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Original article

INTENSIFICATION OF THE BIOHYDROGEN PRODUCTION PROCESS

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Background. In the last decades, humanity has faced the challenge of finding new ways to obtain renewable, environmentally friendly energy carriers. Hydrogen is one of such energy carriers; however, the current methods of its production require fossil fuels and are accompanied by significant CO_2 emissions. Consequently, the energy costs needed to obtain hydrogen by electrolysis exceed the amount of energy produced by burning the hydrogen. Simultaneously, the hydrogen yields for alternative ways, such as fermentation, remain low.

Objective. The aim of the work is the development of approaches to intensify the biohydrogen obtaining process from agricultural waste.

Methods. An increase in hydrogen yield was achieved using specifically grown microorganisms of the *Clostridium* spp. A combination of the waste fermentation process with the production of hydrogen in a microbial fuel cell (MFC), which was fed with the liquid fraction after fermentation, was employed.

Results. The yield of hydrogen depends on the component composition of the raw material. Higher lignin content in the raw material reduces the yield of hydrogen. The addition of *Clostridium* spp. to the natural consortium in the amount of 10% of the total inoculum led to an increase in hydrogen yield. The combination of two processes – fermentation and hydrogen production in a MFC – increased the yield of hydrogen by 1.7 times, along with a higher degree of organic raw materials utilization.

Conclusions. The additional introduction of *Clostridium* spp. to the hydrogen-producing consortium leads to a 7–10% increase in the yield of hydrogen, depending on the composition of the raw material. The yield of hydrogen obtained in the fermentation process for the substrate containing corn silage is $12 \pm 1\%$ higher than for the wheat straw. In general, the combination of the fermentation and hydrogen production in the MFC in a two-stage process leads to an overall increase in the yield of hydrogen by $60 \pm 5\%$.

Keywords: biohydrogen; renewable energy carriers; agricultural waste; microbial fuel cell; microbial consortium.

Introduction

Hydrogen is considered to be a promising energy carrier of the future. The incessant development of hydrogen energy is associated with certain advantages such as:

– the environmental safety of using hydrogen as an energy carrier, since the only product of its combustion is water;

– the possibility of its usage as a single-component energy carrier, and in the form of additives to motor fuels;

– unlimited supplies of raw materials, since, in addition to water, from which hydrogen can be obtained by electrolysis, all types of fossil fuels, various types of biomass, solid and liquid industrial wastes containing organic substances, household waste, etc. can serve as raw materials for hydrogen production [1].

Hydrogen is an ecologically clean energy carrier provided it is obtained in processes that do not have a negative impact on the environment. Today, only 4% of hydrogen, produced in the world, is obtained from water by electrolysis. The rest of the hydrogen is produced from fossil fuels such as gas, oil, coal. Waste of various origins can be an alternative source for hydrogen production. Therefore, researches into the biotechnological hydrogen production from various organic wastes are carried out in different countries [2, 3].

The transformation of organic waste into biohydrogen via the fermentation process occurs due to the metabolic activity of a wide range of microorganisms, each of which is characterized by the ability to consume certain substrates and has specific ways of substrates transformation. During the fermentation of raw materials, hydrogen is formed along with other products. The maximum hydrogen yield (4 moles per mole of glucose) in anaerobic fermentation processes can be achieved with the formation of acetate as a by-product. In the case of other types of fermentation and other by-products, the yield of hydrogen decreases [4].

The main problems that accompany the process of obtaining hydrogen through anaerobic fermentation of organic waste are related to:

– probable changes in the biochemical process of hydrogen production with the transition to methanogenesis;

– consumption of released hydrogen by consumers;

– inhibition of the process by metabolites;

– low hydrogen content in biogas;

– slow progress of the biochemical process of hydrogen synthesis.

The yield of hydrogen, obtained during anaerobic fermentation, depends on the type of raw material, methods of pretreatment of raw materials and inoculum, parameters of the enzymatic process [5]. Thus, the simultaneous usage of cellulose-containing waste and waste containing residues of fats and proteins leads to an increase in the yield of hydrogen [6]. The use of manure increases the output of hydrogen, but also increases the probability of the transition of the hydrogen formation process to methanogenesis [7]. Lay *et al.* [8] show that the concentrations of ammonium, phosphate, and ferrum ions in substrate affect the yield of hydrogen. Their optimal concentrations in the growing medium lead to a 3-fold increase in hydrogen yield. This is due to the fact that ammonium and phosphate ions are nutrients crucial for the growth of microorganisms, and Fe^{2+} is a synergist for the biotransformation of food waste. The addition of wastes that have an alkaline reaction and contain divalent metal ions (Ca^{2+}, Fe^{2+}, Mn^{2+}) also leads to an increase in the speed of the process as well as in hydrogen yield [9].

An increase in the concentration of ferrum ions leads to an increase in the yield of hydrogen, since the ferrum ion is a component of hydrogenases and ferredoxin, which participate in the formation of hydrogen in bacterial cells. An increase in the concentration of magnesium ions leads to an increase in the utilization of the substrate (76%) and hydrogen yield to 3.5 mole H_2 per mole of glucose [10].

The use of a two-stage waste processing system with different temperature conditions in the stages, namely, a thermophilic mode during the process of waste hydrolysis and a mesophilic mode during the process of energy carriers production, leads to an increase in the yield of biogas.

At the same time, the conversion of solids increases in the zone of the first reactor depending on the type of raw material to 6.5–28.0 gVSS/dm^3·day and the production of soluble low molecular weight substances increases by 20.9–34.8% (measured based on COD) [11].

In the two-stage process of obtaining hydrogen, the enzymatic decomposition of high-molecular compounds in the first reactor is carried out at low pH value (5.5 ± 0.5). The yield of hydrogen increases in this process due to the increasing rate of raw materials decomposition, as well as due to the neutralization of methanogenic microorganisms, which are the main consumers of hydrogen. Under such conditions (pH 5.5 ± 0.5, mesophilic temperature mode), acid-forming bacteria, the main of which belong to the genera: *Bacillus*, *Micrococcus*, *Pseudomonas*, *Clostridium*, etc., prevail in the consortium. The solution obtained in the first stage is used as a nutrient substrate for the second stage of fermentation, during which hydrogen-rich biogas is produced [12].

In order to increase hydrogen yield during the processing of cellulose-containing waste, food waste and other organic waste, several sequential fermentation processes can be applied. For example, if anaerobic dark fermentation is used in the first stage, then it is possible to use its effluent for anaerobic photo-fermentation or hydrogen production in a microbial fuel cell (MFC) in the second stage.

The use of an MFC provides simultaneous wastewater treatment or organic waste utilization and production of electricity or hydrogen. Hydrogen can be obtained at the cathode of the MFC by applying an additional voltage to the electrical circuit of the MFC. Theoretically, hydrogen can be obtained in the MFC by applying an additional voltage of 0.12 V. This means that the energy required for hydrogen production in the MFC is nearly 0.26 kW h/m^3 H_2.

Taking into account that the amount of electricity required to obtain hydrogen by electrolysis of aqueous solutions exceeds the energy obtained by burning hydrogen (3 kW·h/m^3H_2 or 33.5 kW·h/kgH_2), anaerobic hydrogen production in the MFC is more profitable and characterized by lower energy consumption [13].

The article presents the results of a two-stage process of obtaining hydrogen from agricultural waste using anaerobic dark fermentation and the MFC.

The aim of the work is development of approaches for intensification of the biohydrogen obtaining process from agricultural waste.

Materials and Methods

Microbial consortium isolated from cattle manure was used for obtaining hydrogen via fermentation. Before fermentation the manure was kept for an hour in a water bath at a temperature of 90 °C for the inactivation of methane-producing bacteria. Since methanogenic bacteria do not form spores, they die under such conditions, and only spore-forming types of microorganisms remain in the consortium. The cultivation of the inoculum was carried out in fermenters with a volume of 1 dm^3, under the anaerobic mesophilic conditions. A dry-air thermostat TS-80M was used to keep the temperature at 35 ± 2 °C. The mix of corn silage with poultry droppings was used as a substrate. Resazurin solution (0.15 g/dm^3) was added in the amount of 1 cm^3/dm^3 of medium to monitor the redox-potential of the growing medium in order to control anaerobic conditions. Microorganisms were identified using light microscopy with an XSP-139TP microscope with a 1000× magnification.

To increase the number of hydrogen-producing bacteria in the inoculum, additional *Clostridium* spp. were added to the microbial consortium isolated from cattle manure.

Primary *Clostridium* spp. isolation and selection was carried out on the liquid medium using cattle manure as a source of inoculum [14]. Omelianskyi's liquid nutrient medium used for *Clostridium* spp. selection containing in gram per cubic decimeter of distilled water: $(NH_4)_3PO_4$ – 1.0; K_2HPO_4 – 1.0; $MgSO_4$ – 0.5; NaCl – 0.1; $CaCO_3$ – 2.0; $FeSO_4$ – 2 drops of 1% solution; peptone – 0.6.

Individual colonies of *Clostridium* spp. were isolated in anaerostat using streak plate method. Petri dishes were filled with sterile nutrient medium containing g/dm^3: K_2HPO_4 – 30; KH_2PO_4 – 2; $MgSO_4$ – 1; NH_4Cl – 1; $CaCO_3$ – 0.1; $FeCl_2$ – 0.4; Agar – 15; Microcrystalline cellulose – 10. After sterilization of the growing medium, 2 drops of the indicator Resazurin 0.15 g/dm^3 solution were added for visual control of the redox potential of the medium.

Wheat straw and corn silage were used as a substrate for fermentation. For the preparation of straw and silage, wheat of the Taira variety and corn of the Lyubava variety, grown in the Korsun-Shevchenkiv district of the Cherkasy region, were used. To provide the consortium of microorganisms with microelements and other nutrients, poultry droppings were added to the substrate in a ratio of 9:1 by dry matter. Wheat straw and corn silage were previously grinded to the size of 3–5 mm.

Hydrogen production was carried out periodically under anaerobic mesophilic conditions in a fermenter with a total volume of 2 dm^3, filled with growing medium to 70% of the volume. The total dry matter weight of added inoculum was 2.1 g. Dry matter content of substrate in the growing medium was 10%. During the fermentation, pH was measured with a pH meter (Sanxin SX811). To reduce the partial pressure of hydrogen in the fermenter, the gas phase was periodically (once a day) pumped out of the fermenter. The composition of biogas was determined using a gas analyzer (K-600 Bosean). After the fermentation the effluent was centrifuged to separate the solid fraction. The liquid fraction of the effluent was used to feed an MFC at the second stage of the process.

The second stage of obtaining hydrogen took place in a two-chamber MFC. The MFC consisted of an anode chamber, which contained a brush anode with a biofilm and the cathode chamber, which contained a graphite electrode on which hydrogen gas was released. Anode and cathode chambers were separated by a proton-selective membrane. The volume of each chamber was 0.5 dm^3. The anode chamber was filled with the liquid fraction of the effluent obtained in the fermentation process and operated in a semi-flow through mode with intermittent substrate renewal. The cathode was immersed in the phosphate buffer solution (pH 7). To create anaerobic conditions for hydrogen evolution on the cathode, the cathode chamber was purged with argon. The cathode chamber was equipped with a gas discharge pipe for hydrogen-gas removal into a gasholder. To obtain hydrogen on the cathode an additional voltage must be applied to the electrodes.

The same heat-pretreated inoculum, which had been isolated from cattle manure, was used to form a biofilm on the brush anode made from graphite fibers. Biofilm formation was carried out in the anode chamber for 3 days at a temperature of 20 ± 2 °C using periodic stirring. To start the process, the inoculum and the liquid effluent from the first stage were added to the anode chamber of the MFC. The additional voltage, applied to MFC electrodes, was 3 V during the whole process of biofilm formation.

The substrate was fed into the anode chamber, where it was degraded by microorganisms of the anode biofilm. In the process of metabolism, microorganisms released electrons and protons and transferred them through the cell membrane. Electrons were accepted by the anode and formed the current in the MFC electrical circuit. Protons formed via this process passed into the cathode chamber through the proton-selective membrane and recombined with electrons on the cathode to form hydrogen. The additional voltage applied to the electrodes in the range of 0.6–1.5 V was used to study the process of obtaining hydrogen in the MFC.

A Q-criterion and a Student's test were used for statistical processing of the results. The Q-criterion was used to exclude gross errors. Student's test was used to calculate the confidence interval. The confidence level was 0.95.

Results

Daily hydrogen-rich biogas yields during the fermentation of wheat straw and corn silage are demonstrated in Fig. 1. According to the results, the lag phase is longer for wheat straw. At the same time, the yield of biogas for wheat straw is less compared to corn silage.

To prevent the transition from hydrogen fermentation to methanogenesis during the fermentation process, a voltage of 3.5 V was periodically applied to the graphite electrodes, which were immersed in the medium.

The hydrogen content in biogas changes during the fermentation process. As it is shown in the Fig. 2, the hydrogen content in biogas increased in the first 4 days of the fermentation process. Such dependence was observed for both corn silage and wheat straw. The lower yield of hydrogen during the start-up period can be explained by the fact that it takes some time to reduce the residual oxygen in the fermenter. For the maximum daily yield of biogas, hydrogen content in biogas obtained from wheat straw was 12 ± 1% less than from corn silage. The total yield of hydrogen for 20 days of fermentation was 4.55 dm^3 for corn silage and 2.95 dm^3 for wheat straw as a substrate. After 16 days of fermentation, the yield of biogas and its hydrogen content began to decrease.

The total yield of hydrogen for the entire period of fermentation using the consortium enriched with *Clostridium* spp. and consortium without additional microorganisms is shown in Fig. 3. As can be seen from Fig. 3, the addition of *Clostridium* spp. to the natural consortium in the amount of 10% of the total amount of inoculum led to an increase in hydrogen yield by 7–10%. A 10% increase in the total amount of inoculum resulted in a slight increase in hydrogen yield, but within the margin of error. There is also an increase in hydrogen yield per unit of biomass. For corn silage, the yield of hydrogen reached 0.3 dm^3/g of TOM (total organic matter) when consortium enriched with *Clostridium* spp. was used.

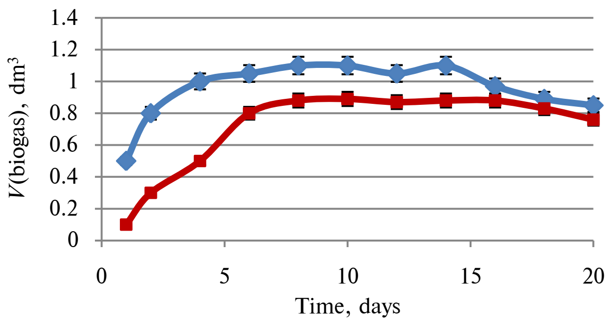

Figure 1: Variation of biogas yield (V(biogas), dm^3) with time during fermentation of the corn silage (blue) and wheat straw (red)

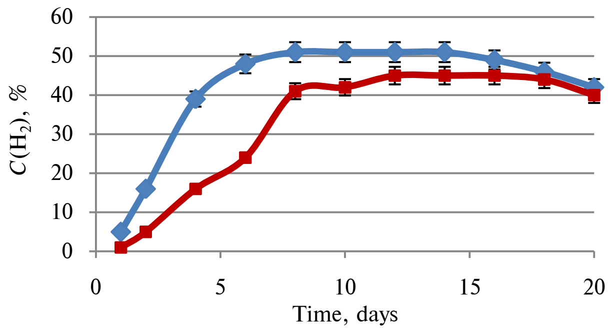

Figure 2: Variation of hydrogen content ($C(H_2)$,%) in biogas during fermentation of the corn silage (blue) and wheat straw (red)

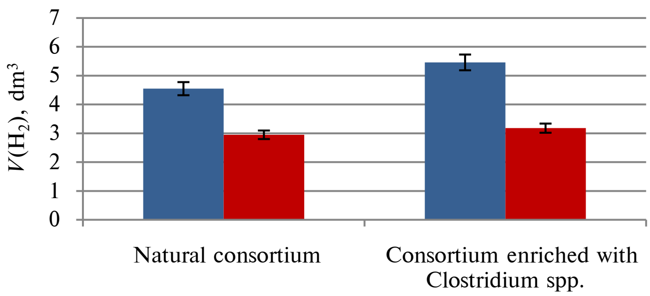

Figure 3: Total hydrogen yield ($V(H_2)$, dm^3) for the fermentation of corn silage (■) and wheat straw (■) using natural consortium and consortium enriched *with Clostridium* spp.

After 20 days of fermentation, the solid and liquid phases of the effluent were separated. The liquid phase of the effluent received after the corn silage fermentation was used in the MFC for the second stage of biohydrogen production.

Hydrogen production in the MFC was carried out at a temperature of 20 ± 2 °C in a periodic mode. Fig. 4 shows the yield of hydrogen in the MFC depending on the additional voltage on the electrodes. Hydrogen yield increased as the applied voltage increased and stabilized at 0.6 V. The value of hydrogen yield varied within the margin of error at an applied voltage of 0.6–1.5 V.

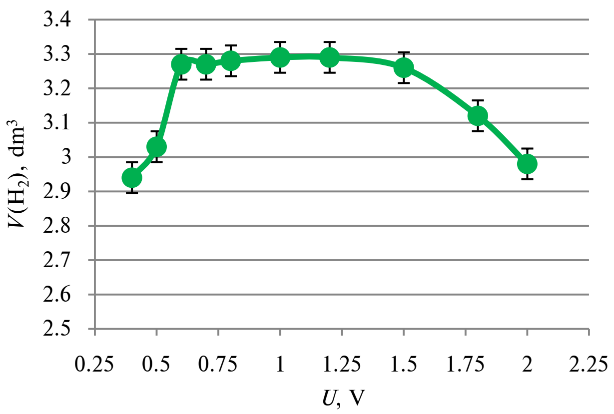

Figure 4: Hydrogen yield ($V(H_2)$, dm^3) with applied voltage (U, V) in the MFC fed with the liquid fraction produced in fermentation process

As it is shown in the diagram (Fig. 5), a two-stage process increased the yield of hydrogen by 1.6–1.7 times compared to the single-stage fermentation process. At the same time, the degree of organic raw materials utilization increased as well as hydrogen yield per unit of its m**ass.**

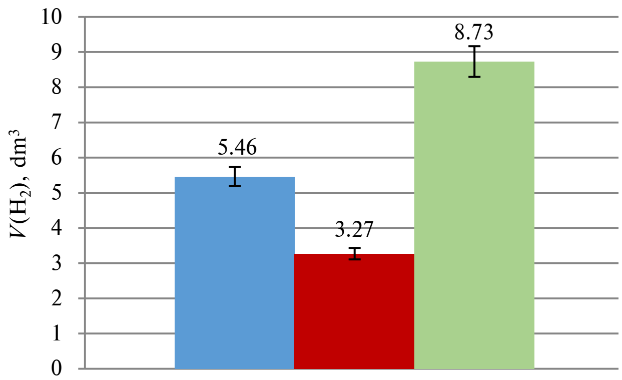

Figure 5: Hydrogen yield ($V(H_2)$, dm^3) for various processes of its production: fermentation (■), hydrogen production in the MFC (■), two-stage process (■)

Discussion

Modern scientific literature describes many pretreatment methods for agricultural raw materials that can be applied to increase the yield of hydrogen and intensify its production by microorganisms [15]. These methods are aimed at destroying the lignocellulosic component of the plant raw material [16]. As a result the access of microorganisms to nutrients is facilitated, and therefore the hydrogen output increases. The influence of micronutrient content [17, 18], medium composition, biomass concentration [19], and inoculum processing methods [20, 21] on hydrogen production is also extensively investigated by various authors. It is known that using of pure cultures of hydrogen producing bacteria as well as bioaugmentation strategies, in which pure cultures of *Clostridium* spp. and co-cultures of other hydrogen producing bacteria are used, leads to an increase in hydrogen yield [22–24].

The method of intensification of the biohydrogen production from agricultural waste, which is proposed in the work, allows us to solve the tasks of increasing both the volumetric flow rate of biohydrogen production and waste utilization degree. It is shown that the additional introduction of *Clostridium* spp. (10%) into the consortium leads to the 7–10% increase in hydrogen yield during the same fermentation period. The increase in hydrogen yield is associated with its higher content in biogas.

To prevent the transition of hydrogen fermentation process to methanogenesis, a decrease in the pH of the medium is often used [25]. But this leads to a decrease in the number of types of microorganisms [26], as a result the hydrogen yield decreases. The method of periodic voltage supply is free from this drawback. Voltage values, which were used as a selective factor in the fermentation process, do not change the pH of the growing medium and do not lead to the release of oxygen, which is an inhibitor of hydrogen production by microorganisms. However, the applied voltage is sufficient to inhibit the vital activity and development of methanogenic microorganisms, which is confirmed by the absence of methane in biogas.

Due to the elimination of microorganisms that consume hydrogen, its content in biogas increases. Taking into account that the content of poultry droppings was the same for the use of both wheat straw and corn silage, it can be assumed that the amount of trace elements, nitrogen and phosphorus available to microorganisms was the same.

Therefore, the yield of hydrogen depended on the content of nutrients in agricultural wastes. The higher content of readily available nutrients in corn silage leads to an increase in the total yield of biogas and an increase in the hydrogen content in biogas up to 12 ± 1% in relation to wheat straw. It is confirmed by the data obtained using mixed [27] and pure cultures [28] of hydrogen producing bacteria. Maximum hydrogen yield obtained through the fermentation of corn silage by the natural consortium with additional *Clostridium* spp. was higher than yield obtained in [27] for the fermentation of cornstalk after acidification pretreatment by mixed culture (0.3 dm^3/g of TOM and 0.15 dm^3/g VS respectively). At the same time, it is reported in [28] that yield of hydrogen obtained through the fermentation of corn silage by pure culture of mesophilic *Clostridium populeti* FZ10 was 0.09 dm^3/g.

The process of biomass fermentation is always accompanied by byproducts formation. Such dissolved additional compounds always remain in the fermentation medium and can be used as a nutrient medium for other types of microorganisms. Sequential application of various metabolic processes that lead to the synthesis of the same product increases the total volumetric yield of this product. The possibility of using various liquid wastes as a substrate for a MFC is well known [29]. In [13] it is shown that the consistent usage of the fermentation process and the microbial fuel cell increases the yield of hydrogen and the degree of wastewater treatment.

The use of the liquid fraction of fermented raw materials that remain after the fermentation process as a substrate for the MFC makes it possible to utilize the residual organic matter and obtain additional pure hydrogen at the cathode of the MFC. Thus, the consistent use of the processes of fermentation and bioelectrochemical hydrogen generation contributes to the intensification agricultural waste utilization as well as to hydrogen production. The combination of the fermentation process and the MFC in a two-stage process leads to a 1.7-fold increase in hydrogen yield compared to simple fermentation without the addition of *Clostridium* spp.

Thus, the use of two-stage process of agricultural waste conversion and the introduction of additional hydrogen-producing microorganisms to the inoculum intensify the microbial mediated hydrogen production process.

At the initial phase of the process, the destruction of the lignocellulosic component of cereal crop waste occurs. Readily available carbohydrates, which are a source of nutrition for the microbial consortium at the initial stage, are also released. Therefore, the curve of dependence of biogas yield on the duration of fermentation has an exponential character with a plateau at a biogas yield value of 630 ± 35 cm^3/dm^3 of medium per day (7.0 cm^3/g of DOM (dry organic matter)) for corn silage and 510 ± 20 cm^3/dm^3 of medium per day (5.7 cm^3/g of DOM) for wheat straw.

The increased yield of biogas in the process of corn silage fermentation compared to wheat straw fermentation is associated with the different lignin content. Corn leaves contain 16–18% lignin and 30% carbohydrates, which can be quickly decomposed by microorganisms. Wheat straw contains 25% lignin and 25 ± 5% decomposable carbohydrates. The content of lignin and carbohydrates also depends on the plants' growing conditions. In addition to its inaccessibility as a source of nutrients, the increased content of lignin slows down the access of microorganisms to carbohydrates, which leads to an increase in the lag phase and a slowdown in the process of hydrogen production. Similar results were obtained by Miftah *et al.* [30].

The decrease in biogas yield after 16 days of cultivation can be explained by the accumulation of metabolic products in the reactors, namely by the high partial pressure of hydrogen, since biogas was pumped out of the reactor once a day.

It is known [31] that an increase in the partial pressure of hydrogen in the reactor environment leads to a change in the metabolism of microorganisms. Thus, a shift from hydrogen biosynthesis to the production of such metabolites as ethanol, alanine, etc. occurs.

The liquid fraction formed in the fermentation process, which contains acids (acetic, butanoic), alcohols and other low-molecular products produced by the microbial consortium during fermentation, was used as a substrate to feed the MFC. To obtain hydrogen in the MFC, the additional voltage was applied to the electrodes during its operation. The highest yield of the hydrogen was obtained at a voltage of 0.6 V.

At lower values of the applied voltage, the yield of hydrogen is lower, because to achieve the evolution of hydrogen at the cathode, it is necessary to reach an overpotential at which the value of the potential of the cathode will be low enough for hydrogen evolution. The quantity of overpotential required by a specific cell depends on electrode material, operational conditions etc. An increase in the applied voltage from 0.6 to 1.5 V does not cause a significant increase in hydrogen yield, and when the voltage is increased above 1.5 V, the hydrogen yield in the MFC gradually decreases. The decrease in hydrogen yield under a high additional voltage can be explained by a change in the metabolism of the anode microbial consortium, which leads to a decrease in the output of protons into the anode space. The aforementioned regularity is confirmed by the data published by Onaran *et al.* [32].

The use of sequential fermentation processes in the fermenter and the MFC allows us to increase hydrogen yield and the degree of raw materials utilization.

The additional introduction of hydrogen-producing microorganisms into the natural consortium for the purpose of hydrogen yield increasing is a new promising direction that requires further research to determine the optimal ratio of inoculum to hydrogen-producing microorganisms and the possibility of additional introduction of several types of microorganisms, etc. To use liquid waste from the fermentation for hydrogen production in microbial fuel cells, it is necessary to optimize the process parameters (retention time, concentration of organic raw materials, pH, influence of activators and inhibitors of the process, structural features of the element). Such steps will make it possible to create a cost-effective industrial technology for obtaining hydrogen.

Conclusions

The yield of hydrogen in the fermentation process is related to the composition of biomass and is 12 ± 1% higher for the substrate containing corn silage compared to the substrate with wheat straw.

Additional introduction of microorganisms of the *Clostridium* spp. into the fermenter during fermentation increases the yield of hydrogen by 7–10%, depending on the composition of the biomass.

The use of a two-stage process for obtaining hydrogen, which combines fermentation and the MFC, makes it possible to increase the yield of hydrogen by 60 ± 5%.

Interests disclosure

Nataliia Golub is the member of the Editorial Council of *Innovative Biosystems and Bioengineering* and was not involved in the editorial evaluation or decision to accept this article for publication. The other authors have no conflicts of interest to declare.

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ІНТЕНСИФІКАЦІЯ ПРОЦЕСУ ОТРИМАННЯ БІОВОДНЮ

Проблематика. Перед людством постала проблема отримання відновлюваних енергоносіїв, які не шкодять довкіллю. Таким енергоносієм є водень. Але способи його отримання на сьогодні потребують викопних видів палива і супроводжуються значними викидами CO_2. Енерговитрати на отримання водню найпоширенішим способом – електролізом – перевищують енергію, яку отримують із водню. В той же час за використання альтернативних методів отримання водню, таких як зброджування, вихід водню невеликий.
Мета. Розробка підходів для інтенсифікації процесу отримання біоводню із сільськогосподарських відходів.
Методика реалізації. З метою підвищення виходу водню зброджування проводили за використання асоціації, в яку додавали спеціально вирощені мікроорганізми виду *Clostridium*. Для підвищення ступеня розкладання сировини використовували поєднання процесу зброджування відходів із одержанням водню в мікробному паливному елементі (МПЕ).
Результати. Вихід водню залежить від компонентного складу сировини. При збільшенні вмісту лігніну знижується вихід водню. Додавання до природної асоціації воденьпродукуючих мікроорганізмів роду *Clostridium* у кількості 10 % від загальної кількості інокулюму підвищувало вихід водню. Поєднання двох процесів зброджування та МПЕ підвищувало вихід водню в 1,7 разу. Водночас збільшувався і ступінь розкладання органічних речовин субстрату.
Висновки. Додаткове введення до асоціації мікроорганізмів виду *Clostridium* підвищує вихід водню на 7–10 % залежно від складу сировини. Вихід водню, отриманого в процесі ферментації субстрату, що містить силос кукурудзи, на 12 ± 1 % вищий, ніж для субстрату, що містить солому пшениці. Поєднання ферментації та процесу отримання водню в МПЕ в двостадійний процес приводить до сумарного підвищення виходу водню на 60 ± 5 %.

Ключові слова: біоводень; відновлювані енергоносії; сільськогосподарські відходи; мікробний паливний елемент; асоціація мікроорганізмів.