

BIOGAS PRODUCTION PROCESS'S KINETICS OF FARM'S POULTRY DUNG

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Abstract. Biogas production process's kinetics of farm's poultry dung (FPD) in thermophylic conditions has been studied. Total gas volume for 29 days of fermentation in control variant didn't exceed 84.5 L, while with addition of 10% and 20% of balanced thermophylic methanogenic microorganisms' association's (BTMMA) inoculate into FPD was 1.7 and 2.1 times higher and amounted to 151 L and 179 L, respectively. It was established that in trial samples maximal methane formation during fermentation process reached to 69.7 and 64.7%, respectively. It was found that in thermophylic fermentation system no bacteria belonging to the Enterobacteriaceae family and other pathogens have been detected.

Key words: biogas, methane, thermophilic methanogenic fermentation, poultry dung.

Poultry farms are producers of not only meat and chicken eggs, and wastes (dung). According to Lysenko V.P. [1] a chicken farm with 400 thousands hens annually produces about 30 thousand tons of poultry dung and over 400 thousands m³ of waste water with high concentrations of organic components. Upon decomposition of such amount of poultry dung about 700 tons of biogas is emitted including 450 tons of methane, 208 tons of carbon dioxide, 35 tons of hydrogen, hydrogen sulfide and ammonia.

It is known that a significant number of areas adjacent to large poultry farms, as a result of long-term uncontrolled disposal of large masses of poultry dung, characterized by violation of the ecological balance in the soil system (abnormal high content of mobile phosphates, a sharp imbalance between nutrients, high content of heavy metals), as well as poultry dung contains a significant amount of weed seeds, eggs and larvae of worms and flies, a lot of pathogens [2].

In this connection, searching of opportunities for the waste utilization as a raw resource and reducing of their negative impact on the environment is an important condition for the preservation of the ecosystem. Thermophilic fermentation of poultry dung with production of biogas and organic fertilizer in biogas reactor is an

environmentally friendly approach for the solution as to clean of the environment and use of organic wastes.

The aim of this work is to study the digestion process of farm's poultry dung by utilization of the BTMMA prepared from the cattle manure.

Materials and methods

Experiments were carried out in 10 L fermenter, where the FPD with an initial pH of 7.4-7.6 and moisture of 89% has been added. An amount of water to hydrate of the FPD was defined by following formula [3]:

$$K_{water} = M_{subs.} \times ((B_{dm}\% - B_{im}\%) : (100\% - B_{dm}\%)),$$

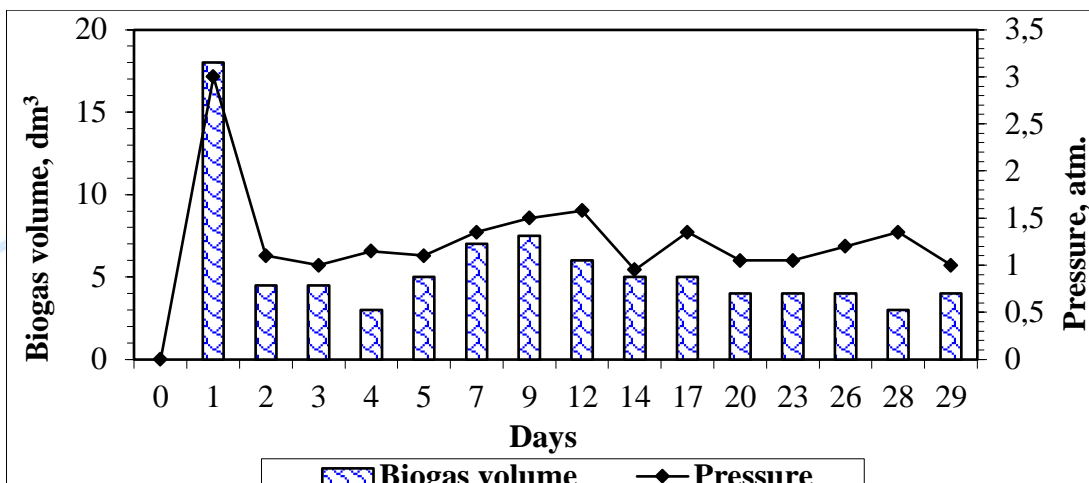
where, K_{water} – water amount in liters, $M_{subs.}$ – mass of the substrate, taken for the preparation of slurry (kg), B_{dm} – the desired moisture content of the substrate, B_{im} – initial moisture of the substrate.

Further, 10% and 20% BTMMA inoculum were added into FPD obtained from cattle manure by stepwise selection at thermophilic conditions (55 ± 1 °C). The total volume of the substrate was 7 L. A natural FPD's association of microorganisms has served as control. Fermentation temperature was kept at 55 ± 1 °C. The total volume of gas was measured by the gas-meter device of PLAZMA GROUP LTD. $Q_{max} = 2,5$ m³/s, $Q_{min} = 0,016$ m³/s, $P_{max} = 3$ vPa. The content of methane, carbon dioxide and nitrogen were determined using gas chromatograph «Chrom-5" (CZ, Prague) with a thermal conductivity detector (detector current 80 mA) using a sorbent "Polysorb-1" (0.1-0.3 mm) and a glass column (1200x3 mm). The rate of carrier gas (He) was 70 ml/min at 20 °C.

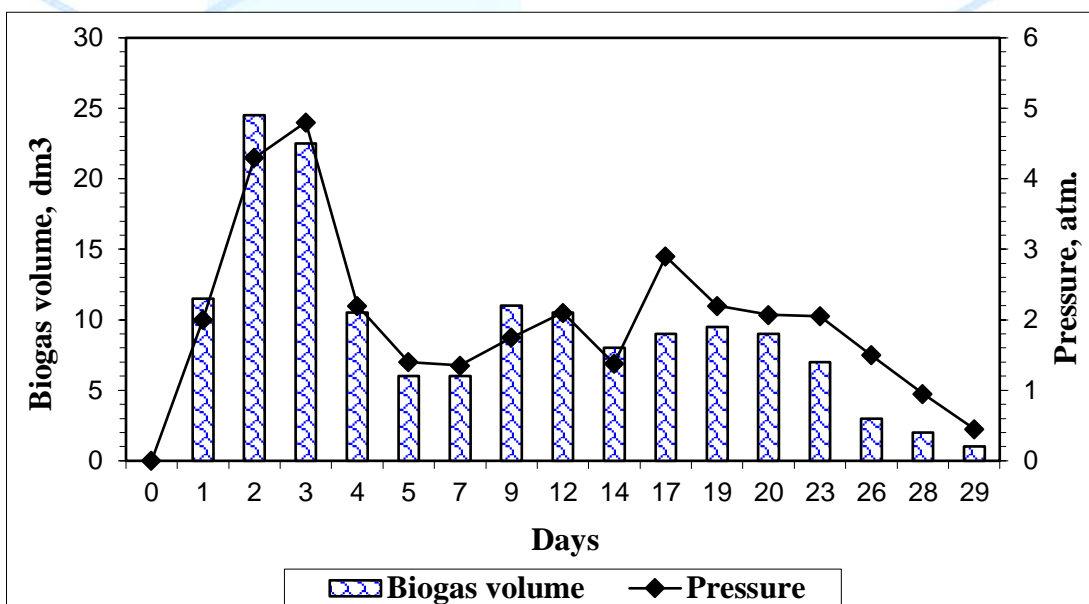
Cultivation and determination of the amount of bacteria specific culture media were used according to Egorov N.S., Zvyagintsev D.G. and Galchenko V.F. et al [4-6]. Cultivation of anaerobic bacteria was conducted in anaerostat with gas phase N₂ and CO₂ in a ratio of 90:10.

Results and discussion.

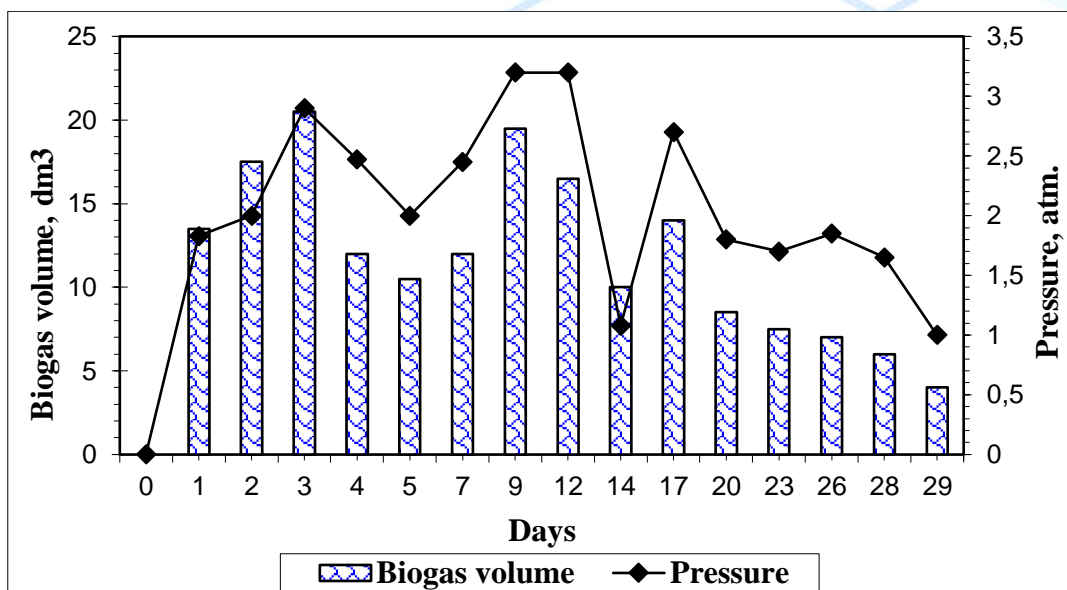
The study of gas-forming process's kinetics of the FPD in thermophilic conditions showed that the capacity of the natural gas-forming microorganisms association as with the introduction of BTMMA and without adding BTMMA characterized by shorter lag-phase. However, in the trial variants with addition into FPD 10% and 20% inoculates of the BTMMA maximum level of gas-formation was 1.36 and 1.14 times higher compared with the control option and was 24.5 and 20.5 dm³, respectively (Fig. 1A, 1B, 1C).



A



B



C

Fig. 1. Gas-formation ability of microbial association of the FPD in thermophilic conditions (55 °C):

- A – control variant (sample №1, natural microflora);
- B – trial variant (sample №2 with 10% inoculate of the BTMMA);
- C – trial variant (sample №3 with 20% inoculate of the BTMMA).

It was established during fermentation process that with addition of 10% inoculate of BTMMA unlike control variant two intensive gas-formation peaks of total biogas have been observed in fermentation system, which corresponds to 2nd and 9th days of fermentation with biogas content of 24.5 and 11 dm³, respectively (Fig. 1B). With increasing concentration of the inoculate to 20%, 3 intensive gas-formation peaks of total biogas have been observed during fermentation, which corresponds to 3rd, 9th and 17th days of fermentation with biogas content of 20.5, 19.5 and 14 dm³, respectively (Fig. 1C).

Comparative characteristics of the duration of FPD's fermentation in all three variants of the experiment (samples #1, #2 and #3) showed that in the trial variants intensive gasification process last for 23-26 days, while in the control variant (sample №1) after 9 day fermentation decreasing of the active gas-formation ability has been observed.

It was established that total gas volume during 29 days in control variant didn't exceed 84.5 dm³ while with addition of the 10% and 20% inoculates of the BTMMA into FPD were 1.7 and 2.1 times more biogas volume which corresponds to 151 dm³ and 179 dm³, respectively. It was measured that biogas pressure in FPD's fermentation dynamics in control variant reached to 3 atm. while in trial variant with addition of 10% of the BTMMA reached to 4.8 atm.

Studies have shown that addition of the BTMMA, prepared from cattle manure, to FPD in thermophilic conditions of fermentation promoted increasing of methane content in biogas background.

The study of methane production process's dynamics of FPD in thermophilic conditions showed that methane production began with the first day of fermentation both with and without BTMMA inoculation (Fig. 2, 3 and 4). However, methane content in biogas background in trial variants (samples #2 and #3) in contrast to the control one (sample #1) was 2.9 times higher within 24 hours of fermentation. It was revealed that the highest degree of methane formation in thermophilic conditions (55 °C) in the process of FPD fermentation by natural microorganisms' association (sample №1) was observed on 29th days of fermentation and amounted to 54.8% (Fig. 2). While in trial samples with addition of 10% and 20% BTMMA inoculate in the FPD (samples #2 and #3) this value was reached on 4th days of fermentation showing 54.1% and 55.5% of CH₄, respectively (Fig. 3 and 4).

Maximum production of methane in the test variants with 10% and 20% of inoculates is observed on 20th and 10th days of FPD fermentation with methane content in the biogas background 69.7% and 64.7%, respectively.

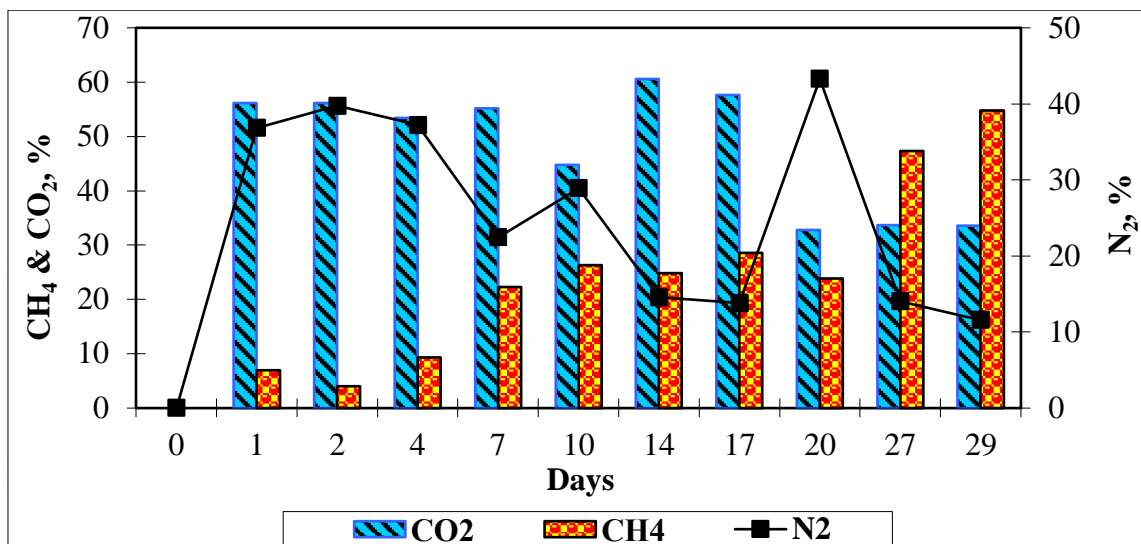


Fig. 2. The content of the biogas (CH₄, CO₂ and N₂) in the FPD's fermentation process in thermophilic conditions (sample #1, control).

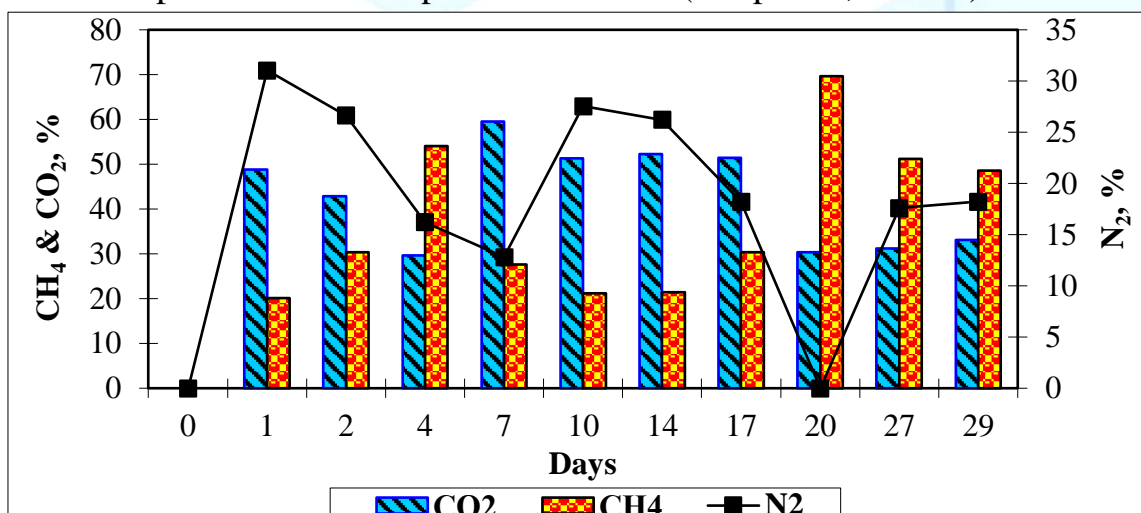


Fig. 3. CH₄, CO₂ and N₂ contents in the FPD's fermentation process in the thermophilic conditions with 10% inoculate of the BTMMA (sample #2).

It should be noted that two peaks of intensive methane formation in the trial #2 supplemented with 10% inoculate unlike control group was observed, which falls on the 4th and 20th days of fermentation with the methane content of 54.1% and 69.7%, respectively (Fig. 3). Sample #3 with the addition of 20% inoculate into FPD showed 3 peaks of intensive methane formation, which is observed at 4th, 10th and 29th days of fermentation with the methane content of 55.5%, 64.7% and 58.7%, respectively (Fig. 4).

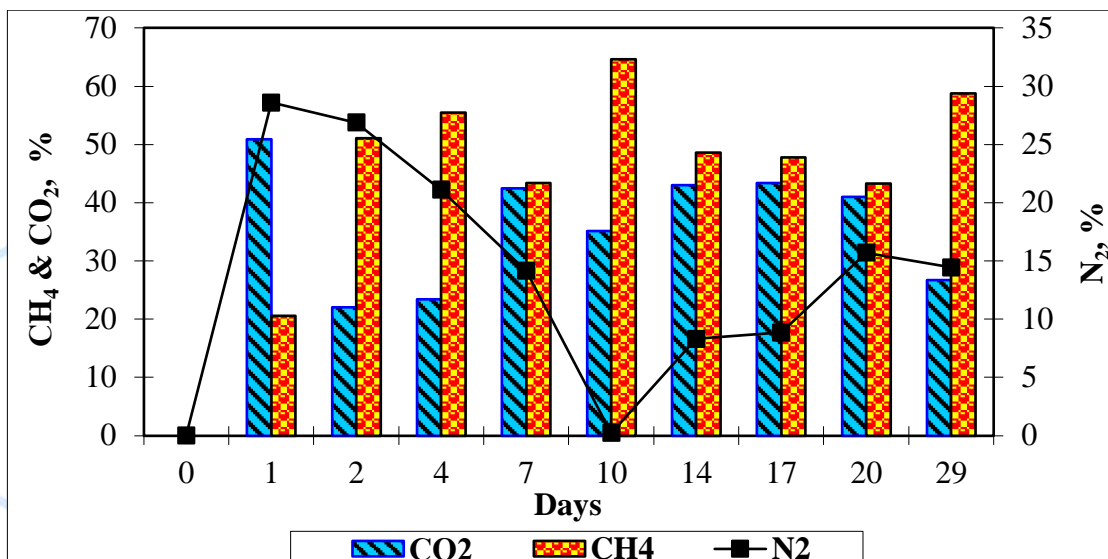


Fig. 4. CH₄, CO₂ and N₂ contents in the FPD's fermentation process in the thermophilic conditions with 20% inoculate of the BTMMA (sample #3).

It is found that there is high content of CO₂ and N₂ in the starting fermentation step as in control and trial variants of the experiment. Also draws attention to the fact that in the FPD's fermentation process as in control and in trial variants of the experiments a stage of more intensive production of the total amount of biogas doesn't correspond to the stage of maximum methane formation.

It is known that with the development of primary and acetogenic microflora during fermentation in decomposition of various organic compounds (formate, acetate, lactate, butyrate, methanol, ethanol) gaseous compounds are produced such as (CO₂, CO, H₂ and N₂) many of which are precursors of methane in a fermentation system [7-9]. Methane formation is the final stage of anaerobic digestion of organic compounds, so the formation of methane depends not only on the activity of methane-producing bacteria as from the previous microorganisms supplying them necessary substrates [10]. Intensive CO₂ formation in the initial stage of the FPD's fermentation with methane production in the next days of fermentation confirmed that methane formation is the final step in decomposition of organic compound (Fig. 2, 3, 4).

During FPD's fermentation process in thermophilic conditions a correlation between reduction of CO₂ and production of CH₄ was revealed. It appears that in the process of FPD's fermentation in thermophilic conditions a possible precursor of methane is CO₂.

In our studies, it was found that in the fermentation system with introduction of 10% and 20% inoculates of the BTMMA into FPD, the highest nitrogen recycling in biogas background was observed on 20th and 10th days of fermentation, which is accompanied by the maximum methane formation (Fig. 3 and 4). It is known that methanogens use ammonia nitrogen or some amino acids as the nitrogen source. Ability to nitrogen fixation was shown for a number of species [11].

Apparently, in thermophilic conditions of the FPD’s fermentation in fermentable system with intensive development of primary and acetogenic micro-flora more available nitrogen sources for methane bacteria may be the molecular nitrogen.

Earlier studies have shown that in mesophilic conditions of the FPD’s fermentation process making both 20% inoculate of the balanced methanogenic microorganisms’ association (BMMA) and without introducing of them methane formation was started with elongated lag-phase [12].

Table 1

**Main physiological groups of microorganisms’ count
in initial and fermented FPD**

Physiological groups of microorganisms	Sample	Cell count, thousands/ml		
		initial	2 nd day	19 th day
Methane-forming bacteria	Control	2,5	25	20
	Trial	2,5	50	25
Cellulose degrading bacteria	Control	25	250	50
	Trial	25	500	250
Anaerobic nitrogenfixing bacteria	Control	0,25	13	25
	Trial	0,25	25	25
Denitrificating bacteria	Control	250	0,025	0,05
	Trial	250	0,05	0,05
Ammonifying bacteria	Control	8600	60	5
	Trial	8600	56	4
Enterobacteriaceae	Control	60000	0	0
	Trial	60000	0	0
Shigella	Control	180	0	0
	Trial	180	0	0
Salmonella	Control	50	0	0
	Trial	50	0	0
E.coli	Control	46000	0	0
	Trial	46000	0	0
Pseudomonas aeruginosa	Control	20	0	0
	Trial	20	0	0
Staphylococcus aureus	Control	2	0	0
	Trial	2	0	0

It was found that, in the mesophilic conditions during the fermentation of the FPD by the natural association of microorganisms, the highest methane formation did not

exceed than 31.5%, but with the addition of 20% BMMA inoculum the maximum methane content was observed on the 15th day of fermentation and did not exceed than 42.5%. However, attention is drawn to the fact that under thermophilic conditions with the addition of 20% BTMMA inoculum into fermentation broth, intensive methane formation was observed from the initial stage of fermentation and on the second day of fermentation the methane content in the gas background was higher than 56%.

It is known that large farms for the maintenance of chickens and their treatment use some antibiotics of a broad spectrum of action [13-14]. Moreover they use various disinfectants for the prevention of premises, which are ultimately in the waste (dung) of farms. Apparently, in the mesophilic fermentation process of the FPD, unlike the fermentation of housekeeping chicken manure (HChM), the revealed delay in gas formation by a natural association of microorganisms with and without the addition of BMMA is possibly associated with the negative effect of antibiotics and disinfectants on microorganisms-hydrolytes that participate in the first stage of anaerobic decomposition of suspended or solid organic matter. According to Vavilina et al. [15], hydrolysis is the limiting stage of the decomposition of organic substances. Obviously, in our studies, a small amount of methane in the fermentation of HChM is due to a delay in the growth of the primary microflora and, accordingly, a delay in the formation of compounds necessary for the growth and development of methane-forming microorganisms.

Previously, it was shown that the use of BMMA in the HChM fermentation process, formation of methane was observed from 6th days of fermentation and the highest methane content (11th days) was 55.2% (Fig. 2B) [16]. A comparative study of the kinetics of the gas formation process of FPD and HChM showed that the addition of 20% BMMA inoculum stimulated the formation of methane by an aboriginal microflora, while the introduction of 20% inoculum into the FPD influenced insignificantly for methane formation process.

Apparently, this is explained by the fact that large farms for maintenance and treatment of chickens use various broad-spectrum antibiotics [17-18] and also use various disinfectants for the prevention of premises. It is known that the action of chlorinated disinfectants shows a violation of the outer membrane of bacterial cells, leakage of vital components and intensive leakage of dipicolinic acid, other spore components and change of structural organization of the spores. At the same time, serious disturbances in the functioning of enzyme systems are observed.

The negative influence of antibiotics is related to their effect on the growth and development of the primary and acidogenic microflora of the methanogenic community, which supply the substrate for methane-forming bacteria.

It is known that under the influence of the primary microflora, the products of their vital activity, such as H₂, CO₂, CO, acetate, formate, butyrate, propionate, ethanol

and methanol are formed in the fermentation [6-9]. It was previously assumed that under mesophilic conditions at 30 °C, low methane content during fermentation of FPD, as with the addition of 20% BMMA inoculum and without the introduction of it, is associated with the delay in the growth of the primary micro-flora [16].

In thermophilic conditions at 55 °C, as a result of the vital activity of microorganisms within the fermentation system, even more heating of the substrate takes place, which negatively affects the structure of antibiotics. As is known, water solutions of antibiotics lose their activity at high temperatures. Apparently, under thermophilic conditions, the effect of antibiotics on the growth and development of primary and acetogenous micro-flora, which supplies substrate for the functioning of methane-forming bacteria, is insignificant.

Thus, according to the results of the study, it was established that in the process of mesophilic fermentation of FPD (with the addition of mesophilic BMMA), unlike the thermophilic fermentation of the FPD (with the addition of BTMMA), the detected delay in methane formation by microbial association is associated with the negative effect of antibiotics on the primary and acetogenous micro-flora that participates in the initial stage of anaerobic decomposition of suspended and solid organic matter.

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