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TWO-STAGE DEGRADATION OF SOLID ORGANIC WASTE AND LIQUID FILTRATE

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The accumulation of solid and liquid organic waste requires their treatment to develop energy biotechnologies and prevent environment pollution.

Aim. The goal of the work was to study the efficiency of the purification of the filtrate from dissolved organic compounds by aerobic oxidation and methane fermentation.

Methods. The standard methods were used to determine pH and redox potential (Eh), the gas composition, the content of short-chain fatty acids, the concentration of dissolved organic compounds counting to the total carbon. The efficiency of two types of microbial metabolism for the degradation of soluble organic compounds of filtrate was compared.

Results. The aerobic oxidation was established to provide 1.9 times more efficient removal of dissolved organic compounds, compared with the anaerobic methane fermentation. However, it provided CH_4 yield 1 L/dm^3 of filtrate (carbon concentration — 1071 mg/L). The necessity to optimize the methods for purifying filtrate to increase the efficiency of the process was determined.

Conclusions. The obtained results will be the basis to develop complex biotechnology providing not only the production of environmentally friendly energy H_2 via the fermentation of solid food waste, but also the purification of filtrate to solve the ecological and energy (CH_4 production) problem of society.

Key words: solid organic waste; soluble organic compounds; environmental biotechnologies; hydrogen; methane; fermentation; aerobic oxidation.

The development of food industries, intensification of agriculture and increasing yields are an indisputable positive aspect of the development of society and the improvement of life quality. However, the intensification of production and the processing of thousands of tons of organic products daily causes an increase in the amount of waste. The problem of degradation of both solid and liquid organic waste is urgent for most countries. Thus, up to 100 million tons is annually accumulated in European countries, and up to 600 million tons is reached in Asian countries [1–5]. They include a wide range of components: food waste, vegetables, fruits, meat, dairy, household, bakery products, agricultural waste (feed residues, crop waste, etc.), restaurant and culinary food residues, etc. [6, 7]. Irrational processing of raw materials and improper use of finished products causes the accumulation of surpluses that pose a threat to the environment and require proper disposal [6, 8, 9].

The accumulation of waste in landfills leads to uncontrolled decay, release of toxic gases and concentrated liquid filtrate polluting the environment [5, 10, 11]. Landfill filtrate is a concentrate of toxic products of microbial metabolism (fatty acids and alcohols), which inhibits plant growth, reduces soil fertility and is toxic to animals and humans [7, 8]. Liquid waste from food and canneries, livestock waste, alcohol bard, etc. are another examples

of dangerous for the environment waste. The volume of such waste is also constantly growing. For example, more than $5.5 \cdot 10^7$ m^3 of wastewater is generated annually in South Korea [12, 13].

Organic waste can be an alternative constantly renewable source for valuable products: molecular hydrogen, methane and purified water. Raw materials for hydrogen production can be food and culinary waste, vegetable residues, agricultural and industrial organic waste, rich in nutrients suitable for the growth of hydrogen-synthesizing bacteria [14]. High efficiency of hydrogen production is achieved by optimization of the process of fermentation of solid organic waste by a community of hydrogen-synthesizing microorganisms. H_2 yield depends on pH, temperature, fermentation time, mass transfer, concentration of organic compounds and microbiome used [3].

To increase the efficiency and profitability of the process, the filtrate formed as a result of fermentation of solid organic waste requires additional purification from dissolved organic compounds. Proper treatment of such waste is a serious environmental problem [6]. Existing physical and chemical methods of filtration, sorption, concentration, etc. are expensive, complex and do not provide effective purification [15, 16].

The liquid filtrate contains easily degradable organic compounds (fatty acids and alcohols), which is a promising substrate for oxidation by microorganisms of a wide range of physiological and taxonomic groups [5, 17].

Accelerated oxidation by aerobic microorganisms to obtain CO_2 and H_2O is the most promising among the biological methods of degradation of dissolved organic compounds [18–20]. Methane fermentation is an alternative to it. Fermentation provides not only the degradation of organic compounds, but also the synthesis of additional energy (CH_4) [18, 21].

To develop an optimal approach to solve the problem of purification of the filtrate, it is necessary to evaluate the efficiency of the aerobic and anaerobic methods, as well as to optimize the process to achieve maximum efficiency.

Therefore, the goal of the work was to study the efficiency of the purification of the filtrate from dissolved organic compounds by aerobic oxidation and methane fermentation.

Materials and Methods

For the purification of the filtrate formed after the fermentation of multicomponent food waste, modular installation with a volume of 40 L (linear dimensions 0.3×0.45×0.3 m) was used (Fig. 1).

The installation is made of plexiglass providing continuous visual inspection of the process dynamics. The installation is divided by two perforated plates (*1*) into three sections. On the one hand, they allowed separating the stages of purification, and, on the other hand, they did not interfere with mass transfer in the installation. Fittings for the sampling of the culture fluid (*2*) and the gas phase (*3*) are installed in the walls of the installation.

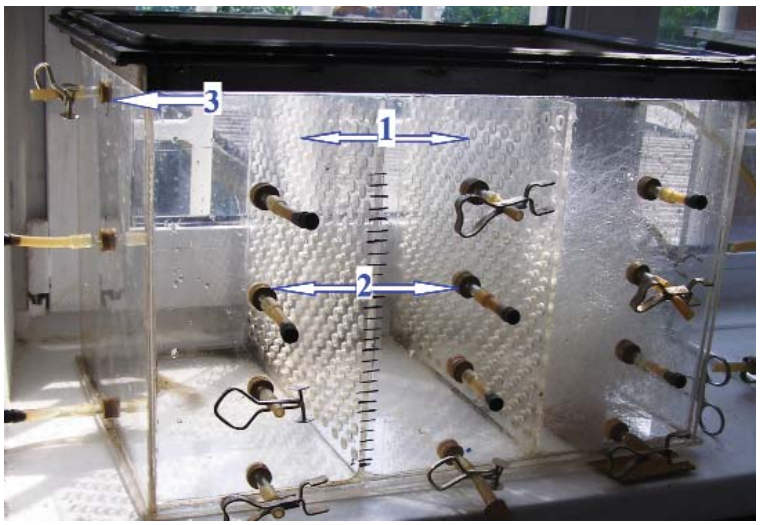

Fig. 1. **The modular plexiglass installation for purification of filtrate:**
1 — perforated plates for division of installation into sections; *2* — fittings for sampling of filtrate; *3* — fittings for sampling of the gas phase

To establish the efficiency of the filtrate purification, two pathways of degradation of dissolved organic compounds were investigated. In the first case, aerobic oxidation was performed. For this purpose, 10 L of the filtrate were added to the installation. The aerators were installed at the bottom of the installation to bubble air over the entire volume of the liquid. The lid was sealed, and air removal was carried out through a water seal. Hoses from the fittings were immersed into the water seal to prevent the spread of bacterial suspension.

In the second case, the anaerobic fermentation of the filtrate by a methanogenic microbial community of the fermented sludge (FS) of methane tank was used. For this, 30 L of the filtrate were added to the installation and 0.5 L of FS was added to the bottom. The installation was sealed and connected to the gasholder.

The dynamics of the process was monitored by changes in pH and redox potential (Eh), the content of dissolved organic compounds in the culture fluid counted on the concentration of total Carbon. The main criterion for completion of the process was the reduction of Carbon concentration, which indicated the purification of the filtrate from dissolved organic compounds. In addition, changes in the composition of the gas phase (CH_4 and CO_2) and the intensity of gas synthesis were evaluated in the anaerobic installation.

The pH and Eh were evaluated by the universal ionometer EZODO MP-103 with remote electrodes (models PY41 and PO50, respectively).

The volume of gas synthesized during the purification of filtrate from soluble organic compounds in the anaerobic installation was determined by the volume of water displaced under the gas pressure from the gasholder to the water seal. The gasholder was refilled with water after each measurement to avoid errors in calculating the composition of the gas phase.

The composition of the gas was analyzed according to the standard method on the gas chromatograph LHM-8-MD [22]. The sterile plastic syringes were used to sample the appropriate volume of gas. The gas composition was calculated by the square of the peaks of its components.

The chromatograph is equipped with two steel columns. The first one (I) in necessary for the analysis of H_2, O_2, N_2 and CH_4. The second column (II) is required for the analysis of CO_2. Column parameters are as follows. First column: l = 3 m, d = 3 mm, with molecular sieve 13X (NaX). Second column: l = 2 m, d = 3 mm, with Porapak-Q carrier. The column temperature is +60 °C. The evaporator temperature is +75 °C. The detector temperature (catharometer) is +60 °C. The detector current is 50 mA. Argon is used as a carrier gas. The gas flow rate is 30 cm^3/min.

The concentration of dissolved organic compounds counting to the total Carbon was determined using the permanganate method [23]. For this, a 5 mL sample of culture fluid was centrifuged in an OPn-8 centrifuge at 3 000 g for 15 min. The supernatant (1 mL) and 0.1 mL of 10% sulfuric acid (H_2SO_4) were added into a chemically pure test tube. The mixture was heated on a boiling water bath. Then 0.05 mL of 0.1% solution of $KMnO_4$ was added. The solution was titrated until a light purple color specific for the permanganate ion appeared. The concentration of Carbon in the sample correlates with the amount of oxidant $KMnO_4$ required for complete oxidation of dissolved organic compounds in the sample. The total content of dissolved organic compounds (mg/L) was calculated after determining the volume of the solution $KMnO_4$ spent for the titration of the test solution, according to the formula:

$$[C] = 0{,}11 \times V_{KMnO_4}\,(\mu L) - 12.168.$$

The content of short-chain fatty acids in the filtrate was determined by gas chromatomass spectrometry on the device Agilent 6890N/5973inert (Agilent Technologies, USA), capillary column DP-FFAP (30 m×0.25 mm×0.25 μm), (J&W Scientific, USA). The separation was performed with a temperature gradient of 10 °C/min from 60 °C to 230 °C. The flow rate through the column is 1 mL/min. The identification of the compounds of individual substances was performed using libraries of mass spectra NIST02 and standard solutions of short-chain fatty acids.

For the analysis, 5 mL of filtrate was sampled. Aliquots of samples were added to 1.5 mL Eppendorf microtubes and frozen at −20 °C. The batch of samples was given for analysis to Kharhota M. A. to the Laboratory of Biological Polymer Compounds of the Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine.

The efficiency of the filtrate purification was evaluated by the duration of the process, the efficiency of degradation of soluble organic compounds, as well as the efficiency of methane synthesis by anaerobic purification.

The experiments were carried out in triplicate. Statistical analysis was conducted via

Excel and Origin 8.5.1 software determining the standard deviation of the data.

Results and Discussion

The study of the efficiency of molecular hydrogen obtaining by the fermentation of organic compounds has been attracting the attention of scientists and representatives of the industrial sector for several decades. The prospects and benefits of this area are in the opportunity to combine energy and environmental biotechnology increasing the efficiency and the profitability of the process [6, 24, 25]. To achieve the maximum yield of H_2, it is necessary to optimize the key fermentation parameters such as pH and Eh of the culture fluid, conditions of mass transfer, the ratio of solid and liquid phases, etc. Thus, the series of experiments resulted in the shortening of the fermentation duration (*T*) to 2–5 days. The yield of molecular hydrogen (VH_2) reached 50–100 L/kg of waste in terms of total solids (TS). The efficiency of waste destruction (*Kd*), i.e. the multiplicity of reduction of their weight, reached 85–95 [26–28].

The results of dark hydrogen fermentation of solid organic waste were promising. However, obtained fermentation filtrate required further purification. The filtrate formed after fermentation contained the following fatty acids: hexanoic (caproic), 3-methylbutanoic (isovaleric), butanoic (butyric), propane (propionic) and acetic. The concentration of dissolved organic compounds reached 1 071 mg/L. In high concentrations, they are toxic to the microbiome of soils, water reservoirs, and lead to the disruption of ecosystems [27, 29–32].

Therefore, the application of an effective method for the degradation of dissolved organic compounds is necessary to develop environmentally friendly biotechnology for hydrogen synthesis and waste degradation. In addition, the purified filtrate is suitable for the reuse for solid food waste fermentation, avoiding the need for new portions of water and increasing the profitability of the process.

To study the dynamics of the aerobic oxidation of organic compounds, 10 L of the filtrate after fermentation of solid food waste were loaded into the plexiglass installation. The initial parameters of the filtrate were as follows: Eh = +80 mV; pH = 6.32; carbon concentration [C] = 1 071 mg/L. To provide the continuous aeration, bubblers were installed at the bottom of the installation. Under such conditions, the filtrate microbiome oxidized organic compounds, providing purification of the culture fluid.

The dynamics of oxidation of organic compounds without regulation was performed. As a result, the concentration of total Carbon 19-fold decreased from 1071 to 56 mg/L (Fig. 2).

The following patterns were established

There was a decrease in pH values from 6.32 to 5.74 at the initial stages of oxidation (during the first 4 days). It could take place due to the hydrolysis of unfermented small solid particles of waste that may have been untreated. As

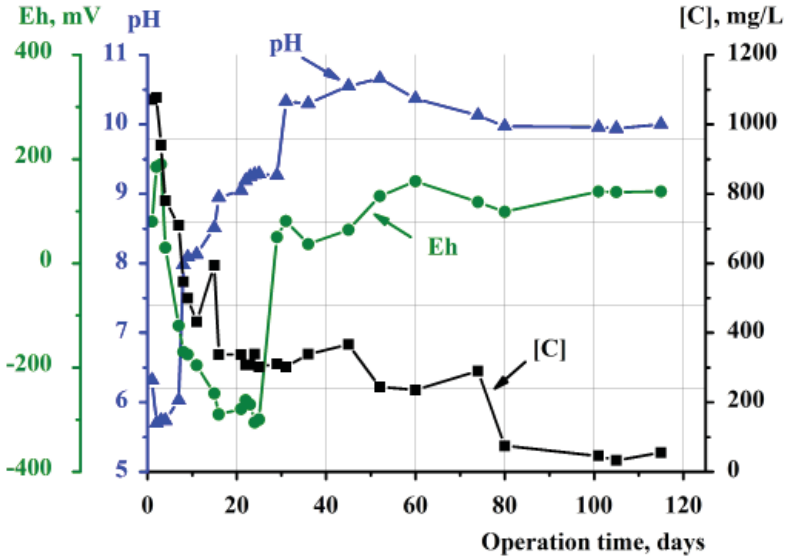

Fig. 2. **The dynamics of metabolic parameters during aerobic oxidation of dissolved organic compounds of filtrate**

a result, the accumulation of hydrolysis products (fatty acids and alcohols) appeared at the initial stages. Further, the pH values were shown to increase from 5.74 to 10.66 over the next 48 days of cultivation. This may indicate the stage of degradation of low molecular weight soluble organic compounds, such as organic acids, alcohols, amino acids, etc. The increase in pH may indicate complete oxidation of the compounds and accumulation of ammonium ions that alkalized the medium. Subsequently, the pH stabilized at about 10.0, which could indicate a decrease in the rate of degradation and completion of the process.

The dynamics of the Eh values also testified to the active growth of microorganisms. Thus, during the first 3 days, the microbiome adapted, as evidenced by the increase in Eh from +80 to +190 mV. Over the next 22 days, the Eh values decreased to −300 mV. Despite intense aeration, the redox potential reached negative values, indicating the growth of microorganisms and oxidation of the substrate. Further, growth and stabilization of Eh about +130 mV correlating with pH stabilization indicated the completion of the process.

The decrease in the concentration of dissolved organic compounds is the main criterion for the efficiency of the process. The concentration of carbon was decreased within 16 days from the beginning of cultivation the most intensively (3-fold from 1071 to 337 mg/L). Subsequently, from 16 to 80 days, the process slowed down, and the concentration of carbon decreased from 337 to 75 mg/L (4.5-fold decrease). Over the next 35 days, the concentration of soluble organic compounds decreased 1.3-fold (from 75 to 56 mg/L).

Thus, as the result of the conducted research aerobic oxidation was shown to be promissing for the purification of the filtrate after fermentation of solid food waste from the dissolved organic compounds. The study of the dynamics of the process distinguished 3 stages. During the first one, the microbiome adaptation and rapid degradation of organic compounds took place. It was evidenced by rapid changes in pH and Eh, as well as a rapid decrease in Carbon concentration. The second stage was characterized by a decrease in the efficiency of degradation of organic compounds and stabilization of pH and Eh. In the third stage, the oxidation of residual concentrations of organic compounds took place and the process was completed. The total duration of the process without optimization was 115 days.

The dynamics of the degradation of dissolved organic compounds of the filtrate due to methanogenic fermentation was investigated. For this purpose, 30 L of filtrate and 0.5 L of FS as inoculum were added to the installation.

As a result, methane fermentation of dissolved organic compounds was shown to 10-fold reduce the concentration of Carbon from 1071 to 105 mg/L (Fig. 3).

The decrease in pH during the first day of cultivation from 6.34 to 5.65 indicated the hydrolysis of unfermented detritus. During

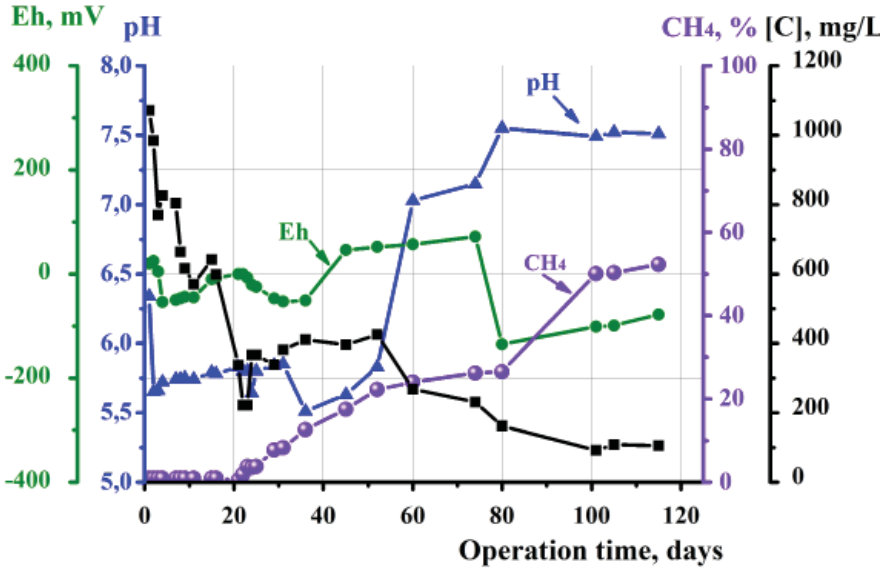

Fig. 3. **The dynamics of metabolic parameters during methane fermentation of dissolved organic compounds of filtrate**

the next 50 days of fermentation, the pH values remained in the range of 5.6–5.7. Subsequently, for 30 days, the pH increased to 7.5 and did not change until the end of the process. Such changes in the pH showed that the degradation of organic compounds and possible salinization of the medium due to the accumulation of alkaline NH_4^+ was achieved during methane fermentation.

The redox potential values remained in the range of −50...+70 mV for 74 days of fermentation. Subsequently, Eh decreased to -135 mV and remained within negative values. Since the regulation of microbial metabolism and optimization of cultivation conditions did not occur, this may indicate the adaptation of microorganisms to the created conditions.

The efficiency of complete degradation of dissolved organic compounds to gaseous products was evidenced by the release of methane after 21 days of cultivation. The most intensive accumulation of methane was observed after 80 days of cultivation. The concentration of CH_4 increased from 26 to 52% in the gas phase.

The most intensive degradation of organic compounds occurred within 23 days from the beginning of cultivation. Thus, the Carbon concentration 4.8-fold decreased from 1 071 to 221 mg/L. Over the next 92 days, the Carbon concentration was halved to 105 mg/L. As a result, the concentration of organic compounds during methane fermentation 10-fold decreased.

Thus, anaerobic degradation by methane fermentation was shown to be useful for effective purification of the filtrate. Two stages of the process were identified. During the first one, an intensive decrease in the Carbon concentration was observed. During the second stage, there was a gradual fermentation of organic compounds and methane synthesis. The total duration of the process was 115 days, with a CH_4 yield of 1 L/dm^3 of filtrate.

Therefore, methane fermentation of organic compounds can be effective for waste degradation. However, the speed of the process is low. It can cause an imbalance in the accumulation of waste and the rate of their treatment. In addition, further industrial implementation requires optimization of fermentation conditions, as methanogenic microorganisms are sensitive to cultivation conditions and competitive microbial communities [33, 34].

Hydrogen fermentation of multicomponent organic waste is a promising area for hydrogen production with simultaneous degradation of waste [24]. It is confirmed by the literature data. Thus, for the fermentation of a wide range of substrates, the yield of hydrogen varies: swine manure with the addition of glucose produces up to 26–30 L/kg of substrate [35], molasses — 585 L/L, dairy waste — 31.5 L/kg of solids [36], corn starch — 150 L/kg, sugars (hexose) — 311 L/kg [37, 38], multicomponent solid food waste — 50–100 L/kg [26–28]. Naturally, carbohydrate-rich foods and wastes (potatoes, cereals, etc.) are fermented faster and more efficiently than meat containing more protein and fat [6]. In addition, the use of hexoses increases the yield of hydrogen, but significantly increases the cost of biotechnology. Therefore, the optimization of the fermentation process of common cheap substrates (food waste) requiring the disposal is promising [24, 28].

The approach for treatment of solid waste is actively being developed and optimized. However, no information was found about the methods for treatment filtrate accumulated after fermentation. The paper compares the efficiency of two types of microbial metabolism for the degradation of soluble organic compounds of filtrate: aerobic oxidation and methane fermentation.

Based on theoretical calculations, aerobic oxidation of the substrate is more promising. Thus, the energy output reaches −2 877.0 kJ/M providing greater speed and efficiency of the process [18–20]:

$$C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O,$$
$$\Delta G_o = -2\,877{,}0 \text{ kJ/M}.$$

Methanogenesis provides lower energy yield, so the degradation of organic compounds under such conditions can occur for a long time reducing the efficiency of biotechnology [18, 21]:

$$HCO_3^- + H^+ + 4H_2 = CH_4 + 3H_2O,$$
$$\Delta G_o = -135{,}6 \text{ kJ/M}.$$

Although aerobic oxidation of organic compounds is the most energy-efficient, its industrial application is limited due to the imbalance in the ratio of electron donor (organic compounds) and acceptor (molecular oxygen). During the oxidation of organic compounds, the rate of oxygen consumption by microorganisms in the liquid phase significantly exceeds the rate of its diffusion into solution. Therefore, to provide an efficient process, it is necessary to use compressors to inject air into a bioreactor. It causes the need to use additional equipment and increase the cost of biotechnology [18].

On the other hand, the use of obligate anaerobic methanogenic microorganisms does not require the application of additional electron acceptors and process equipment [39, 40]. Low molecular weight fatty acids and alcohols are the main products of the fermentation of solid food waste. They are the precursors of monocarbon compounds that serve as substrates for methanogenesis (formate, methanol, methylamine, etc.) [21, 41]:

$$4CH_3OH + 2H_2O \rightarrow 3CH_4 + CO_2 + 4H_2O;$$
$$4HCOO^- + 4H^+ \rightarrow CH_4 + 3CO_2 + 2H_2O;$$
$$4NH_2CH_3 + 2H_2O \rightarrow NH_3 + 3CH_4 + CO_2.$$

This method is common today for the degradation of polymeric organic waste, both agricultural (manure, plant residues) and industrial (excess biomass of activated sludge of aeration tank) [39, 40]. However, it is a complex process that requires control and regulation, as methanogenic microorganisms are sensitive to toxic metals (Cu^{2+}, Co^{2+}, Hg^{2+}, etc.) and competitive processes, including sulfate reduction [42, 33].

Thus, aerobic degradation of dissolved organic compounds can provide a high rate of oxidation of the substrate, but requires additional technological equipment. Methane fermentation is a longer process that can provide the degradation of organic compounds without the need of additional electron acceptors. But its application requires careful control and regulation of the process to maintain the efficient functioning of the methanogenic microbial community.

Two methods were used to purify the filtrate. According to thermodynamic calculations both of them are promising. These suggestions were confirmed experimentally. The efficiency of purification of the filtrate by aerobic method was shown to be higher than anaerobic for the same period of time (115 days). Thus, during aerobic oxidation the concentration of dissolved organic compounds 19-fold decreased from 1 071 to 56 mg/L. Methane fermentation provided 10-fold decrease of total Carbon concentration from 1 071 to 105 mg/L. However, it provided CH_4 yield 1 L/dm^3 of filtrate.

As a result of the conducted research, the fundamental opportunity to apply both aerobic and anaerobic methods to purify the filtrate was shown. The next step is to increase the efficiency of the process by the regulation of microbial metabolism and optimization of the parameters (pH, Eh, mixing, composition of microbial communitiess, etc.).

The heterogeneity of waste composition as a substrate hinders the development of stable and predictable biotechnologies for now [1, 6, 7]. The most efficient are carbohydrate-rich wastes of fruits, vegetables, bakery products, cereals, etc. [6]. However, despite the heterogeneity of the composition, food waste remains promising for industrial use, as it is a cheap, constantly renewable substrate.

Conclusions

The aerobic oxidation was established to provide 1.9 times more efficient removal of dissolved organic compounds of filtrate, compared with the anaerobic methane fermentation. The necessity to optimize the methods to purify filtrate to increase the efficiency of the process was determined. The obtained results will be the basis to develop complex biotechnology providing not only the production of environmentally friendly energy H_2 via the fermentation of solid food waste, but also the purification of filtrate to solve the ecological and energy (CH_4 production) problem of society.

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ДВОСТУПЕНЕВА ДЕГРАДАЦІЯ ТВЕРДИХ ОРГАНІЧНИХ ВІДХОДІВ ТА РІДКОГО ФІЛЬТРАТУ

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Накопичення твердих та рідких органічних відходів потребує їх перероблення для розвитку енергетичних біотехнологій та запобігання забрудненню довкілля.

Метою роботи було вивчити ефективність очищення фільтрату від розчинених органічних сполук за допомогою аеробного окиснення та метанової ферментації.

Методи. Для визначення pH та окисно-відновного потенціалу (Eh), складу газу, вмісту коротколанцюгових жирних кислот, концентрації розчинених органічних сполук за загальним карбоном використовували стандартні методи.

Результати. Порівнювали ефективність двох типів мікробного метаболізму для деградації розчинних органічних сполук фільтрату. Встановлено, що аеробне окиснення забезпечило в 1,9 раза більш ефективне видалення розчинених органічних сполук порівняно з анаеробною метановою ферментацією, однак вона створила умови для виходу CH_4 1 л/дм^3 фільтрату (концентрація за карбоном — 1 071 мг/л). Визначено необхідність оптимізації методів очищення фільтрату для підвищення ефективності процесу.

Висновки. Отримані результати становитимуть основу для розроблення комплексної біотехнології, що забезпечить не тільки виробництво екологічно чистого енергоносія H_2 шляхом зброджування твердих харчових відходів, але й очищення фільтрату для вирішення екологічної та енергетичної (продукування CH_4) проблеми суспільства.

Ключові слова: тверді органічні відходи; розчинні органічні сполуки; екологічні біотехнології; водень; метан; ферментація; аеробне окиснення.

ДВУХСТУПЕНЧАТАЯ ДЕГРАДАЦИЯ ТВЕРДЫХ ОРГАНИЧЕСКИХ ОТХОДОВ И ЖИДКОГО ФИЛЬТРАТА

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Накопление твердых и жидких органических отходов требует их переработки для развития энергетических биотехнологий и предотвращения загрязнения окружающей среды.

Цель. Изучение эффективности очистки фильтрата от растворенных органических соединений с помощью аэробного окисления и метановой ферментации.

Методы. Для определения pH и окислительно-восстановительного потенциала (Eh), состава газа, содержания короткоцепочечных жирных кислот, концентрации растворенных органических соединений по общему карбону были использованы стандартные методы.

Результаты. Проведено сравнение эффективности двух типов микробного метаболизма для деградации растворимых органических соединений фильтрата. Установлено, что аэробное окисление обеспечило в 1,9 раза более эффективное удаление растворенных органических соединений по сравнению с анаэробной метановой ферментацией, однако она сделала возможным выход CH_4 1 л/дм^3 фильтрата (концентрация по карбону — 1 071 мг/л). Определена необходимость оптимизации методов очистки фильтрата для повышения эффективности процесса.

Выводы. Полученные результаты будут основой для разработки комплексной биотехнологии, обеспечивающей не только производство экологически чистого энергоносителя H_2 путем сбраживания твердых пищевых отходов, но также очистку фильтрата для решения экологической и энергетической (продуцирование CH_4) проблемы общества.

Ключевые слова: твердые органические отходы; растворимые органические соединения; экологические биотехнологии; водород; метан; ферментация; аэробное окисление.