, %
Cellulose	46.7±0.5	53.9±0.5
Hemicellulose	23.0±1.0	10.6±1.0
Water soluble substances	7.7±0.5	11.5±0.5
Lignin	13.8±0.2	14.7±0.2
Resins and fats	2.0±10	2.0±10
Ash	5.4±0.5	5.4±0.5
Other	1.4±0.2	1.9±0.2
Total	100.0	100.0

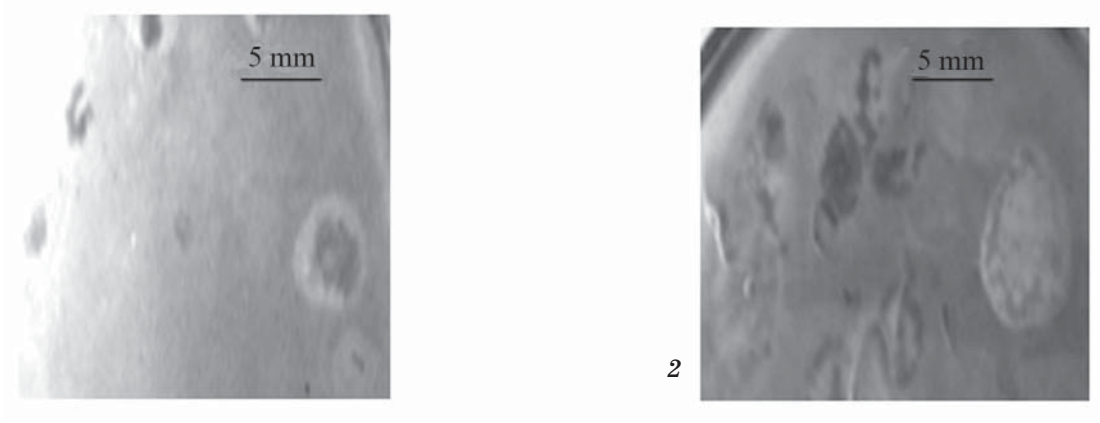

Fig. 2. **Colonies of IFBG C6H 5M (*1*) and IFBG C6H (*2*) on filter paper**

pulp and paper industry, 75% of organic components consist of cellulose, the amount of which after thermobaric treatment slightly increases similarly to switchgrass (Table 1). But savings are depleted in sugars and soluble substances and are preferably used in processes that need cheap raw material containing cellulose.

To study the ability of microorganisms for bioconversion of savings as a substrate, IFBG C6H and IFBG C6H 5M strains were cultured in an environment with different pretreatment (Fig. 4.)

According to the data, strains IFBG C6H 5M and IFBG C6H converted to butanol even raw savings, but the accumulation of the desired product was low, 0.3 and 0.1 g/dm^3 respectively. Grinding of raw materials led to increased production of butanol to 0.6 to 0.8 g/dm^3 respectively, but the preferred method of pretreatment of raw materials was autohydrolysis. In the case of savings processed by explosive autohydrolysis, accumulation of butanol in the culture liquid was 1.6 and 1.1 g/dm^3. It should be noted that in explosive autohydrolysis, furfural formed in small amounts and did not affect the activity of microorganisms.

The results showed the possibility of using lignocellulosic materials as substrate for the production of biofuels and converting cellulose into butanol by microorganism strains IFBG C6H and IFBG C6H 5M. The

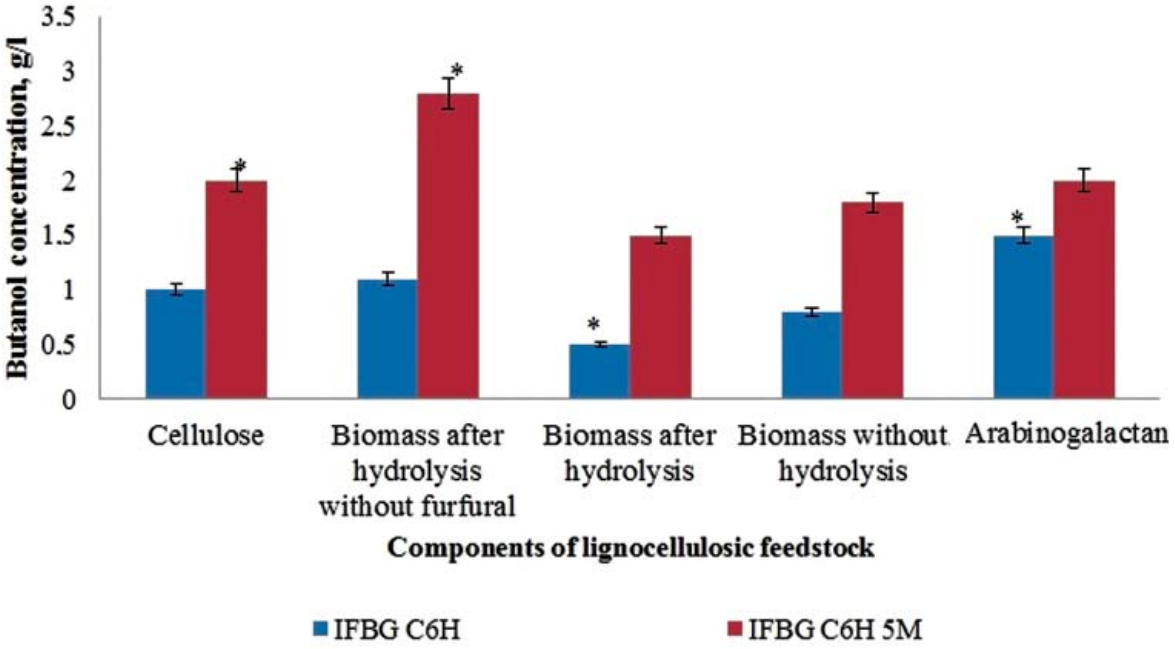

Fig. 3. **Culturing of producer strains on biomass and separate components of lignocellulosic raw materials**
Here and later * signifies the significant difference between strains

Table 2. **Composition of savings after autohydrolysis**

Components	The initial biomass, %	The composition after hydrolysis, %
Cellulose	34.0±0.5	37.0±0.5
Hemicellulose	3.7±1.0	1.5±1.0
Water soluble substances	3.2±0.5	6.9±0.5
Lignin	8.5±0.2	3.5±0.2
Resins and fats	2.3±10	2.3±10
Ash	47.8±0.5	48.0±0.5
Other	0.5±0.2	0.8±0.2
Total	100.0	100.0

increase in accumulation of butanol is 2 times in the case of explosive autohydrolysis as a method of pretreatment of raw materials. It is established that under autohydrolysis only the hemicellulose is partially destroyed to form a large number of water-soluble substances. After autohydrolysis, cellulose and lignin are separate components which can be compared quantitatively with their content in the original raw material. A direct dependence between formation of furfural in the products of explosive autohydrolysis on the amount of hemicellulose in the original biomass is observed. This is because under the hydrolyzing agent at elevated temperatures, sugars can hydrolyze to form furfural and oxymethylfurfural. Withdrawal of furfural and oxymethylfurfural from products of explosive autohydrolysis of switchgrass millet made it possible to achieve an increase in the accumulation of butanol in the fermentation process.

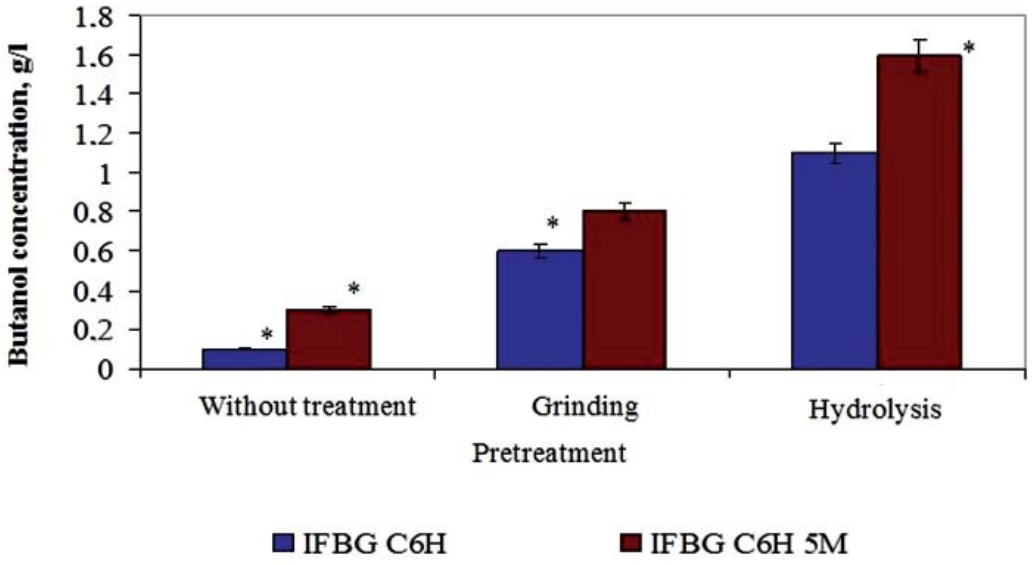

Fig. 4. **Strains cultured on medium with differing pretreatment of savings**

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ЛІГНОЦЕЛЮЛОЗНА СИРОВИНА ПІСЛЯ ВИБУХОВОГО АВТОГІДРОЛІЗУ ЯК СУБСТРАТ ДЛЯ ОТРИМАННЯ БУТАНОЛУ

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Метою роботи було визначення впливу вибухового автогідролізу на лігноцелюлозну сировину (скоп, біомаса дротоподібного проса) з подальшим використанням її як субстрату для отримання біопалива — бутанолу. Для досліджень використовували штами-продуценти бутанолу, біомасу дротоподібного проса *Panicum virgatum* L., компоненти дротоподібного проса після автогідролізу. Паробаричну попередню обробку лігноцелюлозної сировини виконували на спеціально створеному обладнанні. Досліджено вплив вибухового автогідролізу на лігноцелюлозну сировину з використанням надалі для одержання біопалива за допомогою мікробіологічної конверсії. Проведено фракціонування лігноцелюлозної біомаси на складові компоненти після термобаричної обробки. Показано можливість застосування отриманих компонентів після оброблення лігноцелюлози вибуховим автогідролізом як субстрату для мікробіологічного синтезу бутанолу. Встановлено, що перед ферментацією продуктів автогідролізу дротоподібного проса їх необхідно додатково очищати від фурфуролу, який утворюється під час термобаричної обробки і є інгібітором розвитку мікроорганізмів. Підтверджено здатність штамів-продуцентів роду *Clostridium* використовувати целюлозу як субстрат у процесі ферментації. З'ясовано, що в разі використання вибухового автогідролізу для попередньої обробки скопу накопичення бутанолу збільшилось у 2 рази.

Ключові слова: вибуховий автогідроліз, бутанол, лігноцелюлозна сировина, біопаливо.

ЛИГНОЦЕЛЛЮЛОЗНОЕ СЫРЬЕ ПОСЛЕ ВЗРЫВНОГО АВТОГИДРОЛИЗА КАК СУБСТРАТ ДЛЯ ПОЛУЧЕНИЯ БУТАНОЛА

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Целью работы было определение влияния взрывного автогидролиза на лигноцеллюлозное сырье (скоп, биомасса стеблевидного проса) с дальнейшим использованием его как субстрата для получения биотоплива — бутанола. Для исследований использовали штаммы-продуценты бутанола, биомассу стеблевидного проса *Panicum virgatum* L., компоненты стеблевидного проса после автогидролиза. Паробарическую предварительную обработку лигноцеллюлозного сырья проводили на специально созданном оборудовании. Исследовано влияние взрывного автогидролиза на лигноцеллюлозное сырье с дальнейшим использованием для получения биотоплива с помощью микробиологической конверсии. Проведено фракционирование лигноцеллюлозной биомассы на составляющие компоненты после термобарической обработки. Показана возможность использования полученных компонентов после обработки лигноцеллюлозы с помощью взрывного автогидролиза как субстрата для микробиологического синтеза бутанола. Установлено, что перед ферментацией продуктов автогидролиза стеблевидного проса их необходимо дополнительно очищать от фурфурола, который образуется при термобарической обработке и является ингибитором развития микроорганизмов. Подтверждено свойство штаммов-продуцентов рода *Clostridium* использовать целлюлозу как субстрат в процессе ферментации. Установлено, что при использовании взрывного автогидролиза для предварительной обработки скопа накопление бутанола увеличилось в 2 раза.

Ключевые слова: взрывной автогидролиз, бутанол, лигноцелюлозное сырье, биотопливо.