The Influence of Various Factors on the Methane Fermentation Process

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Abstract. The article describes the stages of the methane fermentation process. The phases of methane formation are characterized. The results of the experimental data based on the study of various factors influencing the rate of biogas production and its yield are presented. Such factors as the size of the substrate particles and temperature conditions in the reactor are considered. It is revealed on the basis of experimental data which of the farm animals and poultry excrements are exposed to the most complete fermentation without special preparation. The relationship between fermentation regime, particle size of the feedstock and biogas yield is graphically presented.

Nowadays the problem of waste disposal is very important. In particular the problem of animal wastes and plants residues having a negative impact on the environment should be solved. In different countries this problem is solved in different ways. For example some farmers try to utilize such wastes by spreading them on to their fields as fertilizers. But few farmers pay attention to the fact that the application of fresh manure to land leads to the infection by helminths and other pathogens. The sanitary and epidemiological norms are violated. Furthermore unfermented manure contains a large amount of ammonia and other nitrogen compounds. Getting into the soil, they began to react with carbon dioxide and compounds contained in the soil. As a result, the compounds leading to the formation of nitrous oxide and various substances are formed. Burning the soil, they unfavorably affect it. As these compounds are volatile, a part of them is concentrated in the atmosphere breaking the ozone layer. As a result, the environmentalists have to impose fines and various types of penalties on farmers. Every year these penalties become higher [1-3, 8, 9].

Biotechnology of biogas production and accompanying products is promising from an environmental point of view. Biogas is a product of the microorganisms’ metabolism and it is formed by the decomposition of the organic substrate. The decomposition process can be divided into four stages and at each stage different groups of microorganisms are involved in the process [10]. Methane fermentation process has three phases (Fig. 1).

At the first phase the aerobic bacteria convert macromolecular organic substrates (proteins, carbohydrates, fats, cellulose) using enzymes to lower molecular weight compounds such as sugars, amino acids, fatty acids and water. Enzymes isolated by hydrolytic bacteria attach to the outer wall of bacteria and break organic components of the substrate into small soluble components. The polymers
are converted into oligomers and monomers. This process known as hydrolysis proceeds slowly and depends on extracellular enzymes such as cellulase, amylase, protease and lipase. This process is influenced by the level of pH from 4.5 to 6.0 as well as the time in the tank.

At the second phase of biomethanogenesis acid-forming bacteria are engaged in splitting. Individual molecules enter into the cells of bacteria where they continue to decompose. Anaerobic bacteria are partially involved in this process using oxygen residues and forming anaerobic conditions required for methane microorganisms. At pH from 6.0 to 7.5 primarily fatty acids, low molecular weight alcohols such as ethanol and gases such as carbon dioxide, hydrogen sulfide and ammonia are produced. This phase is called oxidation. At the same time the level of pH is lowered. The intracellular conversion of simple sugars causes the formation of pyruvic acid that is a key intermediate product of metabolism (carbohydrates, glycerol, amino acids). Ammonia appears as a result of amino acid decomposition by bacteria and hydrogen sulfide appears in the case of sulfur-containing amino acids decomposition. Hydrolysis products of fat are used by many kinds of acid-forming bacteria. During the enzymatic reactions glycerol is converted into phosphoglyceraldehyde that then takes part in the carbohydrate metabolism.

**Figure 1 Phases of the methane fermentation process**

At the third phase, the acid-forming bacteria produce the starting substrates such as acetic acid, carbon dioxide and hydrogen for the formation of methane from organic acids. It should be noted that the bacteria reducing the amount of carbon are very sensitive to temperature.

The stage of alkaline fermentation characterizing this phase is carried out by methane-bacteria. During the fermentation of acetic acid and methanol the methane is synthesized as a result of methyl group recovery.

\[
\text{CH}_3\text{COOH} \rightarrow \text{CH}_4 + \text{CO}_2 \quad (1)
\]

\[
4\text{CH}_3\text{OH} \rightarrow 3\text{CH}_4 + 2\text{CO}_2 + 2\text{H}_2\text{O} \quad (2)
\]
Another mechanism of methane formation is a characteristic of those types of methanogenic bacteria which are not capable of utilizing acetic acid and methanol. These bacteria synthesize methane by carbon dioxide recovery:

\[ 4\text{AH}_2 + \text{CO}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O} + \text{A} \quad (3) \]

In the process of methane formation more complex substances such as butyric acid, propionic acid, caproic acid are involved. The conversion is performed according to the type of reaction (3), in which instead of molecular hydrogen the listed organic substrates are involved. For example, while using ethanol is oxidized up to \( \text{CH}_3\text{COOH} \) with simultaneous recovery of carbon dioxide on the type of reaction:

\[ 2\text{CH}_3\text{CH}_2\text{OH} + \text{CO}_2 \rightarrow \text{CH}_4 + 2\text{CH}_3\text{COOH}. \quad (4) \]

These reactions take place at the same time but the methane-forming bacteria are more demanding to the conditions of their existence than the acid-forming bacteria. For example, they require an anaerobic environment and a long time for reproduction. The speed and extent of anaerobic fermentation of methane-forming bacteria depend on their metabolic activity.

A great variety of microorganisms according to some sources up to thousands of different types are involved in this complex set of transformations but the most important of them are methane-forming bacteria. Methane-forming bacteria multiply much slower and they are more sensitive to environmental changes than the acid-forming microorganisms. So initially volatile acids accumulate during the fermentation process and the first stage of methane fermentation is called acid stage.

Further, the speed of acids formation and processing are aligned so that further substrate decomposition and gas formation proceed simultaneously. And it should be mentioned that the intensity of gassing depends on the conditions which are created for the methane-forming bacteria living.

Acid-forming and methane-forming bacteria are found throughout in nature, in particular in animal excrements. It is believed that cattle manure has all the necessary microorganisms for its digestion. Consequently, there is no need to use pure cultures of methane-forming bacteria to cause a fermentation process for biogas production. It’s enough to provide the following conditions for the existing bacteria in the substrate: maintenance of anaerobic conditions in the reactor; maintenance of temperature; the availability of nutrients for bacteria; choice of the right time of fermentation and timely loading and unloading of raw materials; compliance with the acid-base balance; compliance with ratio of carbon and nitrogen; choice of the right humidity of raw materials; regular mixing process and absence of inhibitors.

These parameters influence the different types of bacteria involved in the three stages of methane formation in different ways.

At the last fourth phase methane, carbon dioxide and water are produced. 90% of the total methane is produced in this phase, 70% is formed from the acetic acid. Thus, the formation of acetic acid (3 phase of splitting) is the determinant of the methane production speed. Methanogenic bacteria are anaerobes. The optimum pH is 7.0 and the amplitude of the oscillation can be from 6.6 to 8.0.

Splitting of organic matter into individual components and conversion into methane can take place only in a humid environment, because microorganisms can convert substances only in a solute form.

There are different ways to intensify the process of methane fermentation. Among them are the using of catalysts of different nature, the choice of optimal temperature conditions, grinding of raw materials [4-7].

The aim of this work was to study the composition of farm animals and poultry excrements as well as the study of the methane fermentation process under the influence of various factors.

To achieve this goal the following problems were solved:
- to investigate the chemical composition of farm animals and poultry excrements in various farms of Kemerovo region;
- to investigate the biogas formation speed in accordance with the degree of raw materials crushing;
- to investigate the methane fermentation process of farm animals and poultry wastes at different temperatures.

**Objects of research**
At different stages the objects of research were: cattle manure (national standard 26074-84); pig manure (national standard 26074-84); chicken manure (national standard 26074-84); ferrous sulfate II (national standard 6981-94); biogas produced in the pilot plant.

**Research Methodology**
Experimental studies were carried out in accordance with modern research methodology of complex phenomena by means of conventional, standard and original methods of biochemical, physicochemical, structural and mechanical analysis using the latest achievements in science and technology. Sampling and preparing the samples for the analysis were carried out in accordance with national standard 26712-85. The mass fraction of moisture and solids in manure was determined according to national standard 26713-85. The mass fraction of ash was determined according to national standard 2671-85. The mass fraction of organic matter in manure was determined according to national standard 27980-88 by thermogravimetric method. The pH indicators of the object and the environment were determined in accordance with national standard 27979-88. The mass fraction of total nitrogen in terms of dry matter was determined in accordance with national standard 26715-85. The mass fraction of phosphorus in terms of dry matter was determined in accordance with national standard 26717-85 by photometric method. The mass fraction of potassium in terms of dry matter was determined in accordance with national standard 26718-85 by flame photometric method. The mass fraction of ammonium nitrogen in terms of dry matter was determined in accordance with national standard 26716-85 by the Kjeldahl method.

**Results and discussion**
In cattle-breeding complexes of the Kemerovo region the keeping of cattle without litter is used more often and the slurry is used to remove excrements. In spite of this, the content of the straw in manure is from 5 to 15% of the total weight. The average yield of excrements per one head of farm animals of different categories is presented in Table 1.

| Table 1 - The average daily amount of farm animals’ excrements (per head) |
|-----------------|-----------------|-----------------|------------------|
| Cattle          | Excrements yield, kg | Livestock of pigs | Excrements yield, kg |
| Sires           | 40               | Breeding boars   | 11.1             |
| Cows            | 55               | Weaned piglets   | 2.4              |
| Calves from 4 to 6 months for fattening | 7.5                          |
| Young replacement cattle, months. 6-12 | 14       | Sows: barren     | 8.8              |
| 12-18 and heifers | 27       | bred             | 10.0             |
| Store cattle, months. 4-6 | 14       | milking with litter | 15.3           |
| 6-12            | 26               | Fattening pigs and young replacement pigs of live weight, kg |     |
| older than 12   | 15               | up to 40         | 3.5              |
|                 |                   | 40-80            | 5.1              |
|                 |                   | more than 80     | 6.6              |
The most common way to keep pigs is to use straw bedding (in some cases - sawdust). The percentage of straw in manure is usually from 20 to 40%. Poultry is also kept with the help of the bedding materials such as straw, sawdust, rarely sand. They are mixed with manure and make up 50% of manure weight.

Table 2 shows the average data on the chemical composition of livestock manure and poultry droppings contained in various types of Kemerovo region farms.

| Parameter                                                                 | Unit          | Test results          |
|---------------------------------------------------------------------------|---------------|-----------------------|
|                                                                           | Cattle manure | Pig manure            | Poultry droppings |
| Moisture content                                                          | %            | 85.5                  | 72.9              | 38.6              |
| Ash content                                                               | %            | 13.3                  | 8.6               | 16.0              |
| Mass fraction of organic matter                                           | %            | 86.7                  | 91.4              | 84.0              |
| pH                                                                        | pH unit       | 7.3                   | 6.3               | 7.2               |
| Total nitrogen (in terms of dry substance) not less than                  | %            | 1.95                  | 2.37              | 3.15              |
| Mass fraction of phosphorus (in terms of dry substance) not less than     | %            | 0.84                  | 2.83              | 5.35              |
| Mass fraction of potassium (in terms of dry substance) not less than      | %            | 0.82                  | 0.15              | 0.94              |
| Mass fraction of ammonia nitrogen (in terms of dry substance) not less than| %            | 0.78                  | 0.49              | 0.61              |

To produce biogas the most important indicator of the suitability of the soil is the presence of organic substance in it. According to research of livestock manure (Table 2) in all kinds of livestock and poultry manure there is enough organic substance to ensure the normal activity of microorganisms involved in the production of biogas. Another important criteria is the pH level. While both hydrolyzing and acid-forming bacteria in the acidic environment with pH level from 4.5 to 6.3 reach the optimum of its activity, bacteria forming acetic acid and methane can live only at neutral or slightly alkaline level with pH from 6.8 to 8. So, the most enabling substrate for biogas yield is livestock and poultry manure, which has a slightly alkaline reaction.

Besides, the humidity of the environment has a great importance for normal metabolism of aceto- and methanogenic microorganisms. Methane-oxidizing bacteria can live and reproduce when substrates are soluble in water (at least 50% of the composition of water). The most enabling humidity for the development of methanogens is from 85 to 92%. In livestock manure this requirement is maintained (humidity is 85.5%), so this fact significantly reduces the cost of additional moisturing of the substrate.

For further study we have chosen bedding and non-bedding liquid cattle manure that was brought from Kemerovo region farms. Duration of one experiment has lasted for 25 days. For the purity of the experiment additional portions of raw materials were not added to the samples under investigation. Taking into account the harsh continental climate prevailing in the Kuzbass, we selected two modes of fermentation. They are mesophilic (30-35 °C) and psychrophilic (15-20 °C) modes.

The size of the solid particles of substrate is essential. The larger the interaction area for bacteria and the more fibrous substrate the faster it decomposes under the bacteria's action contained in the cattle or poultry manure. As in the selected material for the study there was a bedding material which composed 12-15% by weight of the substrate, it was milled to the size of 20-25, 30-35 and 40-45 mm before introducing the substrate into the fermenter. The size of particles depended on the time of milling. The size of the solid particles was measured by a ruler and caliper after they were washed under running water through the sieve and after drying the dry solid in air.
The impact of particle size on the biogas-release rate from cattle manure at mesophilic and psychrophilic fermentation modes are shown in Figure 2.

![Image of Figure 2 showing the effect of particle size on biogas yield]

Figure 2. The effect of the particle size of the dry components of manure on the rate of release of biogas

The analysis of the results presented in Figure 2 showed that the first portions of gas at mesophilic fermentation mode is released on the third day, what is more with the particles size of 20-25 mm there was observed maximum biogas yield from 3 to 6 days. With further fermentation the amount of generated biogas was significantly decreased. This can be explained by the fast decomposition of the substrate affected by the bacteria.

The maximum biogas yield was observed at mesophilic mode and with the use of particles sized 30-35 mm. Moreover the period of the gas production increased compared with the first variant for 7-10 days. With the size of particles 40-45 mm the period of active gas production was 19 days but there was minimal amount of released biogas. This is probably due to the fact that solid particles of larger size make redistribution of bacteria and intermixing substrate more complicated and worsen gas yield.

At the psychrophilic fermentation mode the beginning of the gas production was noted on the fifth day, and the optimal size of the solid particles was 20-25 mm. The increasing of the solid particles size to 30-35 mm and 40-45 mm reduces the process of the gas outlet to 10-20 ml/l of substrate. The first portions of the gas at psychrophilic fermentation mode and the amount of solid particles of 30-35 mm were observed on the seventh day which is four days longer than it was during the mesophilic fermentation mode. The increasing of the solid particles size to 40-45 mm affected the speed and volume of gas production.

Thus, for optimal psychrophilic fermentation mode the size of the solid particles in the manure was 20-25 mm and for mesophilic mode it composed 30-35 mm.

For further studies we defined the task to compare the amount of produced biogas from excrements of cattle, pigs, and its mixture in a ratio of 1:1 at mesophilic and psychrophilic fermentation modes.
and the same humidity of raw material at 88%. The process of gas yield from various substrates is shown in Figure 3.

Figure 3. Biogas yield per 1 g of dry organic substances in accordance with the type of substrate

The analysis of the results of this study showed that the gas yield per unit of time at first increased rapidly, and then, after reaching a maximum, it gradually reduced both at mesophilic and psychrophilic fermentation mode. It should be noted that biogas outlet from cattle manure with adding of bedding was larger by a mean of 10.32%.

On the fifth day already the biogas yield in accordance with the used substrate at mesophilic fermentation was 120 cm$^3$ for cattle manure with adding of bedding, 100 cm$^3$ for cattle manure without adding of bedding, 132 cm$^3$ for pigs manure, 127 cm$^3$ for a mixture of manure from cattle with adding of bedding and pig manure in the ratio 1:1.

Dynamic equilibrium of substances is determined by the ease of substrate splitting. It should be noted that the inclusion of the litter materials in cattle manure subjected to further processing (grinding to the optimum size) resulted in a greater yield of biogas than the use of pure cattle manure as a substrate. This can be probably explained by the fact that there was a fast decomposition of organic substances by microorganisms in the pure manure at the first stage (hydrolysis) and there was a sharp decrease in pH level from 6.3 to 4.2 due to the activity of acid-forming bacteria that is unfavorable environment for other groups of bacteria.

The optimum pH for the acid-forming and hydrolyzing bacteria is from 4.5 to 6.3 when the bacteria reach their optimum. As for the methane-forming bacteria the optimum environment is neutral or slightly alkaline environment. In addition, the excess concentration of produced substance leads to stunted growth of the bacteria group which produces it. Ideally, a dynamic equilibrium should be established between the splitting phases in concentrations of substances, that is, between the splitting of the nutrients by bacteria and their intake. Thus, the more complicated is the structure of the substrate, the longer splitting of the substrate can be. The maximum yield of biogas from pigs manure was observed that can be explained by the composition of the substrate and the presence of less recalcitrant substances such as lignin in it.

At psychrophilic fermentation mode a significant biogas yield was observed with the eleventh day of the experiment and lasted up to 37 days. The rate of the gas formation largely depended on the temperature. The
higher is the temperature, the faster is the decomposition of the substrate and the intensity of gas formation. Unlike mesophilic fermentation mode under psychrophilic conditions the gas formation was more intense when a liquid cattle manure without bedding and pig manure were used as a substrate. However, the overall yield of gas at psychrophilic mode was lower by 21%. During fermentation, there was an increase in the internal temperature of the substrate by 1.5-3 °C that indicated the activity of microorganisms.

Conclusions
It can be concluded that the livestock manure is accessible and a good raw material for the biogas production. A number of experiments determining the influence of some factors on the yield of biogas were carried out. It is noted that the maximum yield of biogas was observed at mesophilic mode and particle size of 30-35 mm. At psychophilic fermentation mode the beginning of the gas formation was noted on the fifth day and the optimal size of the solid particles was 20-25 mm. With the increase of the size of the solids to 30-35 mm and 40-45 mm the gas formation process becomes slower and gas yield is reduced by 10-20 ml/l of substrate. A comparison of the amount of generated biogas from cattle and pig excrements and their mixture in a ratio of 1:1 under psychrophilic and mesophilic fermentation conditions and the same moisture of raw material (88%) was done. Biogas yield from cattle manure with adding of bedding was more by 10.32% on the average.

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