III. ENVIRONMENTAL BIOTECHNOLOGY

GASEOUS FUEL OBTAINING VIA FERMENTATION OF ORGANIC LANDFILL WASTE

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Abstract. Fossil carbon-containing fuel is currently one of the most common in industry and economy. The rapid depletion of reserves of this fuel makes it necessary to search for the alternative one. Landfills are a place where methane is spontaneously synthesized due to the decay of organic waste. Controlled and regulated fermentation of the landfill organics can provide biomethane as well as environmental bioremediation. The aim of the work was to study the patterns of methane fermentation of multi component organic waste and optimize the process to increase the efficiency of biomethane synthesis and waste decomposition. Colorimetric and potentiometric methods were used for pH and Eh measurement. Volumetric and chromatographic methods were applied to control volume and composition of synthesized gas. Fermentation parameters were calculated with the use of mathematical and statistical ones. The achievement of high efficiency of methane fermentation of organic waste due to the process regulation was shown. The modeling of unregulated fermentation of organic waste in landfills showed low efficiency of the process. It took 69 days. Weight of waste decreased only 5 times. Hydrogen yield was 5 L/kg of waste. Methane was not synthesized. The regular mass transfer, regulation of the process and waste grinding showed the greatest efficiency. Weight of waste decreased 20 times during only 14 days. Hydrogen yield was 27 L/kg, methane yield was 12 L/kg of waste. Thus, the absence of regulation caused long term decay of waste. The high efficiency is achieved due to regulation of the fermentation process. The results will serve as a basis for the development of industrial biotechnology for the utilization of organic waste to reduce the volume of existing landfills and produce methane energy. This will further allow bioremediation of contaminated areas, obtaining an alternative to fossil fuel biomethane.

Keywords: methane, hydrogen, multi component organic waste, fermentation, landfills, environmental biotechnologies.

INTRODUCTION

Carbon-containing fossil fuel (natural gas, coal, etc.) is currently one of the most common in industry and economy. Rapid population growth and development of manufacturing lead to the depletion of reserves of these resources. An alternative to this is the use of biomethane obtained by methane fermentation of organic compounds [1]. Such methane can be used to generate heat and electricity [2].

Along with this, the problem that needs to be solved at the global level is the accumulation of huge amounts of organic waste (food, agricultural, etc.). Every year the weight of such waste is more than 1 billion tons [3]. As a result, huge amounts of organic waste accumulate in landfills [3, 4]. Landfills are the place of spontaneous and uncontrolled decay. As a result, toxic filtrates and gases are released, which are explosive and flammable. Due to the ability of landfills to burn [5], there is a risk of fires due to improper operation, which leads to poisoning of the environment with dioxins, furans and others [6]. As a result of organics decay on landfills, methane is also synthesized [7], which can be used in industry or transformed into electricity [8].

In contrast to the long-term process of composting or environmentally hazardous incineration, methane

fermentation of organic waste [1] is a promising way to solve environmental and energy problems [9]. Currently, both small and large-capacity installations for the fermentation of waste to produce methane are actively used. The use of small installations is widespread in both Asia and Europe [4]. However, small installations are unprofitable to generate electricity and can meet the needs of small farms. This aim requires the recycling of much larger volumes of waste using large installations. Large installations require complex technological equipment and high operating costs. Currently, the rate of waste accumulation significantly exceeds the rate of their disposal in such installations [4]. That is why an integrated biotechnological approach is needed to solve economic and environmental problems during the operation of such installations, which will include the development of the optimal design of facilities and the fermentation modes.

Considering the negative impact of the landfill on the environment, as well as the potential energy value of organic compounds from landfills, the task of collecting and utilizing biogas in landfills is of considerable interest to both science and industry. This will allow to achieve a gradual bioremediation of contaminated sites, as well as to obtain methane - valuable energy carrier [1].

Methane synthesis by fermentation of organic waste requires coordinated functioning of hydrolytic and methanogenic microorganisms. The greatest efficiency is achieved by fermentation using diversified microbial communities, which are able to ferment a wide range of organic compounds contained in multicomponent organic waste [10]. That is why, the composition of the microbial community is one of the most important values for high process efficiency.

The development of biotechnologies for the controlled microbial fermentation of organic waste from landfills will increase methane yield, waste destruction efficiency, as well as reduce the volume of landfills and improve the environmental situation.

Thus, the aim of the work was to study the patterns of methane fermentation of multi component organic waste and optimize the process to increase the efficiency of biomethane synthesis and waste decomposition.

MATERIALS AND METHODS

Installations to model waste fermentation process in landfills

The designed installations were used to study the patterns of waste destruction in landfills (Fig. 1). There were vertical columns made of organic glass providing visual control during the experiments. The linear dimensions of the columns were $0.2 \times 0.2 \times 0.9$ m. The volume of each column was 36 L.

Fig. 1. Installation to model the process of fermentation of organic waste in landfills: a: 1 – working chamber; 2 – a lid with the built-in fittings for sampling of a gas phase and the manometer for pressure control; 3 – fittings for sampling the culture fluid and the addition of regulators; b: 1 - working chamber; 2 - gasholder.

Organic and inorganic multi component waste was loaded into the working chamber of the installation (1). The lid of the column (2) was hermetically closed to accumulate the synthesized gas and transfer it to the connected gasholder. The gas accumulated in the water-filled gasholder pushed the water into the water seal. Fittings for the gas phase sampling were installed in the lid. In addition, the built-in manometer provided control of possible increase of pressure to prevent the emergency situations. Samples of culture fluid were collected through the fittings mounted in the vertical wall of the column (3).

The composition of the waste loaded into the fermentation columns simulated landfill waste.

Thus, waste with a total weight of 10 kg was loaded into the column. Among them 7 kg contained organic waste: potatoes, carrots, cucumbers, beets, bananas, onions, bakery products, meat waste in equal weight ratios. The remaining 3 kg were inorganic waste: glass, plastic bags, bricks, textiles [2]. As the part of the model mixture, about 70% of waste was organic waste, and 30% contained non-fermentable waste.

To accelerate the process of waste fermentation, the use of an additional inoculum was investigated. For this purpose, a modification of the granular microbial preparation (GMP) applied. It was based on the prepared and optimized microbial community of

the fermented sludge of methane tank. In addition, the GMP contained a mixture of easily digestible substrates to initiate the growth of microorganisms, as well as regulators of microbial metabolism to maintain optimal cultivation conditions.

Modes of fermentation of organic waste in columns

A solid waste mixture simulating landfill waste was loaded into the fermentation column. Thus, 10 kg of waste was placed in layers in a column, alternating organic and inorganic, and poured 11 liters of water. The column was sealed and connected to the gasholder, where the synthesized gas was accumulated (Fig. 1). Cultivation was performed for 3.5 months at 30°C. Depending on the experimental variant, GMP (0.5 kg) was added to the columns.

The dynamics of organic waste destruction was studied in three variants. In the first variant, waste fermentation in the absence of regulation was studied providing the patterns of process in the landfill without optimization and mass transfer. In the second variant, the effect of periodic regulation of microbial metabolism was investigated, maintaining the pH value = 6.0-7.0, and applying of the GMP to increase methane yield. In the third variant, the use of GMP was supplemented by grinding of organic waste to increase the surface of waste, active regulation of microbial metabolism, as well as mass transfer of the components of the fermentation mixture.

Regulation of fermentation was performed by periodic addition of the regulator of microbial metabolism 1 (RMM-1) consisting of a complex of water-soluble compounds.

To maintain optimal fermentation and mass transfer conditions, the culture fluid was periodically drained from the column and RMM-1 was added to neutralize microbial exometabolites. During the loading of the neutralized culture fluid to the column, the exometabolites were washed away from the waste surface, which ensured mass transfer.

To provide high efficiency of fermentation the following conditions were maintained: pH = 6.0-7.0; Eh = -350... -380 mV; grinded waste with a rib length 0.5-1 cm; periodic mass transfer the need for which was determined by the pH decrease.

To assess the dynamics of fermentation, samples of culture fluid and gas phase were periodically taken. The completion of the process was evidenced by the stabilization of the pH and redox potential (Eh) of the culture medium, the destruction of solid

waste (reduction of their volume), termination of gas synthesis and reduction of methane concentration.

Control and regulation of fermentation parameters in columns

The study of the fermentation dynamics of the model mixture of organic waste was performed by the change of the following parameters: pH, Eh of the culture fluid, volume and composition of the synthesized gas, concentration of soluble organic compounds in terms of total carbon concentration.

The values of pH and Eh were determined by potentiometric method measuring them with the ionomer EZODO MP-103. The combined electrodes with BNC connectors (models PY41 and PO50) were used to control pH and Eh, respectively.

The volume of gas synthesized was determined by measuring the volume of water expelled from the gasholder to the water seal under gas pressure. Each time the gas volume and composition were measured, the gasholder was refilled with water to avoid errors in calculating the synthesis gas concentration.

The composition of the gas phase was determined on a gas chromatography [11]. The chromatograph is equipped with two steel columns – one (I) for the analysis of H₂, O₂, N₂ and CH₄, the second (II) – for the analysis of CO₂. Column parameters: I – l = 3 m, d = 3 mm, with molecular sieve 13X (NaX); II – l = 2 m, d = 3 mm, with Porapak-Q carrier; column temperature +60°C, evaporator temperature +75°C, detector temperature (catharometer) +60°C, detector current – 50 mA. Carrier gas – argon, gas flow rate – 30 cm³/min. The gas concentration was calculated from the peak square of the gas phase components.

The total concentration of dissolved organic compounds in terms of carbon concentration was determined using the permanganate method [12]. A 5 mL sample of culture fluid was centrifuged in an OPn-8 centrifuge at 3000 g for 15 minutes. The supernatant (1 mL) and 0.1 mL of 10% sulfuric acid (H₂SO₄) were added to a chemically pure test tube. The mixture was heated on a boiling water bath, adding aliquots (0.05 mL) of standard 0.1% solution of KMnO₄. The solution was titrated until a light purple color characteristic of the permanganate ion appeared. The concentration of carbon in the sample correlated with the amount of oxidant KMnO₄ required for complete oxidation of dissolved organic compounds in the sample. Carbon concentration was determined by the calibration curve.

Calculation of fermentation parameters to evaluate the efficiency of model organic waste decomposition in columns

The main indicators allowed assessing the efficiency of fermentation of organic waste in the columns were the coefficient of solid waste destruction (Kd), energy yield of hydrogen and methane (VH₂ and VCH₄) and their maximum concentration, as well as the duration of the process (T).

The degree of waste fermentation was evaluated by determining the coefficient of waste destruction (Kd). It was calculated as the ratio of the initial and final weight of solid waste (in terms of absolute dry weight). To calculate the Kd, the coefficient of conversion of wet weight to dry (Km) was determined for the initial mixture of waste and the remained unfermented residues.

The coefficient Km was calculated by the formula:

$$Km = m_1 : m_2,$$

where m₁ is the weight of the original wet waste, and m₂ is the weight of dry waste.

Before fermentation, a portion of the waste mixture was weighed, dried at 105°C to constant weight, cooled to room temperature, and then weighed again.

After fermentation, the unfermented solid waste particles were washed in distilled water, dried to constant weight at 105°C and weighed.

The coefficient of waste destruction (Kd) was determined by the formula:

$$Kd = m_2 : m_3,$$

where m₂ is the initial weight of dry waste; m₃ is the weight of dry unfermented residues.

Energy yield (VH₂, VCH₄, L/kg of waste) was defined as the number of liters of synthesized hydrogen and/or methane from 1 kg of organic waste in terms of their absolute dry weight.

The duration of the fermentation cycle (T, days) was calculated as the time from loading of waste to installation until the moment of their maximum destruction and termination of the fermentation process [13].

Determination of the studied fermentation parameters was performed in triplicate. Statistical analysis of the data was performed using the software package Excel and Origin 8.5.1. They were used to plot and determine a standard error of no more than 5% (not illustrated on the graphs).

RESULTS AND DISCUSSION

Existing methods of processing both organic and inorganic waste do not provide their fast and complete destruction. Most of them are buried in landfills causing the increase in landfill volumes, uncontrolled decay of organic compounds and environment pollution [14-16]. At the same time, organic waste can be used as a substrate for valuable products, including biomethane [2, 3] and biohydrogen [17, 18]. Special attention is paid to technologies to obtain biomethane using methanogenic microbial communities. They are promising to improve the efficiency of methane obtaining from landfills and to accelerate the destruction of waste.

We consider the destruction of organic compounds as related binary redox reactions. In these reactions, organic compounds are electron donors, and electron acceptors can be oxygen, nitrate, sulfate, H⁺, CO₂, etc. In these reactions, microorganisms serve as biocatalysts that provide the rapid flow of the process [16, 19-21]. The application of the method of thermodynamic prediction allowed evaluating the efficiency of methanogenic fermentation of waste by microorganisms. Thus, methanogenesis can occur in the following ways:

The yield of free energy, which determines the efficiency of the process, in these reactions varies and can reach ΔG_o = -135.6 kJ/M [22-25]. This indicates the prospects for the application of methanogenic microbial communities for the destruction of organic waste and increase the yield of biomethane in landfills [26].

Inhibition of waste fermentation in the absence of process regulation

To establish the patterns of organic waste fermentation by indigenous microbiome of landfill without optimization and regulation of microbial metabolism is one of the key stages of the study its destruction. This will allow determining the stages requiring further regulation and monitoring to improve the efficiency of waste decomposition and the yield of biomethane.

The first variant of the experiment simulated the process of spontaneous fermentation of waste in landfills. Under such conditions, organic waste was mixed with plastic, glass, paper, etc. They were tightly

pressed and impregnated with water. At the same time, mass transfer was limited and could occur only due to the infiltration of water (rainwater, etc.) from the landfill surface to the lower layers. Such conditions of spontaneous waste fermentation due to the indigenous waste microbiome were modeled in the first fermentation column. In this case, waste and water was loaded. Waste was not subjected to pre-treatment (grinding, heat treatment, etc.). The ratio of waste and water weight was about 1:1.

Under such conditions, there was a significant inhibition of microbial metabolism activity and the

process of waste fermentation in general (Fig. 2). The pH values decreased from 5.8 to 4.8 within 3 days from the beginning of fermentation. In the future, the pH remained in the range of 5.0-5.3 throughout the cycle. This is due to the high initial concentration of organic compounds in the installation, the hydrolysis of which caused the rapid accumulation of microbial exometabolites and acidification. In the absence of mass transfer and regulation of microbial metabolism, the pH remained almost unchanged.

Fig. 2. Inhibition of waste fermentation by indigenous microbiome in the absence of regulation of microbial metabolism

The lack of regulation also had a negative effect on the decrease of the redox potential of the culture fluid. Thus, at the beginning of fermentation $Eh = -23$ mV. However, within 6 days its values increased to +97 mV and subsequently (within 48 days) fluctuated within +50...+110 mV. This indicates the creation of non-optimal conditions for the growth of anaerobic microorganisms that produce energy (biohydrogen, biomethane). Decrease in the Eh values to -11...+40 mV after 50 days of cultivation may indicate the completion of the process and lysis of microbial biomass evidenced by the simultaneous increase in the concentration of dissolved organic compounds and a decrease in the concentration of hydrogen.

Therefore, low pH values = 5.0-5.3 and high $Eh = +50...+100$ mV showed that the created conditions were extremely unfavorable for the growth both of hydrogen-producing and methanogenic microorganisms.

Hydrolysis of waste polymers with the formation of soluble organic compounds was observed. This was evidenced by the increase in their concentration, calculated in terms of the total carbon content in the culture fluid. Thus, in the initial stages of fermentation, the concentration of soluble organic compounds was about 100 mg/L. Its gradual growth to values of 800-1040 mg/L over 50 days indicated partial hydrolysis of polymers by primary anaerobes. However, the absence or imbalance of the process prevented the further destruction of organic compounds by hydrogen-synthesizing and methanogenic microorganisms with H_2 and CH_4 synthesis.

As a result, no methane synthesis was observed during the entire cultivation period due to the lack of conditions necessary for methanogenic metabolism. However, the synthesis of hydrogen was observed. Its maximum concentration reached 39% in the gas phase after 53 days of fermentation. Hydrogen synthesis began 1 day after the start of cultivation. After 3 days,

its concentration reached 22%. However, the lack of regulation of microbial metabolism and suboptimal pH and Eh values led to a gradual inhibition of hydrogen synthesis over the next 18 days. Probably, the adaptation of the microbiome to the fermentation conditions subsequently led to the resumption of H_2 synthesis, although its concentration fluctuated significantly (within 10-35%). The final inhibition of H_2 synthesis was observed after 55 days.

Thus, the fermentation of organic waste by an indigenous microbiome without optimization and regulation of microbial metabolism was shown to cause the strong suppression of the process of waste destruction and energy carrier synthesis. Thus, there was inhibition of microorganism growth and fermentation process in general during the cultivation period ($T = 69$ days). It was manifested in the stabilization of the pH, the absence of visual changes in the destruction of solid waste, decrease in the concentration of H_2 and absence of CH_4 synthesis. Under such conditions, the efficiency of waste destruction was low. The coefficient of destruction, ie the ratio of initial and final weight of waste, was very small, $K_d = 5$. The yield of molecular hydrogen was only $V_{H_2} = 5$ L/kg of waste in terms of absolute dry weight.

Therefore, in order to achieve high efficiency of organic waste destruction and methane yield, it is necessary to optimize the process and regulate microbial metabolism. The synthesis of hydrogen under such suboptimal (in terms of redox potential) conditions can be explained by the ability of Clostridia to locally autoregulate (reduce) the redox potential on the surface of solid waste in biofilms. Only the creation of optimal conditions for the growth of microorganisms will accelerate the fermentation and increase the yield of valuable products.

Regulation of waste fermentation as an effective tool to increase the efficiency of the process

Absence of optimization and regulation of fermentation of organic waste caused complete inhibition of the process. In this case, self-preservation of organic waste occurs leading to their accumulation in landfills and environment pollution. Optimal conditions for the growth of microorganisms are required to neutralize the accumulated toxic microbial metabolites, accelerate the destruction of waste and obtain industrially promising volumes of methane.

Therefore, in the second variant of the experiment, the pH of the culture medium was periodically optimized, and the granular microbial preparation (0.5 kg) containing methanogenic microbial community was added as the inoculum. The model mixture of ungrinded waste was loaded into the fermentation column and filled with water in a weight ratio of about 1:1. The pH optimization was performed irregularly, only with strong acidification of the culture medium. As a result, increase in the efficiency of waste destruction and methane production was shown (Fig. 3).

The pH values naturally decreased from 5.8 to 4.3 during 2 days of cultivation due to the hydrolysis of waste polymers and the accumulation of soluble acidic microbial exometabolites in the culture fluid. To reduce their negative impact, the pH was adjusted to 5.0. As a result, such values remained unchanged for 19 days. A further gradual decrease in pH to 5.5 over the next 27 days indicated a continuation of the hydrolysis of solid organic waste, which required optimization of the pH to values of 6.0-7.0. As a result of pH optimization, the growth of microorganisms was not inhibited due to the accumulation of exometabolites, which contributed to the hydrolysis of waste polymers.

Fig. 3. Increase in the efficiency of methane fermentation of waste by regulation of microbial metabolism (arrows show the addition of regulators)

The development of the microbiome was also evidenced by the change in the values of the redox potential of the culture medium. Thus, Eh decreased from +54 to -30 mV during the first 2 days. Further increase of Eh to +100...+110 mV within 20 days could be caused by inhibition of microbial growth due to accumulation of products of waste hydrolysis. Further, a gradual decrease in the redox potential to -60...-75 mV in liquid phase indicated the growth of anaerobic microorganisms accompanied by the synthesis of hydrogen and methane. However, the uneven decrease in the values of Eh may indicate instability of the process and unbalanced growth of microbial community. Therefore, there is a need to ensure more accurate regulation and optimization of cultural indicators in future.

The destruction of solid organic waste was evidenced not only by the visual reduction of the volume of waste in the column, but also by the change in the concentration of dissolved organic compounds. Thus, it increased from 244 mg/L to 1060 mg/L in terms of carbon within 4 days. Over the next 20 days, the concentration of dissolved organic compounds ranged from 620 to 1390 mg/L. It evidenced the simultaneous hydrolysis of solid organic waste and the involvement of soluble organic compounds in the synthesis of H_2 . Further, there was a decrease in the total carbon content in the culture fluid to the concentration of 250 mg/L, due to the involvement of low molecular weight soluble organic compounds in the process of methane synthesis.

As a result of the application of additional inoculum (GMP), optimization and regulation of microbial metabolism, a natural substitution of hydrogen synthesis by the accumulation of methane in the gas phase was observed. H_2 synthesis began on the second day of cultivation (22% H_2 in the gas phase). However, due to the accumulation of microbial exometabolites, an intensive increase in its concentration was recorded only after 21 days from 4 to 28%. After 37 days of cultivation, a decrease in hydrogen concentration from 20% to trace values was observed. This can be explained by a shift in microbial metabolism and the start of intensive growth of methanogenic microorganisms that use hydrogen and CO_2 for methane synthesis.

The beginning of CH_4 synthesis was recorded after 32 days of cultivation (1.4%). Intensification of methane synthesis (27% in the gas phase) was observed 50 days after the start of fermentation. This coincided with the optimization of pH values and the decrease of the redox potential to negative values.

The maximum concentration of CH_4 was reached after 78 days (53%).

Thus, the regulation of microbial metabolism was shown to significantly increase in the efficiency of methane fermentation process. Thus, the coefficient of waste destruction (K_d) increased 2.2 times from 5 to 11. The yield of hydrogen (V_{H_2}) increased 4 times from 5 to 20 L/kg of waste. The yield of methane (V_{CH_4}) reached 16 L/kg of waste. The total duration of fermentation (T) was 103 days.

Thus, modeling methane fermentation of solid organic waste in landfills, the optimization of this process was shown to be a necessary and promising direction to accelerate the destruction of waste and obtain industrially promising volumes of CH_4 .

The increase in the efficiency of methane fermentation of solid organic waste via the optimization their size, mass transfer and regulation of microbial metabolism

Uncontrolled decay of waste causes the stagnation of the process and is unacceptable for cost-effective industrial application. Periodic regulation of microbial metabolism provides 2-4 times increase in the efficiency of waste destruction, hydrogen yield and methane production.

The next steps are the need to optimize the key fermentation parameters to increase the efficiency of the process. Such parameters are waste particle size, regular maintenance of optimal pH values, mass transfer to ensure uniform distribution of fermentation components, regulators, etc.

Therefore, a study of the efficiency of methane synthesis during the destruction of grinded waste particles, mass transfer and regulation of microbial metabolism was conducted (Fig. 4).

Waste particle size is one of the key parameters that determine the efficiency of solid organic waste fermentation. Hydrolysis of waste polymers occurs at the surface of contact of microorganisms with solid particles. If the waste particle is large, it possesses the small surface area. As a result, waste fermentation is inefficient. On the other hand, excessive grinding of waste also negatively affects the efficiency of the process. It takes place due to the fact that too small particles stick together and prevent the flow of liquid, impairing the mass transfer and access of microorganisms to the surface of the waste. Under such conditions, microbial exometabolites cannot be effectively washed away by water, which causes the appearance of local zones of acidification and inhibition of microbial growth. Therefore, the optimal grinding of waste was found to be 0.5-1 cm of a particle size. In this case, a large

surface area of the waste is created for contact with microorganisms. At the same time, water can circulate freely between the particles, ensuring the leaching of

microbial exometabolites from the waste surface and their neutralization, as well as the effective functioning of regulators of microbial metabolism.

Fig. 4. Optimization of methane fermentation of solid organic waste due to their grinding, mass transfer and regulation of microbial metabolism (arrows show the addition of regulators)

The composition of the microbial community is one of the most important values for high process efficiency. The synthesis of methane by fermentation of organic waste requires the coordinated functioning of hydrolytic and methanogenic microorganisms and occurs in several stages, which include:

- 1) hydrolysis of polymers to monomeric compounds (proteins, polysaccharides, lipids);
- 2) fermentation of monomers (monosaccharides, amino acids, fatty acids) to short-chain fatty acids (acetic, propionic, butyric, valeric, etc.), alcohols and gases H_2 and CO_2 [27];
- 3) the actual methanogenesis [1].

Hydrolytic bacteria involved in the destruction of biopolymers include both aerobes and anaerobes. Representatives of the genus *Bacillus* (*B. subtilis*, *B. mesentericus*, *B. megatherium*, *B. licheniformis*, etc.), which have amylolytic and pectolytic activity, are especially common among hydrolytic bacteria [28]. *Bacillus* spp. utilize oxygen and reduce the redox potential of the medium to -100... -150 mV, providing anaerobic conditions for the anaerobic microorganisms growth. Primary anaerobes, such as members of the *Clostridium* genus [29], ferment monomers to form H_2 and CO_2 [30]. The fermentation products of monomers are involved

in the metabolism of methanogens that synthesize CH_4 [27]. Considering the complex multi-stage process of methanogenesis, there is a need to regulate microbial metabolism in the fermentation of organic waste to ensure optimal functioning of main stages of methanogenic process.

The intensive regulation at the initial stages was shown to provide high efficiency of waste fermentation (Fig. 4). Naturally, the grinding of waste caused their more intensive hydrolysis accompanied by the accumulation of microbial exometabolites and rapid decrease in pH from 6.4 to 5.65 for 1 day. Therefore, control and regulation were necessary during 7 days of fermentation. Further, the intensity of hydrolysis decreased, the pH stabilized in the range of 5.7-6.0. Thus, the regulation of microbial metabolism was not necessary after 22 days of cultivation.

The values of the redox potential of the culture fluid decreased from +197 mV to -20 mV during 8 days. The lowest values (-140 mV) were reached 15 days after the start of fermentation. Subsequently, Eh stabilized -40...+40 mV.

The rapid increase in the concentration of dissolved organic compounds from 745 mg/L to 2300 mg/L during 6 days was the confirmation of the high intensity of hydrolysis of solid waste polymers in the initial stages of fermentation. After

that, the decrease in their concentration indicated the involvement of low molecular organic compounds in the metabolism of hydrogen-synthesizing and subsequently methanogenic microorganisms. Thus, the concentration of dissolved organic compounds was further in the range of 60-260 mg/L.

As a result of the use of grinded waste, regular maintenance of the optimum pH and mass transfer, conditions were created for rapid hydrolysis of solid waste. According to visual assessment, the amount of waste decreased 10 times during 14 days of fermentation (Fig. 5).

Fig. 5. High efficiency of destruction of solid organic waste within 14 days due to their grinding, mass transfer and regulation of microbial metabolism: a - the volume of waste before fermentation; b - the volume of waste 14 days after the start of fermentation

Intensive hydrogen synthesis began on the second day of cultivation (34% in the gas phase). The maximum concentration of 39% was reached 7 days after the start of fermentation.

Subsequently, the synthesis of hydrogen was substituted by methane synthesis. Thus, its synthesis began 17 days after the start of fermentation (0.6%). Relatively intensive synthesis (28%) was observed after 36 days, and the maximum (62%) was reached after 54 days.

Thus, as a result of optimization of key parameters (particle size of waste, mass transfer, regulation of microbial metabolism) and application of GMP as inoculum containing methane-synthesizing microorganisms, high efficiency of fermentation was achieved. Thus, the total cycle was 54 days. At the same time, the time of waste destruction T was only 14 days, which is 4.9 times faster compared to the spontaneous fermentation without regulation (69 days). The coefficient of destruction $K_d = 20$, ie the efficiency of destruction increased 4 times compared to the unregulated process. The yield of hydrogen V_{H_2} reached 27 L/kg of waste, and methane $V_{CH_4} = 12$ L/kg of waste.

Therefore, regulation of microbial metabolism and the process optimization are the key to increase the efficiency of methane fermentation of organic waste.

Methane fermentation is promising to provide waste destruction, as well as to obtain a valuable energy source methane. Unbalanced and unregulated fermentation of solid organic waste in landfills causes strong stagnation of the process. As a result, the rate of waste accumulation exceeds the rate of their utilization. This leads to an increase in the amount of landfills, which threatens an environmental disaster [31, 32]. Therefore, it is urgent to develop cost-effective technologies that will provide not only rapid decomposition of waste to eliminate landfills, but also to obtain a valuable energy source methane [3, 27].

Optimization of waste fermentation is necessary to accelerate waste destruction and increase methane yield. The obtained results showed that the grinding of waste, regulation of microbial metabolism and mass transfer significantly increased the efficiency of fermentation of waste (Table 1).

Table 1. Comparison of the efficiency of organic waste fermentation

№	Fermentation conditions	<i>T</i> , day	<i>Kd</i>	<i>VH₂</i> , L/kg of waste	<i>VCH₄</i> , L/kg of waste
1	Without regulation	69	5	5	0
2	With the addition of GMP and periodic regulation of microbial metabolism	103	11	20	16
3	With the addition of GMP, periodic regulation of microbial metabolism, waste grinding and mass transfer	14 – destruction of solid waste; 54 – destruction of hydrolysis products	20	27	12

Thus, inhibition of microorganism growth was observed without regulation of microbial metabolism due to acidification ($\text{pH} = 5.0\text{-}5.3$). In the absence of grinding, the efficiency of solid waste hydrolysis, which occurs mainly on their surface, was low. The absence of mass transfer did not allow to provide effective neutralization of microbial exometabolites and their washing away from the waste surface. Under such conditions, the waste fermentation process was inhibited, as a result of which the efficiency of destruction was low, and only slow hydrogen synthesis was observed. Similar processes occur in the body of landfills. At high concentrations of organic compounds, the products of their hydrolysis accumulate rapidly during their uncontrolled fermentation. Absence of mass transfer also strongly reduces the efficiency of destruction. As a result, landfills remain unchanged for decades. In addition, due to the high rate of waste accumulation, which exceeds the rate of their destruction, landfills are only growing. Under such conditions, methane synthesis takes place in extremely unoptimal conditions. As a result, its yield and process profitability are low [33, 34].

Process optimization and regulation of microbial metabolism allowed 2-4 times increasing the efficiency of solid organic waste destruction, 4-5.4 times increasing of hydrogen yield and achieving methane yield (12-16 L/kg of waste). Grinding of waste provided a larger surface area for hydrolysis by microorganisms. Regulation of microbial metabolism and mass transfer provided neutralization and leaching of toxic exometabolites. As a result, high efficiency of methanogenesis during the destruction of organic waste was achieved.

Organic waste is a promising substrate for CH_4 synthesis [35]. Carbohydrates, proteins and lipids that are part of them can be fermented to fatty acids, acetate and hydrogen. They serve as substrate for methanogenesis.

Literature confirms that methane contained in biogas synthesized from a wide range of organic wastes is a promising strategy for their processing and energy production [36, 37]. Such organics include waste from fruit, vegetable and oil processing enterprises, waste from the meat and dairy industries, brewing wastes and livestock wastes, etc. They have different contents of carbohydrates, proteins and lipids [38], but their concentration is promising for methane production.

High methane yields from tomato or grape cake have been demonstrated as vegetable and fruit processing waste, adding dry bovine manure and green waste compost as co-substrates, reaching a solids content of 28%. Fermentation of tomato cake made it possible to obtain CH_4 201.61 L/kg of waste [39]. Fermentation of sweet sorghum allowed obtaining CH_4 107 L/kg [40]. Alcohol bard waste yielded CH_4 554 L/kg of organic compounds. Low-carbohydrate meat processing waste can also be used to produce CH_4 . However, to increase the yield of methane, to pre-treat or ferment them with additional substrates was proposed. Thus, whey fermented with cattle manure gave a biogas yield of 621 L/kg [36]. Fermentation of molasses, sugar cane waste, provided production of CH_4 1.48 L/L reactor per day [41]. Studies of the yield of methane during the fermentation of vinase, waste liquor production showed that the yield of H_2 was 14.8 mL/g of substrate, and the yield of CH_4 was 274 L/kg of substrate [2].

It is shown that the addition of mechanically grinded organic waste into the model landfill allowed to obtain an industrially promising methane yield of 478 L/kg of organic compounds, which can be used in landfills to accelerate the destruction of solid waste and CH₄ [42].

Methane concentrations in landfills have been intensively monitored in recent decades [43-44]. Obtained results show significant methane emissions in municipal waste disposal areas [45-47]. Different types of surface coverage conditions are used to reduce the impact of methane emissions on the environment [46-48]. In some cases, the purpose of such covers is not only to protect the environment from toxicants, but also to collect biomethane, which is a high-energy fuel [47, 49]. Such technologies are common in Southern Spain [47], USA, Malaysia [49] etc. However, the potential for transporting upgraded biogas for off-site exploitation as well as the optimal ways of increasing of biomethane yield from landfills are yet to be explored.

CONCLUSIONS

The absence of regulation caused long term decay of waste. The high efficiency is achieved due to regulation of the fermentation process. The results will serve as a basis for the development of industrial biotechnology for the utilization of organic waste to reduce the volume of existing landfills and produce methane energy. This will further allow bioremediation of contaminated areas, obtaining an alternative to fossil fuel biomethane.

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