Ministry of Education and Science of Ukraine
Black Sea Universities Network

ODESA NATIONAL UNIVERSITY OF TECHNOLOGY

International Competition of Student Scientific Works

BLACK SEA SCIENCE 2022

PROCEEDINGS

Black Sea Science International Competition of Student Scientific Works by Odesa National University of Technology

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Odesa National University of Technology, 2022.

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INTRODUCTION

International Competition of Student Scientific Works “Black Sea Science” has been held annually since 2018 at the initiative of Odesa National University of Technology (formerly Odesa National Academy of Food Technologies) with the support of the Ministry of Education and Science of Ukraine. It has been supported by Black Sea Universities Network (the Association of 110 higher education institutions from 12 countries of the Black Sea Region) since 2019, and by Iseki-FOOD Association (European Integrating Food Science and Engineering Knowledge into the Food Chain Association) since 2020.

The goal of the competition is to expand international relations and attract students to research activities. It is held in the following fields:

- Food science and technologies
- Economics and administration
- Information technologies, automation and robotics
- Power engineering and energy efficiency
- Ecology and environmental protection

The jury includes both Ukrainian and foreign scientists. In the 4 years that the competition has been held, the jury included scientists from universities of 24 countries: Angola, Azerbaijan, Benin, Bulgaria, China, Czech Republic, France, Georgia, Germany, Greece, Israel, Italy, Kazakhstan, Latvia, Lithuania, Moldova, Pakistan, Poland, Romania, Serbia, Slovakia, Switzerland, Turkey, USA.

At the same time, every year the geography has expanded and the number of foreign jury members has increased: from 46 jury members representing 25 universities from 12 countries in 2018, to 73 jury members of the 46 universities from 19 countries in 2022.

More than a thousand student research papers have been submitted to the competition from both Ukrainian and foreign institutions from 25 countries: China, Poland, Mexico, USA, France, Greece, Germany, Canada, Costa Rica, Brazil, India, Pakistan, Israel, Macedonia, Lithuania, Latvia, Slovakia, Romania, Kyrgyzstan, Kazakhstan, Bulgaria, Moldova, Georgia, Turkey, Serbia.

The interest of foreign students in the competition grew every year. In 2018, the students representing 15 institutions from 7 countries have submitted 33 works. In 2021 the number of submitted works increased to 73, authored by the students of 40 institutions from 18 countries.

The competition is held in two stages. In the first stage, student research papers are reviewed by members of the jury who are experts in the relevant fields. In the second stage of the competition, the winners of the first stage have the opportunity to present their work to a wide audience in person or online.

All participants of the competition and their scientific supervisors are awarded appropriate certificates, and the scientific works of the winners are included in the electronic proceedings of the competition. Every year the competition receives a large number of positive responses from Ukrainian and foreign colleagues with the desire to participate in the coming years.

5. ECOLOGY AND ENVIRONMENTAL PROTECTION

MODIFICATION OF ANAEROBIC DIGESTION USING RICE HUSK

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Abstract. *The current rate of development of grain-processing and meat-processing industries makes effective and cost-effective waste disposal more actionable. The reprocessing of waste from the meat processing industry, which includes waste water, formed directly in the production of meat and from cattle manure, requires special attention. Biotechnological methods of processing are considered a promising form of recycling food production wastes. One such method is anaerobic digestion, which is a continuous multi-component process that transforms organic material into finished products, such as biogas containing methane, water vapor and carbon dioxide, through microbial activity.*

The aim of this study was to investigate the joint utilization of rice husks, manure and sewage from a meat processing plant by anaerobic digestion in a laboratory anaerobic batch bioreactor for 10 days. During the study, parameters of influence of the substrate composition on the efficiency of decomposition of organic matter, biogas and methane yield were identified. A series of experiments investigating anaerobic fermentation of substrates was carried out with different weight ratios of manure, waste water and rice husks at mesophilic temperature conditions (36 ± 1°C). After benchmarking fermentation mono-substrates and mixtures, the use of mixed substrates for methane was proved to increase the degree of biodegradability of organic substance waste.

Keywords: *wastewater, anaerobic sludge, rise husk, cattle manure, biogas.*

I. INTRODUCTION

Requirements of the modern market dictate the implementation in production processes of technologies with low power, resource and capital intensity. Such technologies enable high-quality and competitive products. One of the strategic directions of development in the food and processing industry is providing the population with food products in a necessary quantity, while also actively greening the industry. Today, upgrades of production capacities in the move towards closed cycle production is considered as one of the fundamental directions to take in solving the problems of rational use of natural raw material resources and environmental protection. Gradually, producers are considering their accumulated world experience of waste recycling, understanding that it creates by-products, and thereby provides an additional source of financial income and also improves their ecological image. Given world growth rates in the prices of raw materials for food, questions of further reduction and effective utilization of food waste and the emergence of processing industries acquire increasing relevance in ecological and economic plans. Manufacturers pay considerable attention to these aspects as their products are oriented to the European consumer market with strict requirements for environmental protection. The choice of methods for waste recycling considers the physical and chemical parameters of waste, the features of the manufacturer, and indicators of and energy efficiency.

In this work, the joint recycling of meat-processing waste and waste from rice cleaning is considered. For the period 2014-2016, 3884 thousand cattle a year were produced in Ukraine's livestock industry. Around 192 thousand were reared in the Odessa region [1]. Manure waste from cattle production for nongrazing time (220-240 days) in Ukraine and the Odessa Region amounts to 34.9 million tons and 1.7 millin tons respectively. Wastewater from the meat-processing entities amounts to 188 800 000 m^3 and 3760000 m^3 a year for Ukraine and Odessa, respectively.

Manure is the mix of excrement and urine from animals mixed with a straw or peat layer. Manure can be categorised as either covering (solid) or bespodstilochny (liquid) manure. The composition of manure depends on parameters such as the breed and age of animals, the type of feed and type of a layer. The second object of the research is wastewater from the meat-processing industry. This wastewater has many components and high level of COD (chemical oxygen demand), content of the weighed substances and fats, increased content of biogenous elements, neutral pH values, temperature within 20-30ºC, and organic substances are in both colloidal and soluble forms.

II. LITERATURE ANALYSIS

According to a statistical year-book of public service of statistics of Ukraine in recent years [1] the productivity of rice in Ukraine was 50 centners per 1 hectare. For Odessa region for the period of 2014-2016, on average 4.5 thousand tons of rice a year were imported by processing plants. This leads to theformation of rice husk waste amounting to 828 tons annually, on average. The rice husk is a by-product of grain cleaning. The husk is characterized by a considerable content of cellulose and mineral substances. There are three main methods of treating rice husks: burning, creating special dumps and conversion to produce silicon. As rather small amounts of rice are cultivated and converted in Ukraine, burning remains by the dominant method of waste treatment of rice and does not conform to modern requirements for low-waste and wasteless technologies. Therefore, to improve the profitability of the grain processing and meat-processing industries, effective waste recycling with the subsequent generation of by-products is the priority direction of development of policy for these industries.

Anaerobic fermentation is considered one of the most efficient paths of utilization of multicomponent effluent. Biochemical and microbiological aspects of the process of anaerobic fermentation depend on the composite conditions. According to the commonly used scheme, anaerobic fermentation consists of four phases: hydrolysis, acidogenesis, acetogenesis and methanogenesis. Courses of each of these phases provide corresponding development of a microbiological community. Bacterial activity results in various products of disintegration of the complicated molecules which are a substrate for microorganisms for the following phase. The bacteria for the reactor are divided into three common groups [2]. The first group includes hydrolytic bacteria, referred to as acidogenic, and which provide initial hydrolysis of a substrate to low-molecular organic acids and other small molecules. The second group is represented by heteroacetogenic bacteria which produce ethanoic acid and Hydrogenium. The third group includes metanogenic bacteria which form a methane. The last group can be divided into subgroups: consumers of Hydrogenium – lithotrophs, and consumers of ethanoic acid – acidtrophs. Synergism of these groups occurs, for example, various growth rates can be explained by common cultivation and appear as a result of interaction between trans-species type of hydrogen transfer. Substrates, which are partly Sulfur and Nitrogen, can cause increases in two additional groups of microorganisms: sulfate-reducing bacteria and denitrifying bacteria [2]. These symbiotic microbial communities can change the characteristics of fermentation, and function as self-regulating systems which maintain pH value, redox potential and thermodynamic equilibrium at optimum levels for growth, thereby providing stability of fermentation.

Courses of anaerobic reactions substantially depend on environmental temperature because of the sensitivity of bacteria to temperature jumps of an anaerobic system. There are three various temperature ranges which affect functioning (activity) of anaerobic bacteria: psychrophilic (18-20 °C), mesophilic (25-40 °C), and thermophilic (50-70 °C). It is known that when keeping a psychrophilic temperature regime, the process of a substrate's fermentation takes place very sluggishly and does not provide a sufficient degree of destruction of organic matter. During the mesophilic mode, stable growth of the metanogenic bacteria occurs, and the biomass increases. This mode positively influences biodegradation of substrate components. Keeping a thermophilic temperature regime stimulates a more intensive course of processes of anaerobic fermentation in the reactor. However, expenses on energy for heating and maintaining a thermophilic temperature regime are not compensated in full.

Activity of bacteria depends on pH level as each group of microorganisms has specific levels of pH for optimum growth. Monitoring of the pH parameter is fundamental to maintaining optimum bacterial growth. It is known that accumulation of volatile fatty acids in the bioreactor causes sharp jumps in pH level, which causes serial atrophy in methanogenic bacteria [3]. It, in turn, leads to a decrease in the effectiveness of removal of pollutants and formation of biogas. For acidogenic bacteria the optimum pH level is from 5.2 to 6.5 pH units, while the optimum pH level for metanogen is in the range of 7.5 to 8.5 pH units. Low pH values promote restitution of a proton to Hydrogenium, then its restitution into methane. A wide range of types of acidogenic bacteria entering the hydrolytic group provide their resistance to changes of conditions of cultivation, partly becoming acidophilic. It is known that the mean time of regeneration for such bacteria is 2-3 hours, which is a rather short time term for anaerobic processes [4]. However, this group of bacteria is negatively influenced by low pH values and a redox potential. In the conditions of a sharp increase of Hydrogenium concentration, microorganisms choose the alternate metabolic path for Hydrogenium removal, thereby regulating its concentration [2]. In cases of increased substrate concentrations in the reactor, a bacterium immediately reacts by formation of an excess amount of Hydrogenium and ethanoic acid, thereby reducing the oxidizing potential and pH. If this process continues further, then the substrate in the reactor "turns sour" and finishes the work. However, acidogenic bacteria use feed-back and choose alternate metabolic paths, such as formation of propionic and butane acids that helps to restore the stability of operation of the reactor. The role of Hydrogenium in management of emergence and consumption of the intermediate products explains the formation of some long-chain fatty acids which leading to accumulation or an expenditure of Hydrogenium.

An important aspect in regulating the operation of the bioreactor is availability of nutrients which are important for efficient anaerobic microorganisms' growth [5]. In addition to the main content of macronutrients, organization of a microbiological community demands availability of micronutrients and minerals, such as Nitrogen, Phosphorus, Sulfur, Potassium, Calcium, Magnesium, Iron, Nickel, Cobalt, Zincum and Copper for ensuring optimum microbial growth. These nutrients have to be present at very low concentrations, however, their absence general negatively influences microbial growth and activity of microorganisms. Existence of ions of ammonium plays an important role in anaerobic fermentation. For an optimum process of anaerobic fermentation, the level of ions of ammonium have to be supported at the level of 80 mg/l. It is known from research that a stable course of anaerobic fermentation processes needs the maintenance of ammonium ions at the level of 50-150 mg/l under mesophilic conditions. Higher concentrations of ammonium lead to serious violations of reactor operation because of the resulting decrease in growth rates and specific activity of methanogenic bacteria [6]. In addition, a sulfide concentration is necessary for stable methanogenic organisms' growth, but it should be noted that depending on the pH level of the reactionary environment, sulfides can show toxic properties. Numerous research has shown that sulfide toxicity in relation to methanogenic bacteria, namely acetate consumers and H_2 consumers, inactivates and suppresses transformations of the intermediate products. This process depends on accumulation of volatile fatty acids that cause a decrease in the exit of methane.

From literary data it is known that the presence of heavy metals on a substrate in many cases causes toxic or inhibiting effects on the process of anaerobic fermentation. However, despite this, their presence at a very low («trace») concentration is necessary for microorganisms' growth. The toxicity of heavy metals (Cadmium, Chlorine, Copper, Nickel and Zincum), have been estimated through results of research. It has been revealed that acidogenic, acetogenic and metanogenic microorganisms are characterized by different resistances to the toxic influence of heavy metals [7].

III. OBJECT, SUBJECT, AND METHODS OF RESEARCH

The laboratory tests are executed by fermentation of a substrate in a UASB bioreactor (Upflow Anaerobic Sludge Blanket reactor) of periodic action with a volume of 50 dm^3 (figure 1, image 1). We investigated anaerobic substrate fermentation with different weight ratios of components in mesophilic conditions with a temperature regime of 36±1°C and pH units of 6-7±5. During the laboratory tests we controlled the amount of the emitted biogas, methane and degree of biodegradation of organic matters on an index of the chemical oxygen demand (COD) and pH. Considering design features of the anaerobic reactor, it should be noted that the effectiveness of the process will be affected by the generation rate of easily settling bacterial floccules. Thus, the waste of rice purification is used as a biological additive in anerobic process of sewage fermentation [8]. At the beginning of each cycle a component of a substrate was moved into the reactor, then the crane was hermetically closed. Working mix substrates were heated to 35-37 °C automatically. A stable temperature of working mix substrates was provided by a water-jacket with a temperature sensor. Hashing of a substrate was carried out by an automatic mixer. Biogas was gathered and measured in a water gasholder with a total amount of 13 dm^3.

The pH meter Hanna HI2210 was used to determine pH units. The pH range was from -2 to 16±0.01 units pH, and the temperature range was -0.9 to 120±0,5 ° C. The amount of methane in biogas was determined using the Signalling-explosimeter thermochemical device CTX-17-90, and measurements range from 00.0 to 99.9%. The COD indicator was determined according to standard gain 211.1.4.021-95. "Methods of determining the chemical oxygen demand (COD) in surface water and wastewater". The amount of total nitrogen was determined according to the standard gain 211.1.4.031-95 "Methods titration determination of total nitrogen in wastewater". The quantity of VFA (volatile fatty acids) was determined in accordance with "Methods of measuring fat mass concentrations by thin layer chromatography (TLC) ".

The aim of this work was anaerobic digestion of wastewater from meat processing enterprises with different substrate weight ratios of components (Table 1). The first three samples were monosubstrates (S1, S2, S3). The first sample consisted of meat processing wastewater after the mechanical treatment stage, the second consisted of cattle manure and the third was rice husks produced as a by-product of rice cleaning. The other substrates were mixtures and consisted of various components and weight ratios of manure and rice husks as follows: 3:1 (S4), 1:1 (S5), 1:3 (S6).

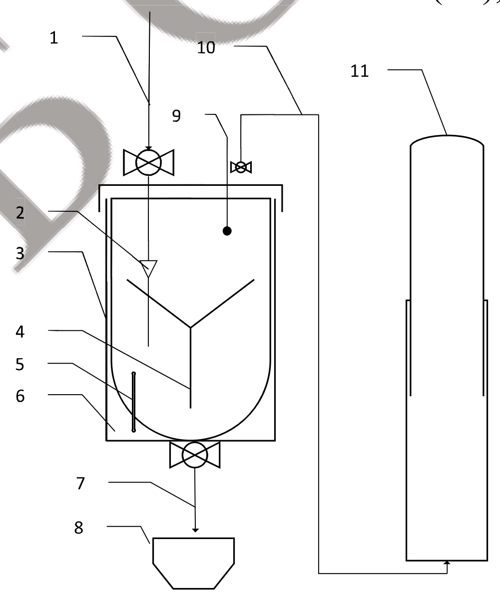


Figure 1. Equipment-technological scheme of anaerobic digestion of sewage, where 1 - supply of waste water; 2 - flow of sewage; 3 - bioreactor; 4 - electric mixer; 5 - heating element; 6 – water-jacket; 7 - sludge effluent: 8 - capacity to collect the sludge; 9 - temperature sensor; 10 - biogas stream; 11 - water gasholder.

Image 1. Photo of laboratory scale anaerobic bioreactor

Each substrate was added to 0.5 l of excess sludge from the previous anaerobic digestion of cattle manure with the addition of ordinary water, which contains the necessary microbial community to start fermentation and decomposition of organic matter. The temperature of the mixture was maintained at 35-36°C in the tests. The value of the redox potential fluctuated in the range of -260-140 mV; conditions for anaerobic transformation of organic matter below this range are not optimal [9]. Fermentation of each of the substrates was carried out for 10 days.

Table 1. Characteristics of substrates

Indicators		Unit	Sewage (S1)	Cattle manure (S2)	Rice husk (S3)	Weight ratio of manure : rice husk		
						3:1 (S4)	1:1 (S5)	1:3 (S6)
Waste	m	kg	-	7,73	-	3,52	2,38	1,37
	r.h.	kg	-	-	5.3	1,28	2,52	3,41
	s	l	28,9	20	20	20	20	20
Active sludge		l	0,5	0.5	0.5	0,5	0,5	0,5
Dry organic matter		%	5,8	11.65	25.2	15,1	18,6	20,4
s - meat processing wastewater sewage, m – cattle manure, r.h. – rice husk								

IV. RESULTS

According to the research, fluctuations in pH during anaerobic digestion of different substrates occurred in the range of 6.2 pH units in the substrate S2 (cattle manure) to 7.3 pH units in the substrate S1 (waste water). All substrates were characterized by optimum pH levels for microbial community functioning.

After the 10 day cycle of fermentation, substrates were observed to decrease in dry matter content by about 2 times the biggest indicator of removal of solids reported in the substrate S1 (sewage) – 56% and in mixed substrate S6 (1m : 3r.h.) – 54%. There was also a marked decrease in COD of substrates before and after fermentation, reflecting the degree of decomposition of organic matter. Figures for COD decrease were as follows: S1 (sewage) – 42%, S2 (manure) – 58%, S3 (rice husk) – 37%, S4 (3m : 1r.h.) – 66%, S5 (1m : 1r.h.) – 64% and S6 (1m : 3r.h.) – 65%.

The amount of total nitrogen in the substrates after anaerobic digestion did decreased slightly, by 5-10% in all substrates. The content of VFA after anaerobic digestion of substrates for S2 (manure), S4 (3m : 1r.h.), S5 (1m : 1r.h.) and S6 (1m : 3r.h.) decreased by 49%, 55%, 61% and 54%, respectively. In substrates S1 (sewage) and S3 (rice husk) VFA accumulation was observed, ie their number increased by 5% and 20%, respectively, indicating a slowing of reactions in anaerobic digestion. Changes in the physical and chemical parameters for the investigated substrates before and after digestion in an anaerobic reactor are given in table 2.

Table 2. Physical and chemical properties of substrates before and after anaerobic digestion

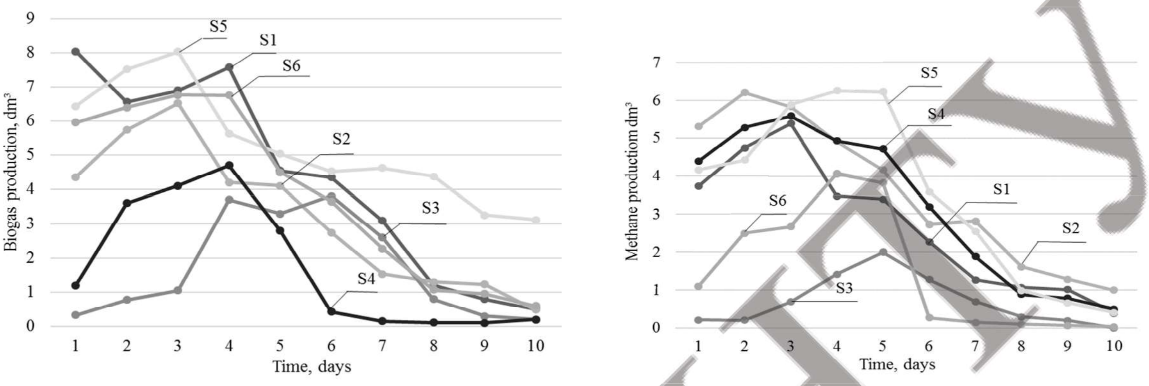
Indicators before and after anaerobic digestion	Unit	Sewage (S1)	Cattle manure (S2)	Rice husk (S3)	Weight ratio of manure : rice husk		
					3:1 (S4)	1:1 (S5)	1:3 (S6)
pH	-	7.27	6,4	6.72	6.41	6.53	6.43
		6.81	6,22	6.65	6.51	6.47	6.41
Dry organic matter	mg/l	5.8	11,65	25.2	15.1	18.6	20.4
		3.3	5,7	11.25	7.9	9.2	11.0
COD	mg/l	1270	4455	1410	2892	2620	2078
		737	1871	888	938	943	935
Total nitrogen	mg/l	102	458,2	108.3	173	228	157
		98.7	439	106	172.5	224.7	156.4
VFA	mg/l	165	427	311.8	328	334.3	372.6
		174	218,4	373	148.5	164	171.7
Degree of biodegradation	%	42	58	37	66	64	65

According to the dynamics of the intensity of production for biogas, the substrate samples S4 (3m : 1r.h.) and S2 (cattle manure) showed the highest level of bacterial activity on the first day, and the production of biogas was 8.04 dm^3 and 6.43 dm^3, respectively.

The low levels of activity of bacteria in fermentation were noted on the first day for S1 (sewage) – which produced1.22 dm^3 of biogas, and S3 (rice husk), which produced 0.33 dm^3 of biogas.Other substrates with a significant share of cattle manure contained methanogenic bacteria (Methanococcus, Methanobacteriales, Methanomicrobiales, Methanosarcina and Methanosaeta) [10].

The best biogas production results I during the 10 day period were S4 (3 m:1r.h.) – 8.04 dm^3 on the first day, S2 (manure) – 8.03 dm^3 on the third day, S5 (1m : 1r.h.) – 6.76 dm^3 on the third day, and S6 (1m:3r.h.) – 6.52 dm^3 on the third day of fermentation. The total amount of biogas produced over the entire period for each substrate was (in order of size): S2 (manure) – 52.53 dm^3, S4 (3m : 1r.h.) – 43.54 dm^3, S5 (1 m:1r.h.) – 38.9 dm^3, S6 (1 m:3r.h.) – 32.2 dm^3, S1 (sewage) – 17.38 dm^3, S3 (rice husk) – 16.86 dm^3.

The results of the tests of the dynamics of biogas formation from various substrates are shown in Figure 2 a). The volume of methane in biogas for the period of study was: S2 (manure) – 35.9 dm^3, S4 (3 m:1r.h.) – 35.2 dm^3, S5 (1m:1r.h.) – 32.1 dm^3, S6 (1m:3r.h.) – 26.7 dm^3, S1 (sewage) – 14.8 dm^3, S3 (rice husk) – 7 dm^3. The dynamics of the intensity of methane as a result of methanogenic bacteria during anaerobic digestion are shown in figure 2 b).



2 a) 2 b)

Figure 2. a) dynamics of intensity of the production of biogas, b) dynamics of intensity of the production of methane in biogas, formed from substrates (S1, S2, S3, S4, S5, S6.)

V. CONCLUSIONS

The aim of the work was to investigate the impact of rice husks on the anaerobic digestion process of industrial meat processing wastewater and cattle manure for 10 days. Changes in the physical-chemical characteristics of substrates showed that the pH during substrates fermentation remained within optimal conditions for methanogenic microorganisms. Dry organic matter content after fermentation in all samples decreased by 2 times, and the amount of total nitrogen in the substrates decreased by 5-10%. A slight accumulation of intermediate components of fermentation wre also noted – volatile fatty acids in the substrates S1 (sewage) – 5% and S3 (rice husk) – 20%, which indicated a failure in the phase change of anaerobic digestion process.

Substrate fermentation was characterized by varying degrees of organic matter decomposition, which depended on the components of the substrate. The quantity of biodegradable organic matter ranged from 37% in the substrate S3 (rice husk) to 66% in substrate S4 (3m : 1r.h.). According to the results of the investigation into substrates' gas-forming properties, during the period of fermentation the largest amount of biogas production was noted for substrates S2 (manure) – 52.53 dm^3 and S4 – 43.54 dm^3. Total methane content in biogas samples was S2 – 35.9 dm^3 and S4 – 35.2 dm^3.

The results of comparative analysis of indicators before and after the fermentation of mixed substrates with different weight ratios of cattle manure and rice husks (S4 – 3:1, S5 – 1:1, S6 – 1:3) show that the effectiveness of organic matter biodegradation in all three substrates was 65 ± 1%; the amounts of collected biogas were: S4 (3m : 1r.h.) – 43.54 dm^3, S5 (1m : 1r.h.) – 38.9 dm^3, S6 (1m : 3r.h.) – 32.2 dm^3. The amounts of methane in the collected biogas were: S4 (3m : 1r.h.) – 35.2 dm^3, S5 (1m : 1r.h.) – 32.1 dm^3, S6 (1m : 3r.h.) – 26,.7 dm^3. Thus, the joint fermentation results of rice husk and meat production wastewater in an anaerobic bioreactor demonstrate the significant efficiency of this recycling method which provides high rates of organic substance decomposition and biogas formation with high methane content.

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