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RAPE SWITCHGRASS BIOMASS (*Brassica napus*) AS RAW MATERIALS FOR BIOBUTANOL PRODUCTION

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The aim of the work was to investigate the accumulation of butanol by *Clostridium* strain producers using meshed green rape biomass as substrate. The accumulation of butanol by producer strains was studied using rape as substrate in the doses of 5–30 g/l. The cells were precipitated in an ultracentrifuge, the supernatant was distilled, and fermentation products were determined. The presence of solvents in the culture fluid was determined by gas chromatography. The biggest accumulation of butanol was produced by the strain *Clostridium* sp. IMB B-7570 on 2.3 g/l mashed switchgrass biomass. The optimal inoculum concentration for maximum accumulation of butanol using switchgrass biomass was 10% of the volume of fermentation liquid. The greatest accumulation of butanol (2.9 g/l) was obtained in optimal culture conditions and at 10 g/l dry switchgrass biomass in the fermentation medium. Thus, the present study showed that mashed switchgrass biomass was assimilated by *Clostridium* sp. strains. The accumulation of butanol depended on *Clostridium* strain, the amount of inoculum, the concentration and degree of grinding of the substrate.

Key words: biobutanol, *Clostridium*, plant biomass, switchgrass.

Lately, producing liquid organic compounds from the renewable raw materials such as plant biomass became a hotter topic once again. Butanol (butyl alcohol) and ethanol are among such compounds [1, 2]. A significant amount of biomass is produced in agriculture [3], which can be used as raw materials for the bioconversion processes [4]. Various microorganisms are capable of growing on a substrate containing lignin and cellulose, producing substances like butanol, ethanol, acetone, etc. [5]. In a classic acetone-butanol-ethanol (ABE) fermentation process, the solvents are generated in a ratio of 3:6:1. Butanol is accumulated in the culture fluid if the concentration of sugar-containing substrate is at least 2 % volumetric. That is caused by the inhibiting influence of butanol on the culture growth and development. The solvent ratio changes if the culture substrates contain lignin and cellulose. The butanol concentration also changes, it depends on the amount of available carbohydrate medium (cellulose and hemicellulose) [6]. The aim of present work was to study the butanol accumulation by the producer strains of the genus *Clostridium* on a substrate of rape raw biomass.

Materials and Methods

The study objects were the strains *Clostridium acetobutylicum IMB B-7407 (*IFBG C6H), *C. tyrobutylicum* IFBG C4B and *Clostridium* sp. IMB B-7570 of the "Collection of strains of microorganisms and lines of plants for food and agricultural biotechnology" of the State institution "Institute of food biotechnology and genomics" of the National Academy of Sciences of Ukraine (henceforth, Collection); green

biomass of rape *Brassica napus (*National Scientific centre "Institute of mechanization and electrification of agriculture", Ukrainian Academy of Agricultural sciences). Samples were cultured in flasks with liquid medium or in Petri dishes. The chosen inoculum medium was glycerol medium as follows (g/l): glycerol (analytical reagent grade) — 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 minutes at 1 atm and used for accumulation and introducing standardized doses of active bacteria to the fermentation medium. The inoculum was cultured during 24 hours, the accumulation of bacteria was evaluated by feculence of culture. After fermentation, the remaining glycerol and alcohol concentration were determined in inoculum [7].

Microorganisms were cultured on solid substrate in an anaerostat "AE 01" (Russian Federation) under nitrogen atmosphere. The anaerostat was placed in a thermostat at 35±10 °C. The rape biomass was dried at 30±10 °C for 48 hr. The dried biomass was mashed in a laboratory mill "Cyclone MSH 1" (Ukraine). The fractions were measured (20, 60, 100, 150, and 200 mesh) with "Millipore RETSCH sieve shakers" meshes (U.S.A.). Moisture of the raw material was evaluated with a RADWAG MA 50/C/1 (Poland) moisture analyzer. The major components of rape biomass were identified using the following normative protocols: lignin [8], cellulose [9], moisture [10], protein [11], hemicellulose [12]. To produce rape biomass mash, 20.0 g of dry biomass were added to 1 l of water and sterilized at 2 atm for 2 hr. To determine the optimal concentration of substrate, rape biomass mash was prepared in concentrations ranging from 5 to 30 g/l with a pitch of 5 g/l. The inoculum was cultured in 500 ml flasks in 250 ml culture medium. The flasks were stopped with concentrated sulfuric acid plugs, weighted and kept in thermostats at 35±10 °C. After fermentation (72 hr of culturing) cells were precipitated using the ultracentrifuge "Labofuge 400R" (Germany) at 13000 rpm for 10 min. After culturing the fermentation products were extracted from the culture fluid. The presence of ethanol, acetone and butanol in the culture fluid was determined using gas chromatograph with flame ionization detector. The 3 m column was packed with carbowax 1500 on N-A-W-DMCS Chromatone (0.20–0.25 mm), the column temperature was 60±2 0C, the temperature of oven 160±5 0C. The flow ratio of Nitrogen to Hydrogen to air was 1:1:10.

All experiments were performed in triplicate. Statistical data analysis was conducted using Microsoft Excel software. Difference between two average values was considered significant at $P < 0.05$.

Results and Discussion

The major components of rape biomass were studied to find the potential Carbon sources in it (Fig. 1). The major components of rape biomass include: 31% protein, 27% cellulose, and 3% hemicellulose. Other components include 13% lignin, which was not assimilated by microorganisms. The obtained results reveal which share of rape biomass can be assimilated by bacteria of the genus *Clostridium*.

Butanol accumulation was studied by the producer strains IMB B-7570, IFBG C4B and IFBG C6H cultured on a substrate of rape biomass (Fig. 2). After the fermentation by producer strains, three main ABE products were identified in the culture fluid (acetone, butanol and ethanol). It is shown that butanol accumulation was the strongest (2.3 g/l) at rape biomass mash as substrate and *Clostridium* sp. IMB B-7570 as the producer strain. In that case, acetone was present in low amounts (0.5 g/l), similarly to ethanol (0.1 g/l). The IMB B-7570 strain was used in further research due to its superior ability to produce butanol on rape biomass substrate.

The effect of grinding the substrate to various mesh sizes on butanol accumulation was analyzed using rape biomass as substrate and IMB B-7570 strain as butanol producer (Fig. 3). It can be seen in Fig. 3 that butanol accumulated in concentrations up to 0.1 g/l if the rape biomass was ground minimally. Butanol concentration in the culture fluid increased with the level of grinding of the rape biomass. The highest butanol accumulation

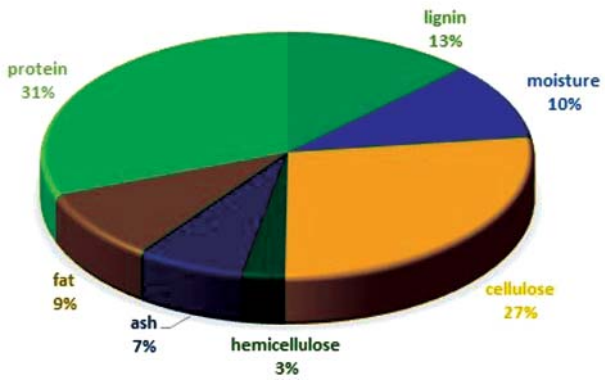

Fig. 1. **Macrocomponent composition of rape biomass**

was observed at substrate ground to 200 mesh (0.076 mm).

The obtained data reveal that the accessibility of raw material increased with substrate milling. That effect can be caused by grinding decreasing crystal zones of cellulose and increasing the amorphic zones which are easily destroyed by enzymes. In bacteria of the genus *Clostridium* the enzymes capable of cleaving cellulose are part of the extracellular multi-enzyme complex, cellulosome [6, 13].

The amount of cellulosomes is proportional to the number of bacteria in the fermentation medium. The number of bacteria in the beginning of fermentation depends on the quality and quantity of the inoculum. That is why, for the standardization of the amount of bacteria introduced into the inoculum, liquid nutrient medium with water-soluble carbon sources is used.

Thus, the effect of the concentration of IMB B-7570 strain inoculum was studied on the accumulation of butanol using rape biomass as a substrate (Fig. 4). As a result, it was shown that the concentration of inoculum introduced into the fermentation medium significantly influences the accumulation of butanol. It was shown that the amount of produced butanol increases proportionally with an increase in the concentration of inoculum material from 5 to 10% of the volume of fermentation mixture.

Increasing the concentration of inoculum to 15–20% caused the accumulation of butanol to drop. Further increasing the inoculum concentration in the fermentation mixture, in general, inhibited the synthesis of butanol in the medium. Inhibition of the butanol synthesis under increasing the inoculum concentration can be due to increased accumulation of primary metabolites of ABE fermentation (butyric, lactic and acetic acids). The optimum concentration of the inoculum added to the fermentation medium was 10%. Then the largest amount of butanol was accumulated, 2.5 g/l. Subsequent studies were conducted at precisely that inoculum concentration.

However, the concentration of inoculum was not the only key factor in the accumulation of butanol. Another important factor that influenced the growth and development of microorganisms and the ABE process was the concentration of available carbon source. The biomass of dried rape without seeds was used as a carbon source.

We studied the effects of various concentrations of rape biomass in a fermentation medium on the accumulation of butanol (Fig. 5). The obtained data indicate that the accumulation of butanol in the fermentation medium increased proportionally to in the concentration of rape biomass (substrate) from 5 to 10 g/l. When the amount of dried milled rape biomass increased from 15.0 to 30.0 g/l, the accumulation of butanol decreased.

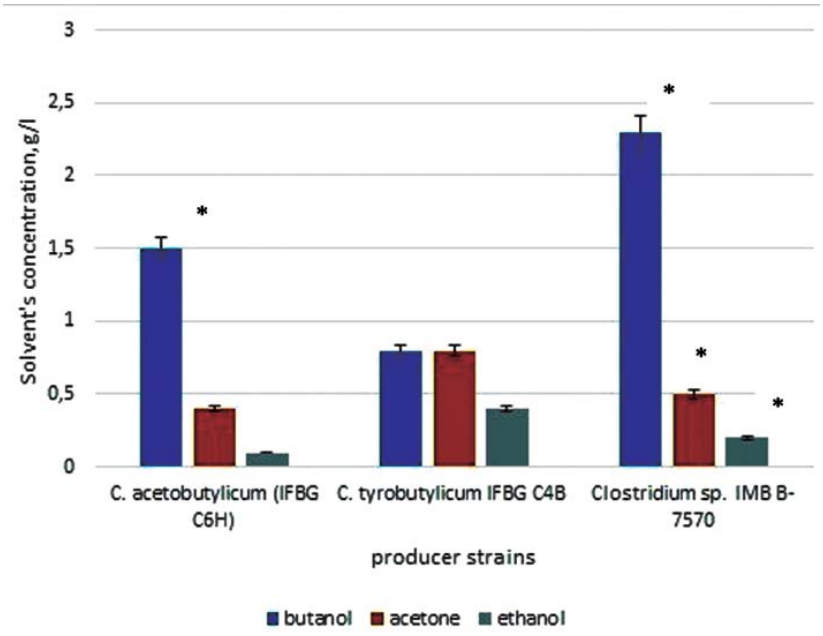

Fig. 2. **Accumulation of solvents by strains producing butanol while using rape as substrate**
**Hereinafter:* $P < 0.05$ compared to control, native medium used as control

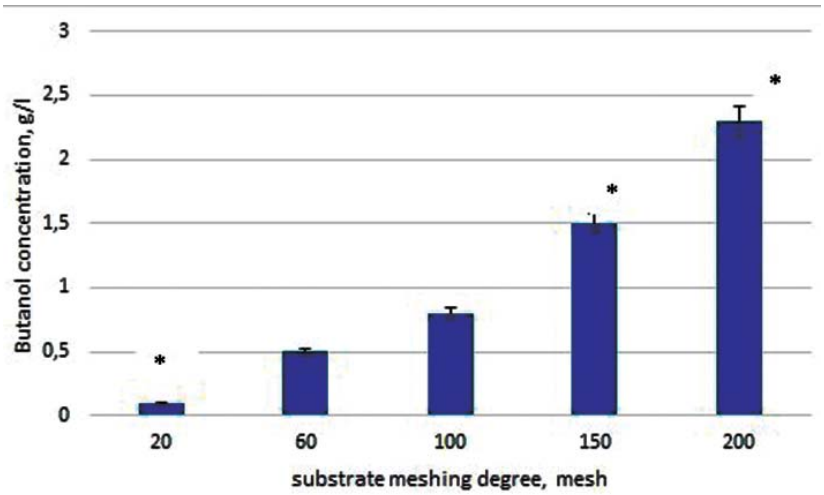

Fig. 3. **The effect of different grinding size on butanol accumulation**

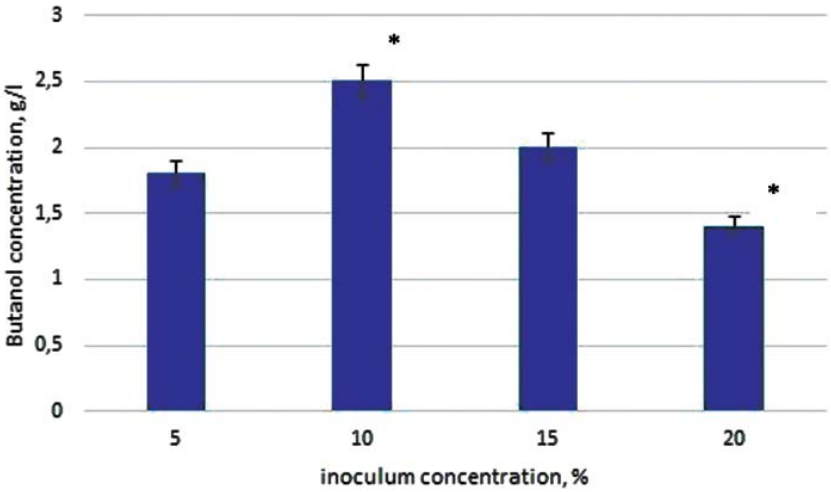

Fig. 4. **The effect of various concentraations of inoculum on butanol accumulation**

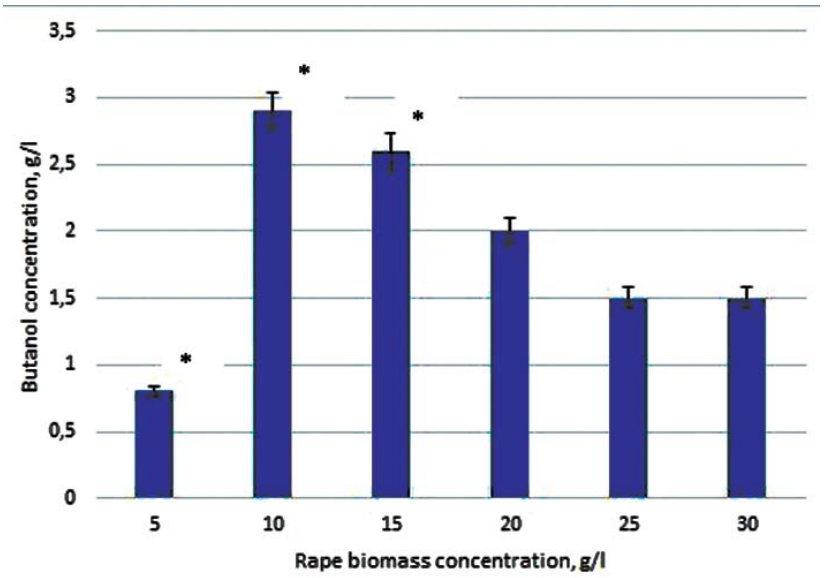

Fig. 5. **The effect of biomass concentration on butanol accumulation**

Our results indicate that increasing the concentration of carbon substrate reduces the bioavailability of the substrate. The largest accumulation of butanol (2.9 g/l) in the fermentation medium was obtained at a concentration of dry milled rape biomass of 10.0 g/l.

Thus according to the obtained results, pre-treated rape biomass was converted by the strains of *Clostridium* sp. The subsequent accumulation of butanol depended on the strain, amount of inoculum, and degree of grinding and concentration of the substrate. The largest amount of butanol (2.9 g/l) was produced by *Clostridium* sp. IMB B-7570 on the substrate of milled 200 mesh (0.076 mm) rape biomass at a concentration of 10.0 g/l.

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БІОМАСА РІПАКУ (*Brassica napus*) ЯК СИРОВИНА ДЛЯ ОТРИМАННЯ БІОБУТАНОЛУ

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Метою роботи було дослідити накопичення бутанолу штамами-продуцентами роду *Clostridium* з використанням подрібненої зеленої біомаси ріпаку як субстрату. Для вивчення накопичення бутанолу штамами-продуцентами наважку біомаси відбирали ваговим методом у діапазоні 5–30 г/л. Клітини осаджували за допомогою ультрацентрифугування, супернатант переганяли та визначали продукти бродіння. Наявність розчинників у культуральній рідині визначали за допомогою газової хроматографії. Найбільше накопичення бутанолу спостерігали за використання штаму *Clostridium* sp. IMB B-7570 та подрібненої біомаси ріпаку (2,3 г/л) як субстрату. Показано, що оптимальна концентрація посівного матеріалу для максимального накопичення бутанолу з використанням біомаси ріпаку становила 10% від об'єму ферментаційної рідини. Виявлено, що найбільше накопичення бутанолу (2,9 г/л) було за оптимізації умов культивування та концентрації 10 г/л сухої біомаси ріпаку у ферментаційному середовищі. Таким чином, проведені дослідження показали, що подрібнена біомаса ріпаку асимілювалась штамами *Clostridium* sp., накопичення бутанолу залежало від штаму, кількості посівного матеріалу, концентрації та ступеня подрібнення субстрату.

Ключові слова: біобутанол, *Clostridium*, рослинна біомаса, ріпак.

БИОМАССА РАПСА (*Brassica napus*) В КАЧЕСТВЕ СЫРЬЯ ДЛЯ ПОЛУЧЕНИЯ БИОБУТАНОЛА

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Целью работы было исследовать накопление бутанола штаммами-продуцентами рода *Clostridium* с использованием измельченной зеленой биомассы рапса в качестве субстрата. Для исследования накопления бутанола штаммами-продуцентами с использованием рапса навеску биомассы отбирали весовым методом в диапазоне 5–30 г/л. Клетки осаждали с помощью ультрацентрифугирования, супернатант перегоняли и определяли продукты брожения. Присутствие растворителей в культуральной жидкости определяли с помощью газовой хроматографии. Наибольшее накопление бутанола наблюдалось с использованием штамма *Clostridium* sp. IMB B-7570 и измельченой биомассы рапса (2,3 г/л) в качестве субстрата. Показано, что оптимальная концентрация посевного материала для максимального накопления бутанола с использованием биомассы рапса составляла 10% от объема ферментационой жидкости. Выявлено, что наибольшое накопление бутанола (2,9 г/л) наблюдалось при оптимизации условий культивирования и концентрации сухой биомассы рапса у ферментационной среде 10 г/л. Таким образом, проведенные исследования показали, что измельченная биомасса рапса ассимилировалась штаммами *Clostridium* sp., при этом накопление бутанола зависело от штамма, количества посевного материала, концентрации и степени измельчения субстрата.

Ключевые слова: биобутанол, *Clostridium*, растительная биомасса, рапс.