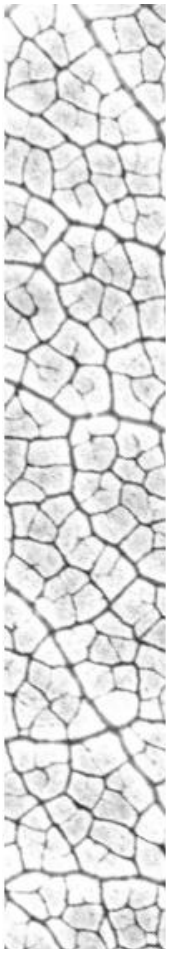

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Anaerobic processing and microbiological analysis of agricultural waste properties for obtaining highly concentrated methane

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Abstract
In this article, the authors analyzed the known and modern methods of obtaining biogas, their advantages and disadvantages, and proposed a method and installation for cleaning biogas from harmful impurities: hydrogen sulfide, CO2 and water vapor. In laboratory units studied the main process parameters: the compositions of nutrient medium, stirring speed (to remove the cork education and improve metabolism), stop, and loading of fresh substrate supply for methanogenic bacteria, and pH.

Keywords: microbiology, analysis, agricultural waste, methane, biogas, enzymes

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INTRODUCTION

A great contribution to the study and development of technologies and technical means of obtaining of biogas and the analysis of technologies and technical means of processing organic waste to produce biogas and its purification to obtain highly concentrated methane made by such scholars as Afanasiev V. N., Kovalev N. G., Imamov S., Gridnev P. I., Maksimov D. A., Afanasiev A. V., Arkhipchenko I. A., Lukin, S. M., Bondarenko A. N., Khmyrov V. D., Malak Y. F., Mironov V., Kaluga B. V. V., Kovalenko V. P., Naidenko V. K. etc. (Aleshina and Chernyshev 2010, Aniskin and 2004, Kaipova et al. 2018, Klimenko and Reutov 2005, Rozhkov and Krivoshchekova 2010).

Technologies for the production of organic fertilizers with the production of highly concentrated methane from agricultural products are complex systems that include a set of separate processes and operations formed in a certain sequence. As shown above, the ratio and choice of processes in the anaerobic process system is determined in each case at the design stage of objects. The sequence of determining the main technical - economic indicators of functioning of systems of anaerobic digestion of agricultural organic wastes, transport them and prepare high quality bio-fertilizers on its basis it is possible will present as: bioreactors – continuous, semi continuous and continuous; bioreactors – single-chamber (vertical single phase or two phase and multiphase), Multicam with biogas cleaning and without cleaning the received gas, and the separated piece bioreactors and separated piece obtained without bio-fertilizer

Analysis of the literature data shows (Angelidaki et al. 2011, Oleskowicz-Popiel et al. 2008, Lübken et al. 2010, Blagutina 2007, Imomov 2011) that the advantages of the method of anaerobic processing of agricultural organic waste (Makhatov et al. 2019) during the cultivation of methane-forming microorganisms are addressed at the maximum concentrations of cells of methane-forming bacteria. Depending on the purpose of using biogas, different methods of purification from individual components of biogas are required.

As you know, when using biogas, it can be upgraded to a variety of highly concentrated gas as a fuel.

Analyzing the above, the biogas produced by the anaerobic method is very closely related to the composition of the original organic waste. Studying the obtained composition of biogas, sh. Imomov comes to the conclusion that the amount and types of fuel obtained from biomass depend not only on the total volume of the reproduced biomass, but also on the quality of the biomass (humidity, composition of organic substances, physical characteristics, etc.) (Imomov et al. 2013). Today, the share of renewable energy sources (RES) in the global energy balance is small-about 14%,

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and the contribution of biomass is about 1.8%. But, as practice shows, even minor fluctuations in the supply of energy resources in the markets cause noticeable changes in prices. This suggests that the role of biomass based on agricultural waste in energy and strengthening stability in the markets of these resources will only grow in the future.

In the structure of biomass based on agricultural energy waste in the world, biomass energy is up to 13%. According to the forecasts of scientists working on biotechnology, the share of biomass based on agricultural waste as energy sources will reach 47.7% by 2040, and the contribution of biomass more than 23.8%.

Recently, special attention has been paid to the energy use of agricultural waste. There are the following arguments in favor of this:

- the use of agricultural waste, provided it is continuously restored (for example, new forest landings after deforestation), does not lead to an increase in the concentration of CO_2 in the atmosphere;

- in the industrialized countries, in recent years, there have been surpluses of cultivated land, which should be used for the cultivation of energy crops;

- the use of agricultural waste (agricultural - livestock, industrial and household) for energy purposes also solves environmental problems;

- newly created technology, the technology of new generation, which.

As is known, during the anaerobic process of the resulting biogas, a large part is diverted to methane CH_4, CO_2 and other related gases. In the anaerobic process, the resulting biogas can be purified or enriched biogas.

When using purified biogas, water vapor, NH_3 and H_2S, as well as solid impurities, are removed from the biogas composition. When biogas is enriched, carbon dioxide (CO_2) is removed from the biogas composition.

Analyzing the composition of biogas, we (Kaipova et al. 2016) studied the composition of biogas obtained from local raw materials-agricultural waste, such as cattle manure. Comparison of the biogas obtained in laboratory conditions turned out to be more methane than obtained by WELtec Bio Power GmbH, (Germany) single-phase biogas plant and two-phase biogas plant operating on agricultural organic waste from UTEC GmbH (located in Lower Saxony), UTS, Doma model, and SBI Bau GmbH/BIOFerm GmbH. In these companies, the obtained CH_4 biogas did not exceed 57.7% (the highest figure), although these plants loaded multicomponent organic waste products, which is not possible in Kazakhstan. When analyzing the above installations during the period of the sequence of works in operation of the installation, faults were noted, such as serious failures in the operation of individual units during the monitoring period. Just before the measurements were made, a four-day failure was noted due to a short-circuit failure of the submersible motor of the mixing device. At the same time, there were non-working days of the biogas plant, as a result of failure of the mixing device (shaft failure). In addition, such downtime leads to unstable operation of biogas plants. Consequence: an important problem is the lack of an additional surface for the colonization of sulfur methanobrazuyuschih bacteria in the test bioreactor. Manufacturers of such biogas plants did not provide for the speed of mixing devices in terms of ensuring microbiological contacts of methanobrazuyuschih strict anaerobic bacteria.

The above installations work on organic waste such as mainly from corn silage (from 70 ... 90%, and sometimes up to 100%). In addition, the organic waste (reads it as the main raw material) includes Turkey droppings (up to 6% of the total volume of the loaded bioreactor) and rye (cereal silage from an entire plant up to 7 %). Sometimes, with the increase in the price of organic waste, it first uses cereal silage for silage made from corn cobs and stalks. In the analysis, there were periods when potatoes were used as an additive to the raw material used for some time. The volume of raw materials loaded weekly has been constantly increasing in accordance with the time of operation of the plant after its commissioning and amounted to an average of 190 tons of substrate per week.

Based on the above, laboratory and semi-production biogas plants and its components used in production cannot be considered separately from each other. Since the fermentation process for obtaining highly concentrated methane by purification of biogas formed during processing of biomass based on agricultural waste is strictly anaerobic and the sequence of the reaction of methane formation cannot be violated from the point of view of Microbiology. When the process of processing organic waste does not have microbiological violations, the resulting biogas has more CH_4 than other related gases. At the same time, the biogas system does not need the concentration of methane and other indicators**.**

At present, the purification of biogas is done in some ways. There are many technologies for cleaning biogas such as: - technology of reactive cleaning with various solvents;

- continuous desulfurization of biogas;
- wet cleaning (absorption);
- pressure adsorption (PSA);
- chemical purification with amines (absorption);
- cryogenic air separation, and others.

To get more environmental benefits, the technology of accelerated (reactive) cleaning with various solvents is used. At the same time, the constant isolation of carbon to the maximum extent and preservation of soil fertilization with N, K and P elements. It makes it possible to use reactive solvents NH_3 and KOH which provide high separation effects of carbon dioxide (CO_2). Residues from biogas CO_2, H_2S, NH_3, N_2, water and

steam are removed by different processes. When removing H_2S and mercaptans from biogas, the technology is used for wet cleaning, air supply to gas tanks, cassette, as well as fine-iron chip filters and cleaning with sodium hydroxide.

In recent years, the world's manufacturing industry has been widely developing the introduction of biotechnologies that preserve the protection of the environment and produce renewable energy. At the same time, reducing greenhouse gas emissions to the surrounding areas. Another problem that is closely related to this problem is the use of waste obtained during anaerobic processing. As you know the resulting processed products are divided into two types: high-quality organic fertilizers and biogas (Blagutina 2007, Imomov 2011; Gammariello, et al. 2016). The latter requires additional cleaning from harmful impurities for use in the internal combustion engine or other needs. When burning, biogas is converted to heat energy, and when using a more complex system, it is converted to electric energy. Biogas can also be considered as a fuel for internal combustion engines. The efficiency coefficient when it is converted to thermal energy is 0.74 - 0.96, and in electric energy-0.3 - 0.4. Crude biogas when used in complex systems strongly affects its technical characteristics (Kaipova et al. 2018).

To prevent the above disadvantages, we have proposed a method and installation for cleaning biogas from harmful impurities.

The developed pilot-industrial filter for biogas purification consists of a metal body with a volume of 2.2 m3, inlet and outlet pipes for purified biogas, as well as automatic pressure meters for each stage of biogas purification (Kaipova et al. 2018). Acceleration of the process of microbiological transformations in them is achieved by intensive removal of the emerging gas and purification of gaseous products. However, their operation has shown that in the end, the performance of these plants is functionally dependent on the gas purity used and the quality of organic fertilizer obtained during anaerobic processing.

In order to obtain the necessary purified biogas and high-quality organic biofertilizers for the process, it is necessary first of all to make the correct selection of an anaerobic process that depends (suitable) for the climatic conditions of the biogas plant (BSU) operation. A biogas plant with a "classic" operating scheme often does not meet the modern requirements of the microbiological process and climatic conditions. The biggest problem that arises when cleaning biogas is the presence of hydrogen sulfide, CO_2 and water vapor in it. In the production of biogas, the most harmful component is hydrogen sulfide. As is known, in biogas plants of small capacity (hundreds of m^3/day), an adsorption ("dry") method for removing H_2S is used due to the formation of sulfides when interacting with iron oxide (ferro oxide filter):

$$Fe_2O_3 \cdot 3H_2O + 3H_2S \rightarrow Fe_2S_3 + 6H_2O$$

It is toxic, has an unpleasant smell, in the presence of moisture and, especially in combination with carbon dioxide, causes corrosion of metal equipment, during combustion it forms oxide and sulfur dioxide, which, interacting with water vapor, turn into sulfurous and sulfuric acids, which have a high corrosion activity. The content of hydrogen sulfide in biogas can reach up to 3%. As noted above, hydrogen sulfide together with water vapor and especially in combination with carbon dioxide has a corrosive effect on the metal surfaces of gas equipment, and the corrosion rate can reach 0.5-1 mm per year. When biogas is burned, hydrogen sulfide is converted to sulfur oxides. They interact with water vapor to form sulfuric and sulfurous acids, which are also corrosive. In addition, H_2S, SO_2, and SO_3 are highly toxic gases (Blagutina 2007).

It is known that without cleaning these impurities, the biogas mixture negatively affects the performance of power plants, such as internal combustion engines, so cleaning the gas from hydrogen sulfide is relevant. If you ignore gas cleaning at all, then the metal structures of the installation are quickly corroded, the nozzles of the heating system quickly become clogged and fail. This indicates that before using biogas, it is necessary to release it from hydrogen sulfide. As is known, there are currently three main methods for cleaning biogas: the method of liquid (wet) and solid (dry) chemical absorption of impurities (absorption and adsorption), the method of membrane separation and freezing (cryogenic method).

The disadvantages of these methods are high energy consumption, which creates the need for primary capital expenditures. Therefore, more acceptable methods are being developed for cleaning biogas from hydrogen sulfide, carbon dioxide, and water vapor.

Analyzing all of the above methods (Kedelbaev et al. 2018a), it is economically feasible to dry clean the biogas from hydrogen sulfide. The method of dry cleaning of biogas is carried out in a scrubber with a solid layer through which the biogas flows. For adsorption, sawdust and metal shavings are used. During the cleaning process, hydrogen sulfide is retained on the metal shavings under the action of the adsorption material, and the sawdust absorbs water vapor.

A necessary condition for obtaining highly concentrated methane by purification of biogas formed during processing of biomass based on agricultural waste is also the purification of biogas – the presence of hydrogen sulfide, CO_2 and water vapor in it. At the same time, anaerobic processing of biomass based on agricultural waste from which biogas is obtained has a kind of adapted methanogenic Association. These conditions should be taken into account when designing the process of obtaining highly concentrated methane by biogas purification.

RESEARCH METHODS AND MATERIALS

Waste analysis: nitrogen and carbon content. The organic and inorganic parts of the waste were analyzed separately using different methods. Since the anaerobic digestion process involves mainly organic waste, from the point of view of biotechnology, it is most effective to carry out the process only with their participation.

Separation of the inorganic component of waste was carried out by calcination in a muffle furnace at a temperature of – 350°C. in the inorganic part, only the content of N, CA, K, Na, P, MD, Fe, i.e. the most important elements for feeding microorganisms, was determined by the method (Kreshkov 1970, Schreiner et al. 1983). The elemental composition of the organic part for nitrogen and carbon content was determined using a mass spectrometer using the method (Mutalieva et al. 2015). The content of fractions (water-soluble compounds, compounds soluble in alcohol, protein, hemicellulose, cellulose, lignin, ash) was determined by the method.

Determination of ash content. To determine the ash content, use the following method for determining the ash content:

1g of the product is weighed in a crucible previously adjusted to a constant weight. Then it is burned in a muffle furnace and weighed again. Ash content is calculated using the formula:

$$\mathrm{P} = \mathrm{P1}/\mathrm{P}.100\% \quad (1)$$

where: P - ash content ,%; P1 - weight of the product after burning, g; P - weight of the product before burning, g.

Moisture determination. RUB the suspension with a mass of about 50 g in a porcelain mortar. In a pre-dried to a constant mass and weighted with an error of no more than 0.001 g, place the 5 g suspension weighed on an analytical balance with an error of no more than 0.001 g. place the suspension And the lid to it in a drying Cabinet preheated to 130°C and dry for 40 minutes at a temperature of 130± 2°C. Then place the desiccator, closed with a lid, for cooling. After cooling, weigh the bux with a hitch and find the humidity. Then calculate the moisture content of the manure using the formula:

$$\mathrm{X} = \frac{m_2 \cdot 100}{m_1}\% \quad (2)$$

where: m_1 - mass of fresh manure, g
m_2 - is the mass of dried manure, g
X – moisture content.

RESULTS AND DISCUSSION

Enzymes play a large role in the life of microbes. They are mandatory participants in a variety of biochemical reactions that underlie the functions of energy required for the functioning of methanobrazuyuschih bacteria, their respiration and reproduction. Results and discussion

Enzymes play a large role in the life of microbes. They are mandatory participants in a variety of biochemical reactions that underlie the functions of energy required for the functioning of methanobrazuyuschih bacteria, their respiration and reproduction.

Before sowing, glass bioreactors (0.5 and 0.2 liters in volume) were dried in a thermostat so that the airbags, along with moisture droplets, could not get inside the bioreactors. The seeded bioreactors were closed, and the free space between the bottom and the rubber cover was sealed with a band-aid to prevent oxygen from entering the bioreactor from the outside. The bioreactors were installed upside down in the thermostat. Fast-growing aerobes, absorbing the oxygen in the Cup, create favorable conditions for the growth of anaerobes.

Before conducting experiments on cow manure, the composition of the manure was checked for suitability for anaerobic treatment. During the first experiments, the manure brought from the cowshed, where they contain milk cows, was found to contain the following antibiotics, which were used to prevent the cowshed and the calf farm:

1. *Chlorotetracycline;*
2. *Bacitracin Zn;*
3. *OxyrtetracyclineNH₄;*
4. *Enramicin;*
5. *Colistin sulfate;*
6. *Neomycine sulfate;*
7. *Salinomycine;*
8. *Monensin;*
9. *Virginomycin;*
10. *LincomycinHCl*
11. *Penicillin*
12. *Sulfathinozol*
13. *Fenbendazol*
14. *Thiomyrine*
15. *Naracine*
16. *Maduramicine*
17. *Apromacine*
18. *Senduramacine*
19. *Kropido;*
20. *Dicrosuril*.

Knowing in the methanobrazuyuschy source material (manure) on the totality of detected in its behavior, the relationship between plasma properties and the place of manifestation of its action produced by a living cell and regulating the metabolism in the body are divided into extracellular and intracellular .Each type of methanobrazuyuschih bacteria (more than 3.5 million species)produces a constant set for it produced by a living cell, some of which break down to varying degrees of complex organic matter and carbohydrates, and

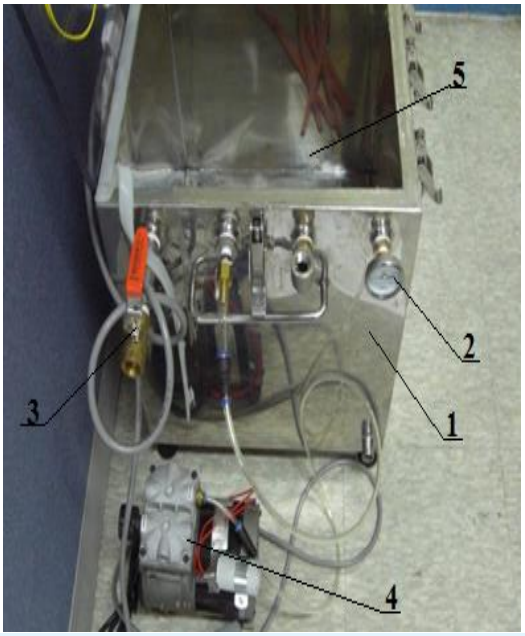

Fig. 1. Anaerostat for the cultivation of methanogenic association.
1 - anaerostat housing; 2 - vacuum meter; 3 - anaerostat oxygen suction taps; 4 - vacuum compressor; 5 - anaerostat cover

Fig. 2. Locations of experimental bioreactors in anaerostat

others cause oxidation and reduction of various organic substances.

Stability of systems is a complex organic substance such as methane-forming bacteria allows the use of biochemical properties in combination with their morphological, cultural and other permanent signs to identify types of methane-forming bacteria.

For normal cultivation we made anaerostat for the cultivation of anaerobic methanogenic bacteria. As you know, an Anaerostat-a device for growing microbes in anaerobic conditions-is a thick-walled glass pyramidal cube with a hermetically screwed lid, which has a vacuum meter and two taps for connecting to a vacuum pump (**Fig. 1**). The anaerostat consists of a housing 1, a vacuum measuring pressure gauge 2, an oxygen suction tap from the inside, and a vacuum compressor 4

Fig. 3. Bioreactors with one cube with organic waste baktiari

Table 1. Prepared nutrient medium

Environment properties	g/l	g/l
$MgSO_4 \cdot 7H_2O$	0,25	0,25
$CaCl_2 \cdot 2H_2O$	0,0238	0,25
KNO_3	-	1,0
Na citrate (CH_2COONa)	0,165	0,165
K_2HPO_4	0,04	0,04
$FeCl_3 \cdot 6H_2O$	0,002	0,002
$ZnSO_4 \cdot 7H_2O$	0,222	0,222
$CuSO \cdot 5H_2O$	0,079	0,079
$MnCl_2 \cdot 4H_2O$	1,81	1,81
$Na_2MoO_4 \cdot 2H_2O$	0,03	0,03
H_3BO_3	2,80	2,80

(MAN TGA, MAN TGS, MAN TGX. 2002-..., SD7H15-6008, 24 volts) and covers for tight closure from the top of the anaerostat.

The latter is known to require a thorough vacuum and oxygen- free condition for the cultivation of anaerobic bacteria in anaerobic conditions. To create anaerobic, the latter is known to require a thorough vacuum and oxygen- free condition for the cultivation of anaerobic bacteria in anaerobic conditions. To create anaerobic conditions in the device, the air was first pumped out of the inside. When the vacuum value reached 0.03-0.04 kg / cm^2 (accuracy clas s 0.4, with a measuremen t error of 0.0001 k g / cm^2), the oxygen suction valv e 3 was tightl y closed. Experimental bioreactors inside the anaerostat (**Fig.2**) placed under the same conditions of the fermentation temperature 54=1°C.

In parallel with the above experience, another experiment was performed to detect a complex organic substance of the studied methanobrazuyuschih bacteria was seeded on special differential diagnostic nutrient media (**Fig. 3**). Before starting the experiments, agar medium was prepared (**Table 1**). The prepared nutrient medium included basic solutions of potassium hydrophosphate salts, potassium chlorides, magnesium, calcium, sodium acetate, yeast, vitamins B_1, B_2, B_6, B_{12}, C, PP, agar-agar. A small amount of manure (one cube of a medical syringe) was placed on the nutrient medium.

Fig. 4. Biomass on baktiari with methane formed by the association

When sown in several laboratory bioreactors the same biomass revealed that all the reactors were anaerobiosis in different ways any one not like the other.

During the bubble process, the formation of fermented manure on the agar tank medium is reduced, the reason for the reduction of bubbles in bioreactors (suppression of the process) is the formation of acidic products of bacterial hydrolysis. Experimental data on measuring the pH of the medium confirmed this. During the maturation of the methane biocenosis, the pH changed from 7.5 at the beginning of the process and increased to 7.8. Therefore, to obtain a stable mode of anaerobic fermentation, the pH of the medium in bioreactors is a sensitive parameter used to determine the stability of fermentation.

From **Fig. 3** it is seen that the ability to break down methane-forming compounds such as organic substances which are usually combined into a single group called sugars common to many pathogenic microbes. Under the action of saccharolytic substances produced by a living cell of bacteria, sugars are apparently split into aldehydes and acids. Their final cleavage products are gaseous substances such as carbon dioxide ($C0_2$) and hydrogen (H_2).

Comparisons of the results of the two types of experience have shown (**Fig. 3** and **Fig. 4**) that the biomass in bioreactors despite the same anaerobic fermentation mode results in bioreactors are different.

This means, each bioreactor has its own adapted methane-producing bacteria. You can't conduct on any ratio or composition from outside on an adapted one or another bioreactor. In addition, methane-producing bacteria can not translate even on a small time from place to another, because they are considered to be anaerobes.

It is characterized by different species, and even species of methanogenic bacteria are treated differently to the same substances.

A. Lablinskaya in her analyses, also giving an example, some bacteria, fermenting lactose, remain neutral in relation to glucose, others, on the contrary, ferment glucose, and others, the most active, cause the splitting of both glucose and lactose.

When gaseous products are formed in the environment, they are pushed up the bioreactors located in the "float", so that an air bubble is collected at the sealed end (where the medical syringe is installed).

To identify gaseous products formed in a liquid medium during the cultivation of microorganisms, it was possible to apply the method of Ya. 3. Zimina (1969) suggested using a foam sponge instead of "floats". However, in our experiments, it is easy to see visually with non-armed eyes.

Based on the above, the use of methods for immobilizing microorganisms (Bitemirova et al. 2015, Kedelbaev et al. 2018b) in order to increase the yield of biogas during anaerobic processing of organic waste is of great importance for improving the efficiency of bioreactors working with mixed composition of manure (cattle) and cow litter. The main task of such bioreactors is to intensify heat exchange and homogenize the fermentation medium, which helps to accelerate methanogenesis by fixing the methanogenic microflora often discharged-by the flow scheme of bioreactors. When cultivating methanobrazuyuschih bacteria, as well as various polymer compositions to justify their use as carriers for the immobilization of microorganisms, in addition, the possibility of using waste biogas production in the form of biofertilizers.

As is well known and is a strict anaerobic methane-producing bacteria have optimum temperature and other technological parameters of fermentation.

CONCLUSION

Thus, when studying saccharolytic enzymes secreted by microbes that take into account not only the phenomenon of the splitting of certain sugars by acid production, but also the depth of the enzymatic process in the presence of nutrient medium, stirring speed(to remove the cork education or to improve metabolism), stop, and loading of fresh substrate supply for methanogenic bacteria, and pH.

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