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INVESTIGATION OF METHANOGENIC FERMENTATION OF FOOD INDUSTRY WASTE

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Purpose. To investigate the joint utilization of rice husk, cattle manure and meat processing plant's waste water by anaerobic fermentation taking to account it is considered as one of the most effective ways of multicomponent substrates utilization, and to determine the optimal weight proportions of mixed waste components as a substrate for fermentation in an anaerobic bioreactor. **Methodology**. We have fermented substrates in a bioreactor with an upward flow of liquid through an anaerobic sludge layer UASB of periodic action with a total volume of 50 dm^3 to study anaerobic fermentation of substrates with different weight ratio of components in mesophilic conditions. We have monitored the indicators of the amount of released formed biogas and methane, the degree of organic matter biodegradation by the indicator of COD and the medium pH. We have performed a number of experiments of substrates anaerobic fermentation with different weight ratio of cattle manure, waste water and rice husk at mesophilic temperature. **Results**. We have identified the parameters of the influence of the substrate composition on the efficiency of the organic matter decomposition, biogas formation and methane yield as a result of anaerobic reactions. It allows to determine the degree of the organic matter destruction of cattle manure and rice husk and evaluated the negative impact of fatty acids on anaerobic reactions. **Originality**. For the first time, we have carried out the integrated research of joint fermentation of rice husk and waste from mixed production in an anaerobic bioreactor. We have proved the efficiency of using mixed substrates for methane production and increasing the degree of the waste organic substances biodegradation in comparison with the fermentation rates of monosubstrates. **Practical value**. We have demonstrated the effectiveness of joint fermentation of rice husk and waste from mixed production in an anaerobic bioreactor, which provides high rates of organic matter destruction in the biological environment with high methane content and stability of similar production reactions.

Key words*:* anaerobic fermentation, waste, rice husk, cattle manure, biogas, joint fermentation, mesophilic conditions.

ДОСЛІДЖЕННЯ МЕТАНОГЕННОГО ЗБРОДЖУВАННЯ ВІДХОДІВ ХАРЧОВОЇ ПРОМИСЛОВОСТІ

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Метою даної роботи є дослідження спільної утилізації рисової лузги, гною великої рогатої худоби стічних вод м'ясопереробного підприємства шляхом анаеробного зброджування в лабораторному анаеробному UASB біореакторі періодичної дії на протязі 10 днів і визначення оптимальних вагових пропорцій сумішей змішаних відходів як субстрату для ферментації в анаеробному біореакторі. Актуальність роботи пов'язана з тим, що анаеробне зброджування вважається одним із найефективніших способів утилізації багатокомпонентних субстратів, якими є відходи харчового виробництва. Під час дослідження виділені параметри впливу складу субстрату на ефективність розкладання органічних речовин, утворення біогазу і вихід метану у результаті анаеробних реакцій. Проведено низку експериментів анаеробного зброджування субстратів з різним ваговим співвідношенням гною великої рогатої худоби, стічних вод та рисової лузги при мезофільному температурному режимі. Для цього ферментували субстрати в біореакторі UASB з висхідним потоком рідини через шар анаеробного мулу періодичної дії для вивчення анаеробного зброджування субстратів з різним ваговим співвідношенням компонентів у мезофільних умовах. В результаті дослідження визначено показники кількості виділеного біогазу та метану, ступінь біодеградації органічних речовин за показником ХПК та pH середовища. Проведено ряд дослідів субстратів анаеробної ферментації з різним ваговим співвідношенням гною великої рогатої худоби, стічних вод і рисовою лузгою при мезофільній температурі. У результаті дослідження визначено ступінь деструкції органічних речовин гною великої рогатої худоби та рисової лузги і оцінено негативний вплив жирних кислот на протікання анаеробних реакцій з метою виділення оптимальних вагових пропорцій складників змішаних відходів у якості субстрату для зброджування в анаеробному біореакторі. Була доведена ефективність використання змішаних субстратів для отримання метану і збільшення ступеня біорозкладання органічних речовин відходів у порівнянні з показниками зброджування моносубстратів.

Ключові слова: анаеробне зброджування, відходи, рисова лузга, гній великої рогатої худоби, біогаз, спільна ферментація, мезофільні умови

PROBLEM STATEMENT. The main provisions of the policy strategy on environmental protection and sustainable development provide a comprehensive solution to the problems of balanced development of the country's economy and improving the environment [1, 2]. The current pace of the grain processing and meat processing industries development makes the issues of efficient and cost-effective ways of the industrial waste utilization more and more urgent. In accordance with the principles of the concept of the raw waste reprocessing, special attention needs to be paid to the utilization of meat processing waste, which includes sewage generated directly during meat production and manure from cattle slaughterhouses [3]. Biotechnological methods of processing are considered to be a promising direction of food waste utilization [4]. One such method is anaerobic fermentation, which is a continuous multi-component process of the organic matter conversion into final products - biogas containing methane, water vapor and carbon dioxide, which is provided by the work of various microbial communities [5].

One of the strategic directions of the food and processing industry development, which provide the required number of people with food, is the active greening of enterprises [6]. Namely, taking to account the global growth rate of food prices, the issue of further reduction and effective disposal of food waste and processing industries are becoming relevant both ecologically and economically. Today, the modernization of production facilities and the transfer of production to a closed cycle are considered as one of the fundamental areas in addressing the issues of natural resources' rational use and environmental protection. The requirements of the modern market dictate the need to introduce into the production process technologies with low energy, resource and capital intensity, which allow producing high quality and competitive products. Gradually, manufacturers are turning to the accumulated domestic and foreign experience in waste disposal, which involves the formation of by-products, thereby providing additional profits and improving the environmental image of the enterprise [7]. Companies, whose products are focused on the European consumer market, give significant attention to these aspects.

According to the statistical yearbook of the State Statistics Service of Ukraine for recent years [8], rice yield in Ukraine was 50 quintals per 1 hectare. During the period of 2014-2016 years processing enterprises have received an average of 4.5 thousand tons of rice per year from the Odessa region, thus ensuring the generation of rice husk waste in an average of 828 tons per year. There are three main ways to dispose of rice husk: incineration, creation of special dumps and processing with subsequent production of silicon compounds. Taking to account the relatively small volumes of rice cultivation and processing in Ukraine, the dominant method of rice waste disposal remains incineration, which does not meet modern requirements of low-waste and non-waste technologies. As for the pace of the meat processing industry and animal husbandry development in general, during the 2014-2016 years the number of cattle in Ukraine has been 3884 thousand heads per year, of which approximately 192 thousand heads are raised and processed in the Odessa region. Thus, the volume of waste from livestock farming and meat processing enterprises in Ukraine and Odessa region is: cattle manure for the stall period (220-240 days) – 34.9 million tons and 1.7 million tons, respectively; the average data of the sewage specific consumption - 188.8 million m^3 and 3.76 million m^3 per year, respectively. Based on this, improving the profitability of grain processing and meat processing enterprises through efficient disposal of waste with the subsequent receipt of secondary products is a priority for the enterprise policy development on waste management.

The choice of disposal methods is carried out taking into account the waste physical and chemical parameters, the enterprise peculiarity, eco- and energy efficiency. Rice husk is a product of cereals husking – rice, characterized by a significant content of fiber and minerals. Cattle manure is a mixture of animal feces with straw or peat litter, which is divided into litter (solid) and litter-free (liquid) manure. The composition of manure depends on such parameters as breed, age of animals, feed and litter type. Sewage from meat processing plants is multicomponent and highly concentrated in terms of COD (chemical oxygen demand), BOD_{full} (biochemical oxygen demand for 20 days), of suspended solids and fats content; it has a high content of nutrients, neutral pH values, temperature in the range of 20-30 ^oC, organic substances in colloidal and soluble forms [9].

Anaerobic fermentation is considered as one of the most effective ways of multicomponent substrates utilization. Biochemical and microbiological aspects of the anaerobic fermentation process depend on the complex conditions that arise during the different microbial communities' development with the simultaneous course of the corresponding biological reactions, which are usually specific to substrates. The main factors influencing the process of substrate anaerobic fermentation in the reactor are temperature, pH level, nutrient content, toxic substances, volatile fatty acids (VFA), heavy metals, lipids and carbohydrates, hydraulic retention time, substrate flow rate and sludge load [10].

The course of anaerobic reactions largely depends on the environment temperature due to the sensitivity to temperature jumps of the anaerobic system microbial communities [11]. There are three different temperature ranges in which the growth and functioning (viability) of anaerobic bacteria is ensured: psychrophilic (18-20 °C), mesophilic (25-40 °C), and thermophilic (50-70 °C). It is known that in compliance with the psychrophilic regime, the process of substrates' fermentation is very slow and does not provide a sufficient degree of the organic matter destruction. During the mesophilic regime, stable growth of methanogenic bacteria is ensured, which increases the biomass growth and has a positive effect on the substrate components biodegradation. Adherence to the thermophilic temperature in the reactor stimulates a more intensive process of anaerobic fermentation, compared to the above; however, energy costs for heating and maintaining the thermophilic temperature are not fully compensated by the biogas formation from the reactor, which provides additional costs.

The microbial communities' activity also depends on the pH level, as each group of microorganisms involved in the process has specific pH levels for optimal growth. Control of the pH parameter is one of the fundamental parameters for maintaining optimal bacterial growth. It is known that the VFA accumulation in the bioreactor causes sharp jumps in pH, which causes the successive death of methanogens, which in turn reduces the efficiency of pollutant removal and biogas formation [12]. The optimal pH level for cytogenic bacteria is from 5.2 to 6.5 pH units, while the optimal pH level for methanogens varies from 7.5 to 8.5 pH units. The optimal pH level of the reactor working medium is considered to be 6.8-7.3 pH units, under such conditions there is no excessive VFA accumulation and significant changes in biomass growth aren't observed.

An important aspect in bioreactor regulating is the presence of nutrients that are important for the efficient anaerobic microorganisms' growth [13]. In addition to the main content of macronutrients, the fine organization of the microbial community requires micronutrients and trace elements such as Nitrogen, Phosphorus, Sulfur, Potassium, Calcium, Magnesium, Iron, Nickel, Cobalt, Zinc and Copper to ensure optimal growth. These nutrients must be present in very low concentrations; however, their absence generally adversely affects the growth and viability of microorganisms.

The ammonium ions presence plays an important role in anaerobic fermentation. For optimal anaerobic fermentation, the ammonium ions level should be maintained at 80 mg/l. Known studies of the stable course of anaerobic fermentation processes containing ammonium ions under mesophilic conditions at the level of 50-150 mg/l; higher concentrations of ammonium led to serious reactor disturbances due to reduced growth and specific activity of methanogenic bacteria [14]. In addition, an adequate amount of sulfide is required for the stable growth of methanogenic organisms, but it should be noted that depending on the pH level of the reaction medium, sulfides can exhibit toxic properties. Numerous studies have shown that sulfide toxicity is manifested against methanogens, namely acetate consumers and H_2 consumers, which inactivate and inhibit the conversion of intermediates to the final ones; this process depends on the accumulation of volatile fatty acids, which further reduces methane yield.

It is known from the literature that the presence of heavy metals in the substrate in many cases causes a toxic or inhibitory effect on the anaerobic fermentation process. However, despite this, their presence in very low ("trace") concentrations is necessary for the growth of microorganisms. Scientists have evaluated the toxicity of heavy metals (Cadmium, Chlorine, Copper, Nickel and Zinc); the study found that acidogenic, acetogenic and methanogenic microorganisms are characterized by different resistance to toxic effects of heavy metals [15].

MATERIAL AND RESULTS. Experimental studies were performed by fermenting substrates in a bioreactor with an upward flow of liquid through an anaerobic sludge layer UASB (Upflow Anaerobic Sludge Blanket reactor) of periodic action with a total volume of 50 dm^3 (Fig. 1). Anaerobic fermentation of substrates with different weight ratio of components in mesophilic conditions at a temperature of 36 ± 1 °C and pH of 6-7 ± 5 was studied. During the experimental study, the indicators of the amount of released formed biogas and methane, the degree of organic matter biodegradation by the indicator of COD and the medium pH were monitored.

At the beginning of the each cycle the substrate components were fed to the reactor, after which the valve was sealed. The working mixture was heated to 35-37 °C using a heating element, which, if necessary, turned on automatically. Stable temperature of the working mixture was provided by a water jacket with a temperature sensor. Mixing of the substrate was carried out using an automatic stirrer periodic action. The released biogas volume was collected and measured in a water gasholder with a total volume of 5 dm^3.

To determine the pH of the reaction medium a pH meter Hanna HI2210 was used with a pH range from -2 to 16 ± 0.01 pH units and temperature from -0.9 to 120 ± 0.5 °C. The methane amount in the biogas was determined using a thermochemical signaling device-explosimeter STX-17-90 with a measurement range of 00.0 - 99.9% LEL. The COD indicator was determined in accordance with the KND standard 211.1.4.021-95. "Methods for determining the chemical oxygen demand in surface and wastewater." The total nitrogen amount was determined in accordance with the KND standard 211.1.4.031-95 "Method of titrometric determination of total nitrogen in wastewater". The amount of VFA was determined in accordance with the "Method of measuring the mass concentrations of fats by thin layer chromatography".

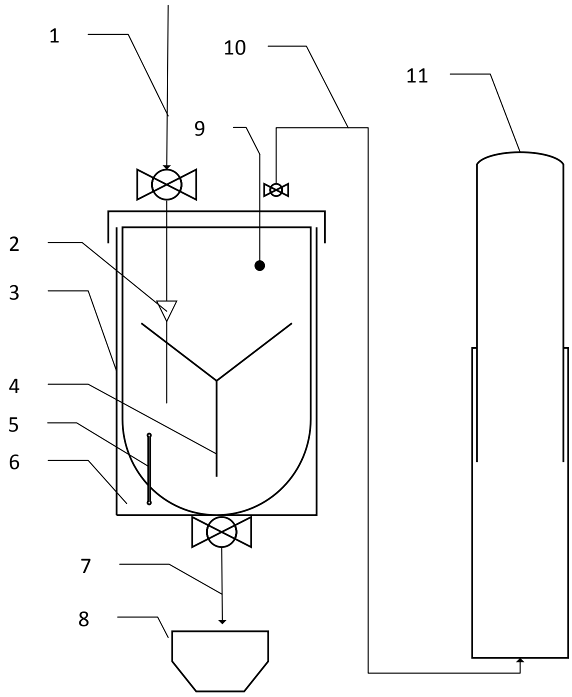

Figure 1 – Apparatus and technological scheme of anaerobic sewage fermentation
1 – supply of sewage; 2 – sewage flow; 3 – bioreactor; 4 – electric stirrer; 5 – heating element; 6 – water jacket; 7 – flow of excess sludge; 8 – capacity for collecting sludge; 9 – temperature sensor; 10 – biogas flow; 11 – water gasholder

In the research course, a study of sewage anaerobic fermentation from a meat processing plant with different weight ratios of substrate components (Table 1) was held. The first three substrates are monosubstrates (S_1, S_2, S_3) and consist separately of the meat processing plant effluent after the mechanical treatment stage and of straw manure with straw litter and of rice husk after rice treatment. Other substrates are mixtures and consist of different components by weight ratio of manure and rice husk 3:1 (S_4), 1:1 (S_5), 1:3 (S_6).

Table 1 – Characteristics of substrates' composition

Indicator		Units	Sewage (S_1)	Cattle manure (S_2)	Rice husk (S_3)	Weight ratio of cattle manure: rice husk		
						3:1 (S_4)	1:1 (S_5)	1:3 (S_6)
Waste	S_1	l	28,9	20	20	20	20	20
	S_2	kg	-	7,73	-	3,52	2,38	1,37
	S_3	kg	-	-	5,3	1,28	2,52	3,41
Excess silt		l	0,5	0,5	0,5	0,5	0,5	0,5
Dry matter content		%	5,8	11,65	25,2	15,1	18,6	20,4

The excess sludge (seed) in a volume of 0.5 l from the previous anaerobic fermentation of cattle manure with the addition of ordinary water was added to each substrate. The excess sludge contained all the necessary microbial communities to begin fermentation and organic matter destruction. Studies were performed while maintaining the temperature of the working mixture at 35-36 °C. The values of the redox potential varied in the range from -260… -140 mV and below; these are the optimal conditions for anaerobic transformation of organic matter [16]. Fermentation of each of the substrates was performed for 10 days.

According to the study results, pH fluctuations during anaerobic fermentation of various substrates occurred in the range of 6.2 pH units in the S_2 substrate (cattle manure) to 7.3 pH units in the S_1 substrate (sewage), all substrates were characterized by the optimal pH level for the microbial community functioning. At the end of the 10-day fermentation cycle, the substrates had a decrease in dry matter content of approximately 2 times, the highest rate of dry matter removal was recorded in the S_1 substrate (sewage) - 56% and in the mixed S_6 substrate ($1S_2$:$3S_3$.) - 54%. There was also a decrease in the substrates' COD before and after fermentation, which reflects the degree of organic matter destruction, namely: S_1 (sewage) - 42%, S_2 (cattle manure) - 58%, S_3 (rice husk) - 37%, S_4 ($3S_2$:$1S_3$) - 66%, S_5 ($1S_2$:$1S_3$) - 64% and S_6 ($1S_2$:$3S_3$) - 65%. The amount of total nitrogen in the substrates after anaerobic fermentation has not changed; there was a slight decrease in total nitrogen in all substrates in the range of 5-10%. The LVH content after anaerobic fermentation in the substrates S_2 (cattle manure), S_4 ($3S_2$:$1S_3$), S5 ($1S_2$:$1S_3$) and S_6 ($1S_2$:$3S_3$) decreased by 49%, 55%, 61 and 54% respectively. In the substrates S_1 (sewage) and S_3 (rice husk) there was an VFA accumulation, namely an increase in their number by 5% and 20%, respectively, which indicates a slowing of anaerobic fermentation reactions. Changes in physicochemical parameters of the studied substrates before and after fermentation in an anaerobic bioreactor are given in table 2.

Table 2 – Physic and chemical parameters of substrates before and after anaerobic fermentation

Indicators	Units	Samples before (a) and after (b) fermentation	Sewage (S_1)	Cattle manure (S_2)	Rice husk (S_3)	Weight ratio of cattle manure: rice husk		
						3:1 (S_4)	1:1 (S_5)	1:3 (S_6)
pH	-	a	7,27	6,24	6,72	6,41	6,53	6,43
		b	6,81	6,22	6,65	6,51	6,47	6,41
Dry matter content	mg/l	a	5,8	11,65	25,2	15,1	18,6	20,4
		b	3,3	5,7	11,25	7,9	9,2	11,0
COD	mg/l	a	1270	4455	1410	2892	2620	2078
		b	737	1871	888	938	943	935
Total nitrogen	mg/l	a	102	458,2	108,3	173	228	157
		b	98,7	439	106	172,5	224,7	156,4
Volatile fatty acids	mg/l	a	165	427	311,8	328	334,3	372,6
		b	174	218,4	373	148,5	164	171,7
The degree of organic matter destruction	%	-	42	58	37	66	64	65

According to the dynamics of the intensity of released biogas by the studied substrates, samples S_4 ($3S_2$:$1S_3$) and S_2 (cattle manure) showed the highest bacterial activity on the first day, biogas release was 8.04 dm^3 and 6.43 dm^3. The lowest activity of bacteria on the first day of fermentation was observed in S_1 (sewage) - 1.22 dm^3 and in S_3 (rice husk) - 0.33 dm^3 due to insufficient bacteria in the substrates at the beginning of fermentation, other substrates contain a significant proportion of cattle manure from available species of methanogenic bacteria (*Methanococcus*, *Methanobacteriales, Methanomicrobiales, Methanosarcina and Methanosaeta*) [17]. The best results of biogas release during the 10-day period are characterized by substrates S_4 ($3S_2$:$1S_3$) - 8.04 dm^3 on the 1st day, S_2 (cattle manure) - 8.03 dm^3 on the 3rd day, S_5 ($1S_2$:$1S_3$) - 6.76 dm^3 on the 3rd day, S6 (1 S_2:$3S_3$) - 6.52 dm^3 on the 3rd day of fermentation. According to the final results, the amount of released biogas for the whole period is: S_2 (cattle manure) - 52.53 dm^3, S_4 ($3S_2$:$1S_3$) - 43.54 dm^3, S_5 ($1S_2$:$1S_3$) – 38.9 dm^3, S6 ($1S_2$:$3S_3$) - 32.2 dm^3, S1 (sewage) - 17.38 dm^3, S_3 (rice husk) - 16.86 dm^3, respectively. The study results of the biogas formation dynamics from different substrates are shown in Fig. 2.

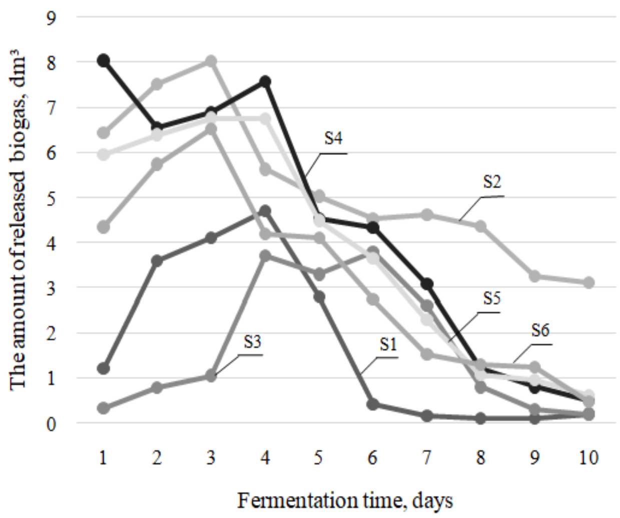

Figure 2 – Dynamics of the biogas production intensity, where the substrate S_1 consists of sewage from the meat processing plant after the mechanical treatment stage, S_2 consists of cattle manure with straw litter, S_3 consists of rice husk after rice treatment, substrates S_4, S_5, S_6 are mixtures and consist of different components by weight ratio of manure and rice husk S_4 - 3:1, S_5 - 1:1, S_6 – 1:3.

The volume of the released methane in the biogas for the study period is: S_2 (cattle manure) - 35.9 dm^3, S_4 ($3S_2$:$1S_3$) - 35.2 dm^3, S_5 ($1S_2$:$1S_3$) - 32.1 dm^3, S_6 ($1S_2$: $3S_3$) - 26.7 dm^3, S_1 (sewage) - 14.8 dm^3, S_3 (rice husk) - 7 dm^3, respectively. The dynamics of the released methane intensity as a result of methanogenic bacteria activity during anaerobic fermentation is shown in Fig.3.

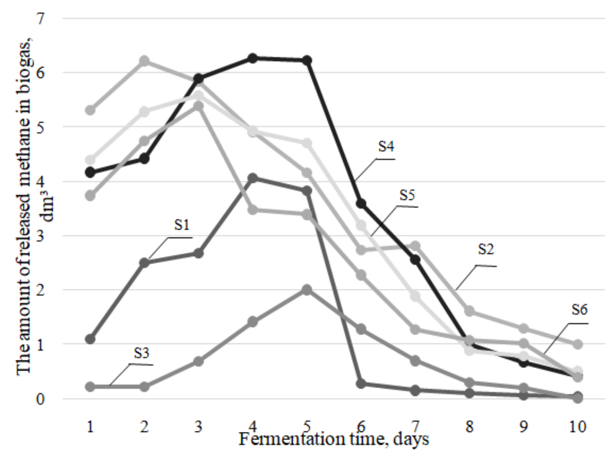

Figure 3 – Dynamics of the intensity of methane production in biogas, where the substrate S_1 consists of sewage from the meat processing plant after the mechanical treatment stage, S_2 consists of cattle manure with straw litter, S_3 consists of rice husk after rice treatment, substrates S_4, S_5, S_6 are mixtures and consist of different components by weight ratio of manure and rice husk S_4 - 3:1, S_5 - 1:1, S_6 - 1:3.

CONCLUSIONS. In the work course, the rice husk influence on the process of anaerobic fermentation of meat processing industry waste in the form of industrial sewage and cattle manure from the animals' slaughter territory for 10 days was performed. Studies of changes in substrates' physicochemical parameters showed that the pH of substrates during fermentation remained within optimal conditions for methanogenic microorganisms, dry matter content after fermentation in all samples decreased by 2 times, the amount of total nitrogen in substrates decreased by 5-10%. There was also a slight accumulation of intermediate components of fermentation - volatile fatty acids, in substrates S_1 (sewage) - 5% and S_3 (rice husk) - 20%, which indicates a failure in the process of changing phases of anaerobic fermentation. Fermentation of substrates is characterized by varying degrees of the organic matter destruction, depending on the substrate components, so the range of organic matter biodegradation varies from 37% in substrate S_3 (rice husk) to 66% in substrate S_4 ($3S_2$:$1S_3$). According to the study results of gas-forming properties of substrates, it was found that during the fermentation period the largest amount of biogas is formed by substrates S_2 (cattle manure) - 52.53 dm^3 and S_4 - 43.54 dm^3, the total methane content in biogas samples was S_2 - 35.9 dm^3, S_4 - 35.2 dm^3.

The results of comparative analysis of indicators before and after fermentation of compatible substrates with different weight ratios of cattle manure and rice husk (S_4 - 3:1, S_5 - 1:1, S_6 - 1:3) show the efficiency of biological composition of organic substances in all other substances 65 ± 1%; amount of released biogas: S_4 ($3S_2$:$1S_3$) - 43.54 dm^3, S5 ($1S_2$:1 S_3) - 38.9 dm^3, S6 ($1S_2$:$3S_3$) - 32.2 dm^3, the amount of methane in the released biogas for the study period is determined by S_4 ($3S_2$:$1S_3$) - 35.2 dm^3, S_5 ($1S_2$:$1S_3$) - 32.1 dm^3, S_6 ($1S_2$:$3S_3$) - 26.7 dm^3. Thus, the study results of joint fermentation of rice husk and waste from mixed production in an anaerobic bioreactor demonstrate the effectiveness of the chosen method of use, which provides high rates of organic matter destruction in the biological environment with high methane content and stability of similar production reactions.

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