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Technological Process of Anaerobic Digestion of Cattle Manure in a Bioenergy Plant

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ABSTRACT

Anaerobic digestion consists of the biological decomposition of organic waste under anaerobic conditions by various types of microorganisms. The purpose of this study was to evaluate the effect of the fermentation starter of methanogenic bacteria on the anaerobic digestion of cattle manure in a bioenergy plant. The effect of various methods (physical, microbiological and chemical) on the digestion of cattle manure was studied under mesophilic (35°C) and thermophilic (50°C) modes. The results of the study showed that the content of volatile fatty acids and the pH of the medium was in the optimal range, the yield of methane biogas (CH_4) during anaerobic digestion at 35°C was 0.45 m^3/kg and at 50°C was 0.58 m^3/kg. The data obtained indicate that the thermophilic mode (50°C) of anaerobic digestion of manure effectively affects the yield of methane biogas. Based on anaerobic digestion in mesophilic mode, a fermentation starter of methanogenic cultures adapted to thermophilic conditions was obtained. According to cultural and morphological characteristics, the cultures were assigned to the genera Methanopyrus and Methanococcus. The results of a study on the effect of the fermentation starter of methanogenic bacteria in fermented manure at 50°C showed that with an increase in the dose of the fermentation starter, the methane-forming ability of anaerobic bacteria increased and the process of methane biogas release intensified (from 0.36 m^3/kg to 0.79 m^3/kg). Besides, the dose of methanogenic fermentation starter based on Methanopyrus and Methanococcus isolates (28 kg) was determined. When the bioenergy plant is launched in thermophilic mode, the release of biogas increases by 2.2 times, and the digestion period decreases to 10 days.

Keywords: biogas, methane, cow manure, temperature, fermentation starter, isolate, Methanopyrus, Methanococcus.

INTRODUCTION

During the last decade, the livestock sector producing sour-milk products has been growing everywhere in the world, attracting close attention from an environmental and economic point of view [Chen et al., 2009; Padula et al., 2012; Ye et al., 2014]. Waste generated in animal husbandry contributes to the eutrophication of surface waters, the growth of pathogenic microflora, and the spread of diseases, and also damages soil and groundwater [Sun et al., 2016; Yeom et al., 2017; Song et al., 2020]. Utilization of animal husbandry waste in the form of manure can be implemented in various ways, of which the most common are traditional methods such as soil fertilization, composting and incineration [Macias-Corral et al., 2008; Astals et al., 2013; Zhang et al., 2019]. Soil fertilization leads to a huge loss of biomass, the release of large amounts of CO_2, the appearance of an unpleasant odor in the disposal sites, and contributes to outbreaks of fungal and viral infections leading to the spread of diseases, which is another serious environmental problem [Araji et al., 2001; Guerra-Rodrıguez et al., 2001; Ni et al., 2015]. Composting requires an appropriate surface area, a well-established control system to control the runoff of rainfall from the application area, leads to losses of available nitrogen, and greenhouse gas emissions, and is poorly suited for dry manure [Demirer and Chen, 2004; Xiao et al., 2013]. Combustion, on the other hand, has low energy value, low efficiency, and serious environmental disadvantages, including greenhouse gas emissions. Thus, it is very important to determine the optimal conditions for the use of the energy value of manure and waste and to avoid the associated environmental and economic problems [Almomani et al., 2017; Almomani et al., 2019].

Anaerobic methane digestion is the most promising technology for processing municipal and other types of organic waste [Eklind and Kirchmann, 2000; Jacobs et al., 2019; Ren et al., 2019]. The use of anaerobic digestion (AD) technology can significantly reduce the environmental burden on the environment and obtain valuable products, biogas, and biofertilizer. Methane, the main component of biogas, can be used to produce thermal and electrical energy [Khalid et al., 2011; Astals et al., 2012; Bolzonella et al., 2012]. Methanogenesis is a microbiological process. The only organisms capable of carrying it out are prokaryotes, i. e. archaebacteria, or archaea, of the genera Methanobacterium, Methanosaeta (Methanothrix), Methanococcus, Methanosarcina, Methanocorpusculum, Methanobrevibacteria and Methanopyrus [Fisgativa et al., 2016]. For them, methane digestion is the main or even the only source of energy [Schnürer, 2016].

Methane digestion includes three stages: hydrolytic (hydrolysis of polysaccharides in an environment of organic origin), acidogenic (digestion of monosaccharides formed in the previous stage to alcohols and then to short-chain volatile fatty acids (VFA), such as formic, acetic, propionic, butyric, and lactic) and the proper methanogenic one (acetoclastic methanogenesis, i.e. splitting of VFA to methane and carbon dioxide; another path of methanogenesis is the reduction of single-carbon molecules (carbon dioxide, formic acid, methanol) with molecular hydrogen, which is also a product of microbial metabolism). Methanogenic archaebacteria carry out only the final stage of the process: hydrolysis and acidogenesis are performed by eubacteria, anaerobic fungi, and protozoa [Moen et al., 2003; Zhang et al., 2008; Ahmadi-Pirlou et al., 2017].

Being a biological process, methanogenesis proceeds only under certain conditions that are most favorable for the microflora. Concerning temperature, there are three types of methanogenesis: psychrophilic (low-temperature), occurring at 10–14°C, mesophilic (medium-temperature) with the most favorable temperature range of 35–37°C, and thermophilic (high temperature), running at 50–55°C. Thermophilic (50–57°C) digestion has several significant advantages associated with an increase in the growth rate of microorganisms and the reactions carried out by them, and a deeper decomposition of organic matter(s) due to an increase in the solubility of organic compounds [Bolzonella et al., 2006; Kim et al., 2006]. The increased temperature of the process makes it possible to achieve disinfection of waste from pathogenic organisms (bacteria, viruses, helminth eggs), which is necessary for the further use of fermented biomass as a biofertilizer [Chen et al., 2008]. The purpose of this study was to evaluate the effect of the fermentation starter of methanogenic bacteria on the AD (AD) of cattle manure in a bioenergy plant (BEP). As a result of the study, a fermentation starter of mesophilic microorganisms adapted to thermophilic conditions will be obtained, which is the basis for the development of new energy-saving technology for the intensification of the methane digestion process.

MATERIALS AND METHODS

Objects of the study

The study was carried out at the Eurasian National University in the Experimental Design Department of the Department of Biotechnology and Microbiology, Astana, Republic of Kazakhstan, from 20.01.2011 to 20.01.2014. The object of the study was cattle manure selected from a livestock farm in the North Kazakhstan region.

Experimental studies were carried out on laboratory equipment, the designs of which provided for obtaining estimated indicators of the working processes of biogas plants under various modes of AD of cattle manure. We studied mesophilic and thermophilic digestion modes without and with the addition of fermentation starter, adapted to thermophilic conditions by methanogenic microorganisms. The technological process of AD of cattle manure can be divided into four main stages (Figure 1). The first stage is the preparation of fresh manure by mixing it with warm water, and homogenization. The second stage is loading fermented manure into the methane tank; creating optimal conditions for AD. The third stage is the process of anaerobic degradation of the fermented substrate. The fourth stage is the production of a by-product in the form of biogas. The fifth stage is the production of organic fertilizer.

Before the experiment, fresh cattle manure was diluted with warm water (70°C) for at least 1 hour to a humidity of 92–93%, to obtain a semi-liquid homogeneous mass. After cooling, the fermented substrate was loaded into the methane tank (1), through the side loading nozzle (2) (Figure 2). The volume of the substrate prepared for digestion was 0.1 m^3, which corresponds to filling 2/3 of the volume of the methane tank. Every day at the same time, the fermented substrate was manually mixed in the methane tank. The 25 mm gas outlet hose (6) was connected to the branch pipe (4) and treated with a sealant. A low-pressure compressor removed air through a gas hose (6) from the methane tank, and an anaerobic environment was formed. The end of the 25 mm exhaust hose (6) was lowered into the watergate (7). The process of formation of biogas in the methane tank was traced by the exit of gas bubbles in the watergate. The joints of the exhaust hose were also treated with a sealant. A 25 mm gas hose (8) was supplied from the watergate and connected to a GSB – 400 gas meter (5). From the gas meter (5), biogas was supplied to the dry gas tank (8), where it was stored and accumulated.

Preparation of laboratory fermentation starter of methanogenic bacteria

At the next stage of the study, a scheme for the preparation and use of a fermentation starter of methanogenic bacteria in mesophilic mode was developed (Figure 3). At the beginning of the process in unit 3, mesophilic methanogens were adapted to thermophilic conditions, and a fermentation starter was prepared that would accelerate the process of anaerobic decomposition of fresh cattle manure. In tank 2, the initial components of manure and water were homogenized, and fresh manure was prepared for processing. Next, an adapted mesophilic additive (AMA) was fed from plant 3 to methane tank 1, and prepared fresh manure was loaded from container 2 to the AMA. The biogas formed in the process (a by-product

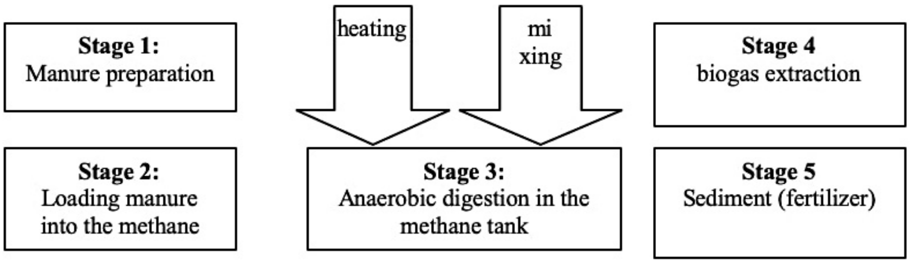

Figure 1. General diagram of the stages of anaerobic manure recycling

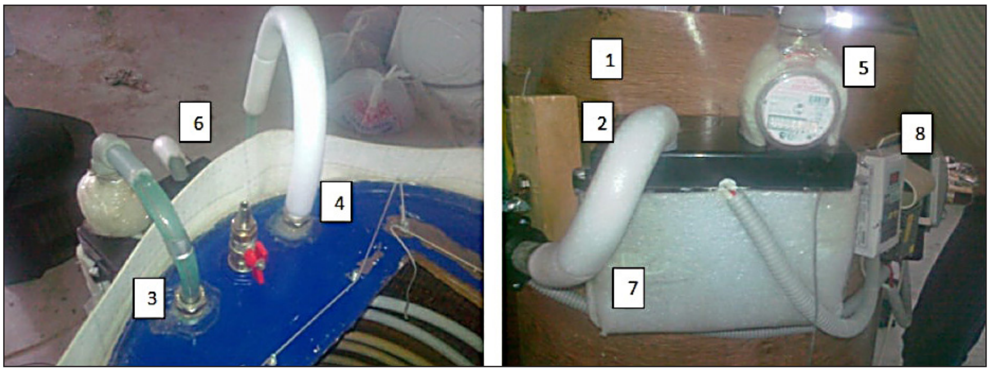

Figure 2. Technological scheme of an experimental biogas plant: 1 - a container for the working substrate (the methane tank); 2 - the heating tape; 3, 4 - the branch pipe for the output of biogas; 5 - the gas meter; 6 - the gas hose; 7 - the watergate; 8 - the dry gas tank

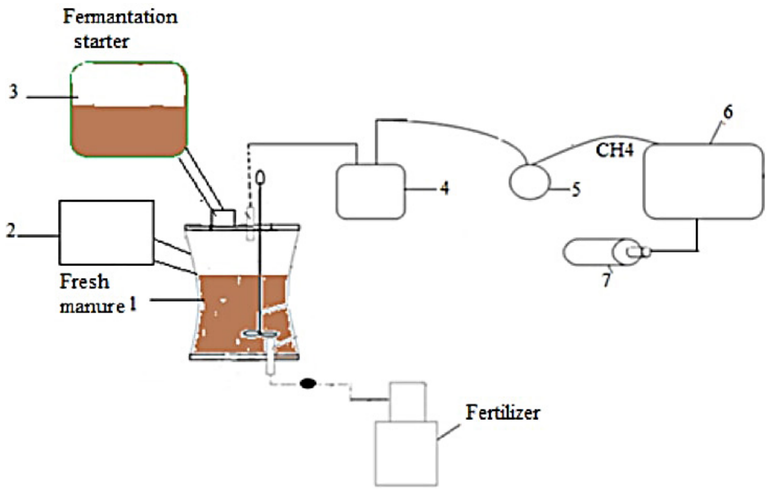

Figure 3. Fermentation starter preparation scheme: 1 - the methane tank; 2 - the container for homogenization of native manure with water; 3 - the adaptation unit; 4 - the dry gas tank; 5 - the filter; 6 - the high-pressure compressor; 7 - the gas cylinders

of AD) was collected in a dry gas tank 4, from where it was sucked by a high-pressure compressor 6. At that time, it passed through a filter with zeolite filler 5 and was cleaned of harmful impurities, such as hydrogen sulfide, carbon dioxide, and water vapor. The purified biogas compressor 6 was loaded into gas cylinders 7 and used as motor fuel. Mixing was carried out with a stirrer daily, at the same hour. The main purpose of mixing is the release of the formed biogas, mixing of the substrate and bacteria, preventing areas of different temperatures inside the methane tank, ensuring uniform distribution of bacterial populations, and preventing the formation of voids and clusters that reduce the effective area of the methane tank.

Method of adaptation of mesophilic microorganisms in a BEP

AMA can be obtained directly in the methane tank itself. The methane tank is started in mesophilic mode, the initial digestion temperature is 35°C. This corresponds to the temperature in the gastrointestinal tract of animals and is natural and comfortable for the growth, development, and production of mesophilic methanogens. When the produced biogas begins to support the process of steady burning, depending on the quality of the flame, we proceed to a gradual increase in the digestion temperature. As a result, gradually, from day to day, increasing by 0.5°C, the digestion temperature is brought to 50°C. Thus, we obtain mesophilic methanogenic microorganisms adapted to thermophilic conditions [De la Rubia et al., 2013; Ho et al., 2013; Nikitina, 2018].

Microbiological research methods:

The morphology of methanogenic bacteria was studied by preparing preparations stained with methylene blue followed by AxioLab microscopy.A1 and Axiolmager D1 (Carl Zeiss, Germany) with a phase-contrast device, in an immersion system with a lens 90 applied with a drop of cedar oil. The methanogenic biomass of cultures was obtained by sequentially subculturing on a Pfennig medium [Pfennig, 1965]. Isolation of pure cultures was carried out using the method of serial dilutions, seeding on a dense nutrient medium (a modified Hungate technique) [Hungate, 1969]. The description of the new bacterium was carried out according to the generally accepted scheme. Cumulative cultivation of methanogens was carried out anaerobically in 100 ml vials. Methanogenic cultures were obtained by adding 900 ml of anaerobic medium to 100 ml of fermented cattle manure. Accumulative cultures were incubated at a temperature of 35°C for 7–10 days.

Chemical methods

The methods for determining humidity and ash content; the moisture content of the fermented manure was taken at 97%. With this humidity, the manure has good fluidity, and the operation of loading it into the methane tank is facilitated. The humidity of fresh liquid manure was 85–86%. To create the necessary humidity of 93%, fresh manure was diluted with warm tap water with a temperature of 70°C. The total dry matter content in the samples was determined after drying to a constant mass at 105°C. The ash residue was determined after burning a dry sample in a muffle furnace to a constant mass at 650°C. The organic matter was calculated as the difference between the dry weight of the sample and the ash residue. The content of gaseous products (H_2, CH_4, CO_2) and VFA was determined on a Chromatek-Crystal 5000.2 chromatograph. The pH was measured using a 320 pH meter (WTW, Germany).

Statistical processing of results

For an objective assessment of the experimental data obtained, their mathematical processing was carried out according to the results of 3–4 repetitions. The studied indicators were processed by methods of mathematical statistics and correlation analysis using computers, using Excel, Statistica 6, and MathCAD software. The reliability of the differences between the mean values was evaluated using the Student's *t*-test ($p \leq 0.05$).

RESULTS AND DISCUSSION

Parameters of the manure utilization process in mesophilic and thermophilic periodic modes

The main prerequisite for the smooth flow of the digestion process of organic waste with a high dry matter content is the complete immersion of solid particles into the liquid. As this liquid, a mixture of fresh manure with water with a humidity of 93–95% was used. Water in manure plays several roles in the production of biogas: (a) it is necessary for metabolic processes [Cox, 1993]; (b) water provides the necessary environment for the transport of nutrients and allows microorganisms to move [Rynk, 1992]; (c) water can displace air from porous spaces, which leads to the formation of anaerobic areas in the material, which improves AD. Consequently, since cow manure (CM) has a high water content compared to other waste studied, the receipt of significant biogas production was confirmed [Agnew and Leonard, 2003].

For the digestion of liquid manure to improve the conditions for the passage of hydrolysis stages, it is advisable to preheat the manure. In the works of Sambo et al. [1995], it was shown that the joint digestion of a mixture of CM and water at 50°C gave an optimal biogas yield, followed by 60 and 40°C. Mahanta et al. [2004] reached the maximum production of biogas from a mixture of manure and water of cattle at 35°C, and then at 45, 30, and 40°C, respectively. In another study [Song et al., 2020], the production of biogas at different temperatures from different substrates was tested. Table 1 shows the main indicators of liquid cattle manure used in experimental studies in mesophilic and thermophilic digestion modes.

After homogenization, the substrate temperature averaged 35–40°C. Therefore, at the initial stage of the start-up in mesophilic mode, no additional heating was required, and in thermophilic mode, the temperature of the methane tank was gradually increased to 50°C and automatically maintained with a deviation of ± 0.5°C. In addition, there was a sufficient concentration of organic substances in liquid manure (4.62; 3.38) for the growth of syntrophic and acetogenic bacteria, which would intensify the process of the development of anaerobic methanogenic microorganisms.

The study of the process of digestion of cattle manure in mesophilic and thermophilic mode

During digestion, dry organic matter breaks down and is converted into biogas. According to the latest scientific works in this field by F.A. Tassew, W.H. Bergland, and others [Dahunsi et al., 2016; Tassew et al., 2020], temperature plays an important role in the process of AD, since it controls the rate of microbial metabolism in anaerobic environments. The results of several studies show that there is a positive relationship between temperature and biogas production [Appels et

Table 1. Indicators of fermented manure at different temperatures

Parameters of fermented manure	Variants of the experiment	
	Mesophilic mode	Thermophilic mode
Substrate humidity, %	93	95
Dry matter content, kg	5.75	4.25
Organic matter content, kg	4.62	3.38
pH value	6.8	7.0

al., 2011]. There are three different temperature ranges in which AD can be processed: psychrophilic (< 30°C), mesophilic (30–40°C), and thermophilic (50–60°C) [Yadvika et al., 2004]. According to recent studies in this area [Xu et al., 2018; Wainaina et al., 2019], the concentration of VFA for CM was in the range of 112 ± 2 to 119± 2 mg/l with 20% propionate, 55% acetate, the rest being butyrate. In solid agricultural waste (SAW), the content of VFA range from 70 ± 2 to 85 ± 2 mg/l. A distinctive feature of these works is the preliminary chemical treatment with $NaHCO_3$, which improves the biodegradability of the substrate compared to the untreated substrate under mesophilic and thermophilic conditions.

We studied the formation of VFA, the content of gaseous products (CH_4, CO_2, H_2), and the pH of the medium in liquid manure at 35°C and 50°C. The results shown in Figure 4 demonstrate that on the 5th day of AD at t-35°C, the formation of VFA, namely acetic, propionic, and butyric acid, is observed. The formation of VFA in the bio plant gradually increased and the amount of acetic acid rose from 0.12 to 0.54 mg·l^{-1}, the concentration of propionic acid from 0.08 to 0.31mg·l-1, and the concentration of butyric acid increased from 0.07 to 0.29 mg·l^{-1} during 35 days of AD. It should be noted that the formation of acetic acid plays the most important role in the speed of the methane formation process. By the end of the experiment, the pH value was maintained at 6.8–7.2. During AD, after the formation of organic acids, the transformation of VFA (acetic, butyric, propionic acid) into precursors of methane occurred.

A gradual accumulation of methane concentration (CH_4) from 0.08 to 0.45 m^3/kg, carbon dioxide (CO_2) from 0.05 to 0.38 m^3/kg, hydrogen concentration (H_2) from 0.01 to 0.08 m^3/kg was observed in the bio plant in the recycled cattle manure. All these reactions in the process of methanogenesis happened simultaneously and the natural microflora of manure played an important role in the formation of methane biogas. In the work of Liu et al. [2018], greater efficiency of biogas production was also observed as a result of the joint digestion of livestock waste and agricultural waste in the mesophilic temperature range. Our further research was aimed at studying the process of AD at t-50°C. As can be seen in Figure 5, the formation of VFA in the thermophilic mode is higher compared to the mesophilic mode.

The amount of acetic acid in the liquid phase ranged from 0.29 to 0.69 mg·l^{-1}, the concentration of propionic acid from 0.13 to 0.52mg·l^{-1}, and the content of butyric acid from 0.08 to 0.36mg·l^{-1} during the 35 days of digestion. On the 35th day of digestion, the pH level increased from 6.2 to 7.3, which is favorable for microorganisms of the methanogenic community. After the decomposition of VFA, products of microbial metabolism were formed [Garmash, 2013; Druzyanova, 2014]. Biogas is a mixture of methane and carbon dioxide in ratios from 30:70 [Kendall et al., 2002] to 60:40 [Huang and Crookes, 1998; Van Herle et al., 2003]. In Francese et al. [2000], the ratio of methane to carbon dioxide reached 73.6:26.4, in Kim et al. [2003], 5,037 liters of biogas released from 1 m3 of medium contained 3,367 liters of methane. The highest methane content in biogas (83–85%) is presented in the work of Filidei et al. [2003]. In addition to methane and carbon dioxide, biogas contains other gaseous products, the concentration of which depends on the composition of the media. In particular, molecular

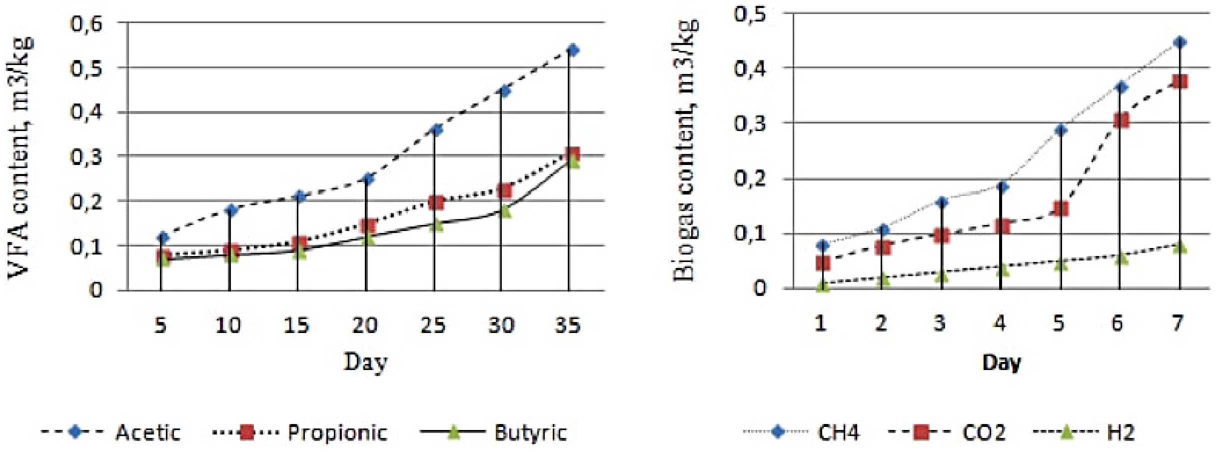

Figure 4. Dynamics of the content of VFA and biogas at 35ºC

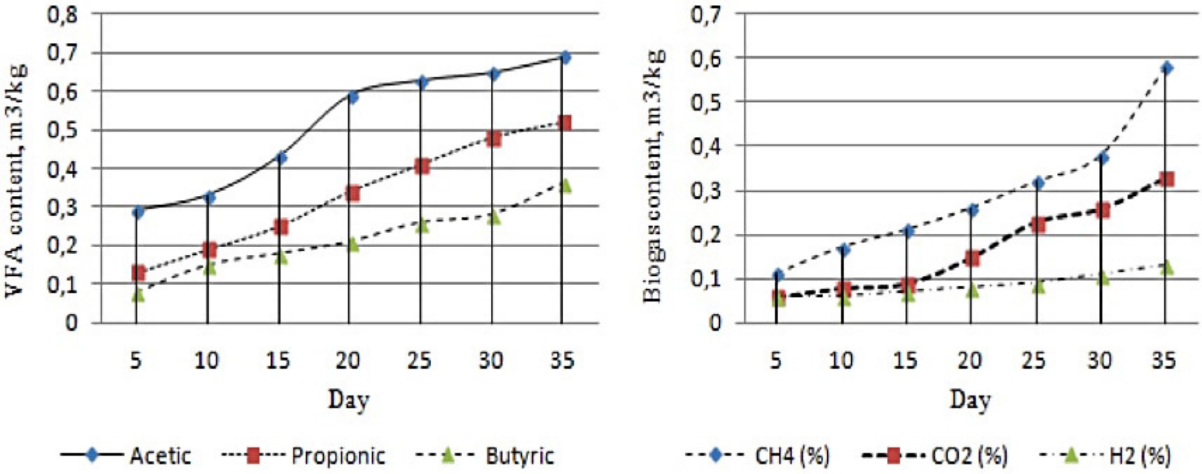

Figure 5. Dynamics of VFA and gas content at 50ºC during 35 days of digestion

hydrogen is an intermediate product of microflora metabolism. On the 5th day of the bio plant launch, biogas appeared in the methane tank. With an increase in temperature, the amount of methane biogas decreased compared to the mesophilic mode. Accumulation of high concentrations of methane (CH_4) from 0.11 to 0.58 m^3/kg, as well as carbon dioxide (CO_2) from 0.06 to 0.42 m^3/kg and hydrogen (H_2) from 0.06 to 0.12 m^3/kg during the 35 days of digestion, were observed in the bio plant. The pH level ranged from 6.3 to 7.1.

In the work of Chun Mei Liu et al. [2018], it was shown that temperature had different effects on the AD of various biomasses, which could cause changes in microbial communities. The relationship between the microbial community and methane production at 35°C, 38°C, 41°C, and 44°C was analyzed during corn straw (CS) AD. The results showed that the daily production of biogas and methane at t-44°C was 16.6–42.4% and 16.2–40.6% higher than at t-35, 38, and 41°C, respectively. O. El Asri et al. [2020] showed that after 30 days of incubation, cow waste released the largest amount of biogas (260 ml/g^{-1}) compared to other studied organic waste (chicken manure, horse waste). In [Yu et al., 2018], AD was carried out using rice straw at various temperatures and the biogas yield was -401.9 m^3/t (dry weight of straw). Thus, there are various technologies for obtaining biogas from various types of waste. A comparison of the results obtained by us on the study of biogas with the potentials proposed by other researchers shows that the objects under study depend on the type of raw materials, methods of their production, and the characteristics of their properties.

Preparation and isolation of fermentation starter of methanogenic bacteria in thermophilic mode

Microorganisms in organic waste play an important role in providing an increase in the reaction kinetics of this process, and they also play a role in the secretion of a hydrolytic enzyme for biodegradation. The co-authors of [Mulka et al., 2016] indicated that bacterial and archaeal communities with the addition of straw during AD of manure at different temperatures (37°C, 44°C, and 52°C) led to an increase in the relative number of methane bacteria. The genera Firmicutes and Bacteroidetes prevailed among the bacteria in all samples. It was also shown in other works that an increase in the operating temperature reduced the species richness for both archaea and bacteria, as well as the uniformity of bacteria. The number of taxonomic units on the samples varied from 12 to 25 for archaea and from 112 to 277 for bacteria.

The results of our microbiological studies show that 2 types of cultures of methanogenic bacteria were detected in the thermophilic mode (Figure 6). The results of microscopic studies have shown that activated cultures of methanogenic bacteria in the final stages of the process are characterized by high biochemical activity and contain a large number of viable cells of methanogenic bacteria, 10^9–10^{10}/ ml. As a result of successive subculturing, two isolates of methanogens were isolated: isolates A and B.

Description of isolated isolates

Isolate A was isolated from semi-liquid cattle manure. Large rods, movable, 0.9×1.8–3.0 microns. Gram-negative, does not form endospores.

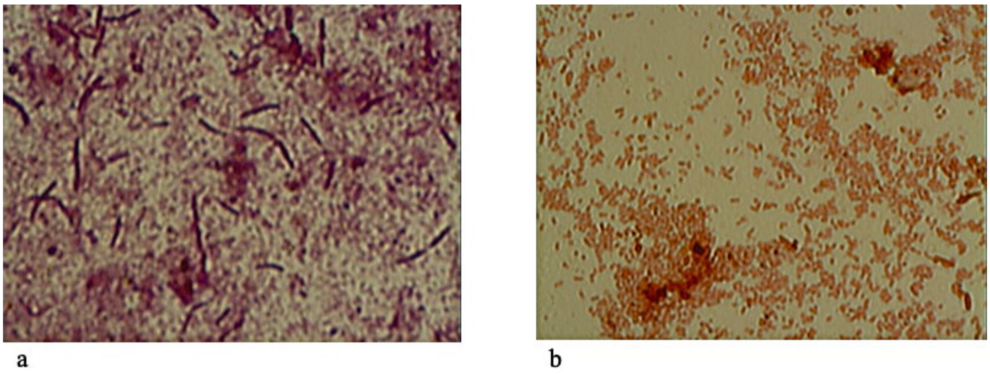

Figure 6. Microstructure of isolates isolated from fermented manure (50ºC)

Receives energy by reducing CO_2 to CH_4, using H_2 as electron donors. Acetate, methylamines, and methanol are not used. The optimal growth temperature is 40–55°C and the pH is 6.1–6.9. Isolate B was isolated from semi-liquid manure with a humidity of 90%. Strictly anaerobic. Cocci of irregular shape. Gram-negative. The substrates for the formation of CH_4 are H_2 and CO_2. The source of nitrogen is ammonia, and the source of sulfur is sulfide. Optimal conditions for growth: temperature 42–50°C and pH 6.6. According to cultural and morphological features, isolate A is assigned to the Methanopyrus genus, and isolate B is assigned to the Methanococcus genus.

Adaptation of mesophilic microorganisms to thermophilic conditions in the bio plant

It has been established that the process of degradation of organic waste mass is stimulated by the addition of special types of bacteria (acetogenic and methanogenic ones) to manure, which intensifies the process by more than 2 times. In several works [Tanimu et al., 2015], AD on food waste (FW) and CM using Pseudomonas aeruginosa as inoculum was evaluated. FW and CM were fermented together as a substrate with bacteria (*P. aeruginosa*). As a result of the study, it was shown that FW from 1 kg of CM produced the largest amount of biogas with a total volume of 88.5 g/kg. The highest concentration of biogas was found in a bioreactor consisting of 2 kg CM and 2 kg FW with a methane content of 52% and 48% CO_2. Our further research was directed to the use of isolated isolates A and B in the form of a fermentation starter adapted to thermophilic conditions at 50°C. To do this, the optimal parameters of the bio plant were determined when an adapted starter was added to the fermented manure of a periodic methane tank in a thermophilic mode (50°C) (Table 2).

In the first variant, biogas appeared on the ninth day after loading manure (Figure 7). On the 15th day, it qualitatively supported the burning process, which indicates the establishment of a stable AD mode inside the methane tank. From that day on, the volume of combustible gas increased for 9 days. On the 25th day of digestion, 0.36 m^3/kg of biogas was formed. From the 30th day of operation, the output of biogas decreased, but did not stop and at the end of the experiment equaled 0.08 m^3/kg. In the second experiment, biogas appeared on the 7th day after loading the substrate. On the 15th day, the biogas burned well. From that day on, the volume of combustible gas increased for 10 days and amounted to 0.57 m^3/kg. From the 30th day, the reduction of biogas began and reached 0.07 m^3/kg. In the third experiment, biogas appeared on the 7th day after loading the methane tank. On the 15th day, the gas burned steadily. From that day on, the volume of biogas increased for 10 days and the output of biogas was 0.65 m^3/kg. From the 30th day, the reduction of biogas began but did not stop. At the end of the experiment, the daily biogas yield was 0.05 m^3/kg. In the fourth experiment, biogas appeared on the 5th day. On the 10th day, it maintained a steady burning. The volume of gas increased for another

Table 2. Experiment scheme

Item	The volume of fermentation starter, kg	The volume of the loaded fresh manure, kg
Variant 1	15	85
Variant 2	19	81
Variant 3	22	78
Variant 4	28	72

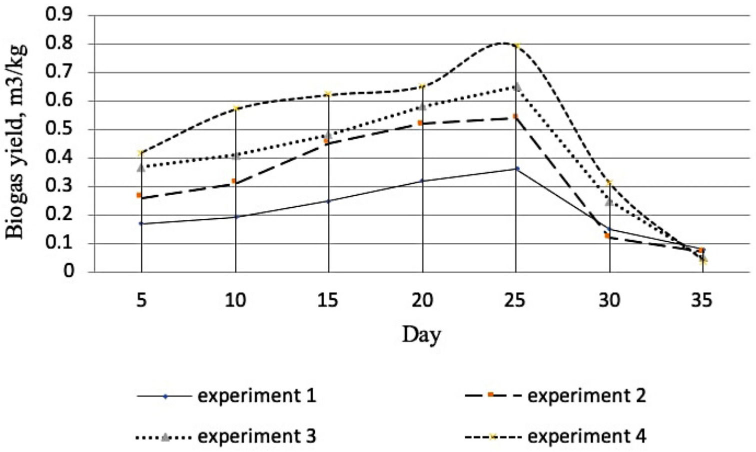

Figure 7. Effect of the dose of the fermentation starter of methanogenic bacteria on the activity of biogas release at a temperature of (50 ± 1)°C: 1 – 15 kg; 2 – 19 kg; 3 – 22 kg; 4 – 28 kg

10 days, and the output of biogas was 0.79 m^3/kg. From the 25th day of work, the daily output began to gradually decrease and on the 35th day, it equaled 0.04 m^3/kg.

Thus, the following results were obtained regarding the increase in the gas formation rate: in the first experiment, the volume of the starter was 15 kg and the yield of methane biogas was 0.36 m^3/kg; in the second experiment, the volume of the starter was 19 kg and the yield of biogas was 0.57 m^3/kg, in the third experiment, the volume of the starter was 22 kg and the yield of biogas was 0.65 m^3; in the fourth experiment, the volume of the starter was 28 kg and the biogas yield was 0.79 m^3/kg during the 25 days of AD. The volume of added fermentation starter proportionally increases the speed of the process of AD of fermented manure, as evidenced by the appearance of biogas in the biological energy plant (BEP). The obtained study results indicate that with an increase in the dose of fermentation starter, the methane-forming ability of methanogenic bacteria increases and the process of biogas release intensifies. The presence of a significant number of relevant microorganisms, such as fermentation starters, allows increasing the rate of decomposition of organic waste, improving biogas production, shortening the start-up time, and making the digestion process more stable.

CONCLUSION

The conducted experimental work allowed us to draw the following main conclusions. It was found that during AD of cattle manure in the mesophilic mode, the content of VFA and the pH of the medium were in the optimal range. The amount of acetic acid was 0.54 $mg \cdot l^{-1}$, propionic acid 0.31 $mg \cdot l^{-1}$ and butyric acid 0.29 $mg \cdot l^{-1}$, methane biogas accumulation (CH_4) equaled 0.45 m^3/kg, CO_2 0.38 m^3/kg, hydrogen level (H_2) was 0.08 m^3/kg. In the thermophilic mode, the amount of acetic acid was 0.69 $mg \cdot l^{-1}$, propionic acid 0.52 $mg \cdot l^{-1}$, and butyric acid 0.36 $mg \cdot l^{-1}$, pH levels ranged from 6.2 to 7.3. The biogas yield was the following: CH_4: 0.58 m^3/kg, CO_2: 0.42 m^3/kg, hydrogen (H_2): 0.12 m^3/kg. Based on AD in mesophilic mode, a fermentation starter of methanogenic cultures adapted to thermophilic conditions was obtained. According to cultural and morphological features, the cultures were assigned to the Methanopyrus and Methanococcus genera. The effect of the fermentation starter of methanogenic bacteria on the AD of cattle manure under the thermophilic mode was studied. The results of the study showed that with a gradual increase in the dose of fermentation starter, the intensity of biogas release increased. In the first experiment, the yield of methane biogas was 0.36 m^3/kg; in the second experiment 0.57 m^3/kg, in the third experiment 0.65 m^3/kg; and in the fourth experiment 0.79 m^3/kg during the 25 days of AD. The obtained study results indicate that with an increase in the dose of fermentation starter, the methane-forming ability of methanogenic bacteria increases and the process of biogas release intensifies. Besides, the dose of thermophilic fermentation starter based on Methanopyrus and Methanococcus isolates (28 kg) has been determined. When the BEP is launched in thermophilic mode, the release of biogas increases by 2.2 times, and the digestion period decreases to 10 days.

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