ULTRASONIC CAVITATION OF LIGNOCELLULOSIC RAW MATERIALS AS EFFECTIVE METHOD OF PREPARATION FOR BUTANOL PRODUCTION

Viacheslav Bratishko[1], Sergey Shulga[2], Olena Tigunova[2], Oksana Achkevych[1]
[1]National University of Life and Environmental Sciences of Ukraine, Ukraine;
[2]Institute of Food Biotechnology and Genomics of NAS of Ukraine, Ukraine
vbratishko@gmail.com, shulga5@i.ua , tigunova@ukr.net, achkevych@nubip.edu.ua

Abstract. Six types of native and ultrasonic cavitation-treated non-cereal plant biomass as a substrate for cultivation of the butanol-producing strain Clostridium sp. IMB B-7570 were studied. During the research, an ultrasonic bath with emitters with a total power of 720 W and an ultrasound frequency of 28 kHz was used. The weighted average size of the straw particles was 78 μm. As a result of the research, the accumulation of butanol in the culture liquid after 72 hours of fermentation was determined. After fermentation of the producer strain Clostridium sp. IMB B-7570 all three products of the acetone-butanol-ethanol process were detected in the culture liquid. It was established that cultivation of the strain on pre-treated biomass significantly increases the accumulation of butanol in the culture liquid. The highest accumulation of butanol (0.7 $g \cdot l^{-1}$) was obtained using crushed rape biomass as a substrate. In this case, ethanol and acetone were present in small quantities – 0.05 $g \cdot l^{-1}$ and 0.02 $g \cdot l^{-1}$, respectively. A significant increase in the yield of biobutanol compared to untreated experiments is the result of the mechanical destruction of the lignocellulosic structure of plant biomass due to the cavitation effect of ultrasound, which is confirmed by numerous experiments on various types of raw materials. A comparative analysis of the research results established that the accumulation of solvents after ultrasonic cavitation of the non-grain part of plants correlates with acid-enzymatically prepared substrate, but ultrasonic cavitation is more effective than hydrolysis, and therefore this method of preliminary preparation of biomass for fermentation can be more competitive in industrial conditions.

Keywords: ultrasound, cavitation, bio-raw material, butanol, lignocellulose.

Introduction

The energy and environmental crises that the world is experiencing force us to reconsider the efficiency of using natural renewable resources [1]. Microbiological conversion of renewable resources of the biosphere in order to obtain useful products, in particular biofuel, is currently one of the urgent problems of biotechnology. Anaerobic bacteria of the *Clostridia* family are known producers of one of the promising types of biofuels – butanol. Microbiological synthesis of butanol during the classic acetone-butanol-ethanol fermentation is currently economically unprofitable. In order to make acetone-butyl fermentation economically profitable, highly productive solventogenic strains are needed, which would use available and cheap raw materials (substrate). One example of such a substrate can be plant biomass [2].

The analysis of plant tissues makes it possible to take into account and determine with sufficient accuracy the substances that are part of plant biomass. Such analysis is the first step in using biomass as a substrate. In order to establish the sources of carbon, which are part of the composition of non-cereal plant biomass, its macrocomponents were investigated and determined [3]. As a result of laboratory studies, it was established that stem plant biomass is rich in components that can ferment, namely cellulose, hemicellulose, and water-soluble substances. However, the composition of biomass includes a lot of lignin, which is difficult to convert by microorganisms [4]. Among those analysed the largest amount of lignin is contained in the non-grain biomass of barley, which indicates its low potential as a raw material for biofuel obtained by fermentation methods, in particular, in biobutanol technologies.

A significant increase in the yield of biobutanol can be achieved by preliminary preparation of bio-raw materials for fermentation [5]. Substrate preparation plays an important role in cultivation. Cultivation of untreated biomass leads to the accumulation of butanol in a low concentration. One of the promising ways of preparing raw materials is the ultrasonic cavitation treatment of suspensions [6]. The effectiveness of such processing is determined by the mechanical destruction of the lignocellulosic structure of plant biomass due to the cavitation effect of ultrasound, which is confirmed by numerous experiments on various types of raw materials [7].

Based on the fact that the increase in the content of available hydrocarbons in the processed suspensions depends on the degree of destruction of lignin, which takes a small part in microbial activity, the question arises about the rational level of destruction of lignin in liquid biofuel technologies, which involve microbial fermentation of bio-raw material. Thus, experiments indicate the possibility of destroying from 50 to 75% of biomass lignin during ultrasonic treatment, respectively, of wheat straw [8] and sugar cane stalks [9] with ultrasound at a frequency of 20 and 24 kHz for 35 and 47 minutes in potassium hydroxide and sodium hydroxide environments, respectively. However, this duration of ultrasonic treatment obviously calls into question the economic feasibility of its use in biofuel technologies.

The experimental data correlate with the results of studies [10], which showed the possibility of increasing the content of available hydrocarbons (increasing the total yield of sugars up to 50%) after the disintegration of a suspension based on defatted soybean flakes with ultrasound at a frequency of 20 kHz, a specific power of 0.3-2.56 $W \cdot ml^{-1}$ for 15-120 s. At the same time, for example, ultrasonic treatment of extruded soybean flakes with a frequency of 20 kHz for 30-60 s [11] did not significantly affect the change in the content of available sugars, as did the ultrasonic treatment of rice husks for 10-60 minutes at frequencies of 40 kHz [12].

Along with this, the issue of comparative analysis of equal methods of preparation of bio-raw materials for fermentation, which can ensure effective destruction of lignocellulosic structures, is important.

Materials and methods

The research was conducted on six types of plant biomass: rapeseed, soybean, wheat, and barley straw, corn, and sunflower stalks. The biomass plant was dried at a temperature of 30 ± 10ºC for 16 hours. Plant biomass consisted of dried and chopped stems and leaves. The dried biomass was crushed using a laboratory “Zyklon” mill. The moisture content of raw materials was determined using a RADWAG MA 50/C/1 weighing moisture analyser. The weighted average size of the crushed particles of raw materials was determined by means of screening laboratory “RLU-3” with a set of laboratory sieves. Plant raw materials were ground to a weighted average particle size of 0.078 mm (passage through laboratory sieve No. 64, coming off sieve No. 67 [13]).

Plant biomass served as a substrate for the cultivation of the butanol-producing strain *Clostridium sp.* IMV B-7570. Determination of the accumulation of butanol in the culture liquid occurred after 72 hours of cultivation.

To confirm the properties of the strain before cellulose fermentation, it was planted on filter paper (Fig. 1). After 72 hours of culture, all samples showed clear zones on the filter paper, and after 120 hours, paper breakdown around the colonies was observed. The results of the study make it possible to assert the presence of cellulases in the culture and the possibility of using cellulose as a carbon source. In bacteria of the genus Clostridium, enzymes that break down cellulose are part of the extracellular multiprotein complex – cellulosomes [14]. The amount of cellulose is directly proportional to the number of bacteria in the fermentation mixture.

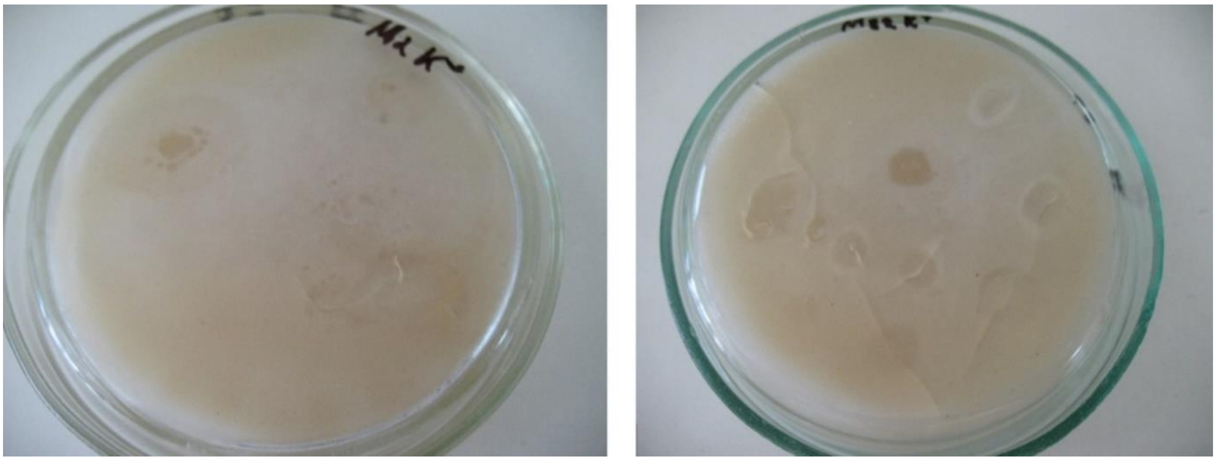

Fig. 1. **Colonies of *Clostridium sp.* IMV B-7570 growing on filter paper**

An ultrasonic bath with emitters with a total power of 720 W and an ultrasound frequency of 28 kHz was used for ultrasonic cavitation treatment. The duration of ultrasonic treatment was 15-25 minutes.

The following parameters were used in the comparative analysis of different methods of raw material pre-treatment for fermentation. Alkaline hydrolysis was carried out using a 1% NaOH solution at a temperature of 100ºC. Acidic hydrolysis was carried out at a temperature of 100ºC with 1% H_2SO_4. Enzymatic hydrolysis of pre-prepared biomass using hydrolyses and a set neutral pH was carried out using a cellulosic enzyme complex of cellulose with *Trichoderma reesei* ATCC 26921 (Sigma, USA) and cellobiase with *Aspergillus niger* (Sigma, USA) and β-glucosidases under optimal conditions recommended by the manufacturer: process temperature – 50 ºC, pH – 5.0 (permissible range 50-65 ºC; pH 4-5). All hydrolysis was carried out within 30 min.

Statistical data processing was performed using Microsoft Excel. All experiments were performed in three repetitions. The difference between two mean values was considered significant at $p < 0.05$. The probability value of the obtained data was calculated using the Student's t-test and was considered reliable at the significance level of $p < 0.05$.

Results and discussion

Six types of native and cavitation ultrasound-treated plant biomass were studied as a substrate for the cultivation of the butanol-producing strain *Clostridium sp.* IMV B-7570. The accumulation of butanol in the culture liquid after 72 hours of fermentation was determined. Cultivation of the strain on pre-treated biomass increases the accumulation of butanol in the culture liquid. After fermentation of the producer strain *Clostridium sp.* IMV B-7570 all three products of the acetone-butanol-ethanol process were detected in the culture liquid.

It was established that the greatest accumulation of butanol in the native raw material (0.7 $g \cdot l^{-1}$) was due to the use of crushed rapeseed biomass (100 $g \cdot l^{-1}$) as a substrate and a strain of Clostridium sp. IMV B-7570. In this case, ethanol and acetone were present in small amounts – 0.05 $g \cdot l^{-1}$ and 0.02 $g \cdot l^{-1}$, respectively [6].

The results of the experimental studies showed the expediency of using ultrasonic preparation of biomass of plant raw materials of all kinds and using it as a substrate for obtaining biobutanol.

In comparison with native (untreated) raw materials, the largest average increase in the accumulation of biobutanol after ultrasonic treatment was observed in the biomass of rapeseed and soybean straw - 1.31 and 1.04 $g \cdot l^{-1}$, respectively (Fig. 2)

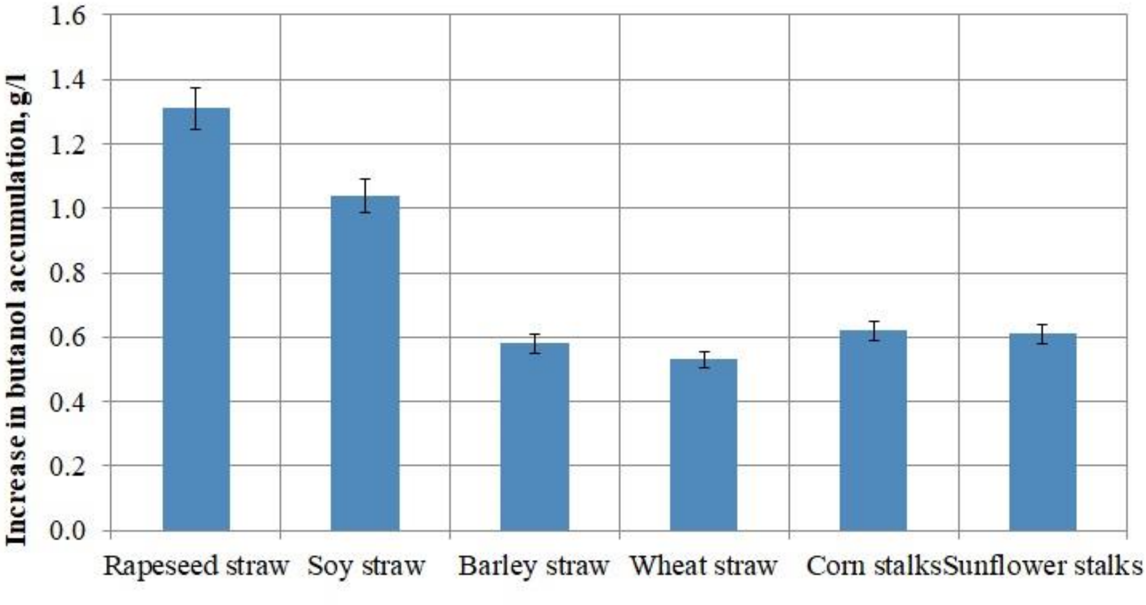

Fig. 2. **Increase in accumulation of butanol by a strain of *Clostridium sp.* IMV B-7570 relative to unprocessed raw materials**

For barley and wheat straw, the increase in butanol accumulation was 0.58 and 0.53 $g \cdot l^{-1}$, respectively. For corn and sunflower stalks – 0.62 and 0.61 $g \cdot l^{-1}$.

Therefore, the straw of leguminous crops, in particular, rapeseed, is the most promising raw material for use in butanol technologies.

For rapeseed straw, experimental studies were conducted on the use of various methods of preparing plant bio-raw material for cultivation, shown in Fig. 3.

According to the results of comparative studies, it was established that the quantitative indicators of solvent accumulation during ultrasonic treatment of the non-grain part of plants are correlated with the acid-enzymatically prepared substrate. At the same time, ultrasonic preparation is economically more profitable than hydrolysis, and therefore this method of preliminary preparation of biomass can be competitive in industrial conditions.

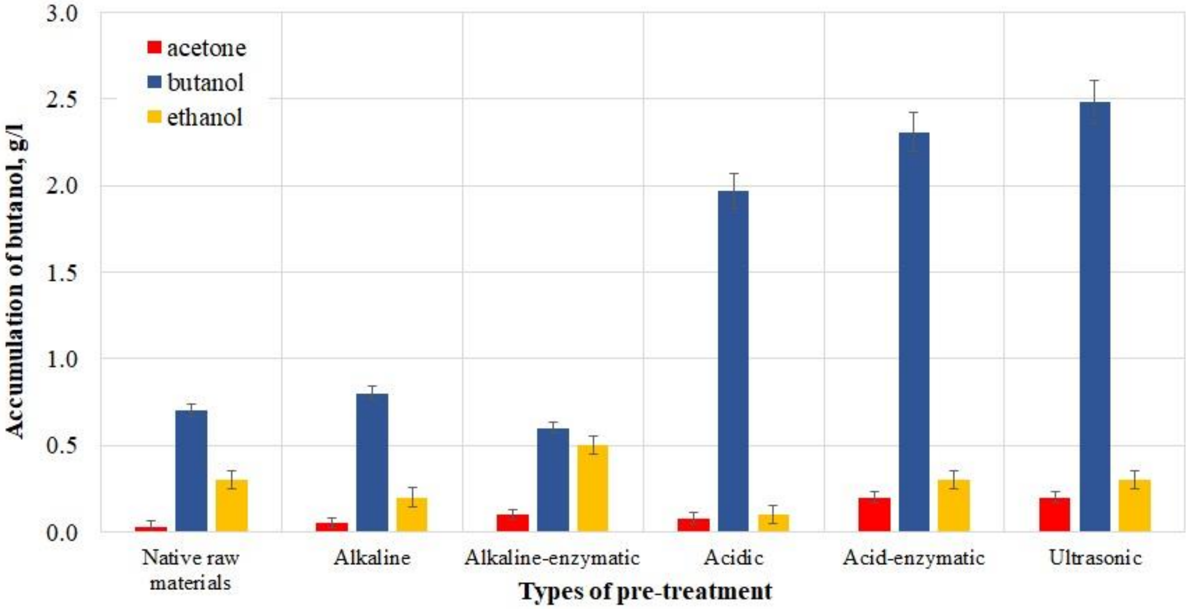

Fig. 3. **Accumulation of solvents during different pre-treatments of rapeseed straw biomass**

Pre-treatment of plant lignocellulosic raw materials using ultrasound has advantages compared to classical biological, physical, and chemical processes of pre-treatment of the substrate followed by enzymatic hydrolysis and production of biofuel. The main advantage of ultrasonic preparation is the reduction of raw material processing time, the lower temperature of the process, the need for smaller amounts of enzyme preparations, and the minimal accumulation of degradation products and inhibitors. The greatest interest for the industry lies in increasing the accumulation of sugars in the process of preliminary processing of raw materials using ultrasound. Obviously, this technology is an additional step to the existing processes of preliminary preparation of raw materials with minimal technological changes. Obviously, the processes of preliminary preparation of raw materials using ultrasound can be combined with other classical methods of preliminary preparation, which represents the potential for further intensification of cultivation.

Conclusions

1. Compared to native (untreated) raw materials, the largest average increase in biobutanol accumulation after ultrasonic treatment was observed in rapeseed and soybean straw biomass – 1.31 and 1.04 $g \cdot l^{-1}$, respectively.
2. A preliminary ultrasonic treatment of raw materials of legume straw, in particular, rape is the most promising raw material for use in butanol technologies.
3. Quantitative indicators of solvent accumulation during ultrasonic treatment of the non-grain part of plants correlate with acid-enzymatically prepared substrate and prevail over other methods of preliminary preparation.
4. Ultrasonic cavitation treatment can be an effective additional step to existing raw material pre-treatment processes with minimal technological changes.

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Author contributions:

Conceptualization, V.B. and S.S.; methodology, S.S., V.B. and O.T.; validation, O.T., V.B. and O.A.; formal analysis, V.B and S.S.; investigation, V.B., S.S., O.T. and O.A.; data curation, O.T. and O.A; writing – original draft preparation, V.B., S.S., O.T.; writing – review and editing, V.B., S.S., O.T. and O.A.; visualization, V.B. and O.T.; project administration, V.B. and S.S.; funding acquisition, V.B. All authors have read and agreed to the published version of the manuscript.

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