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Application of Thermophilic Methanogenic Microbial Consortia in the Fermentation of Poultry Manure

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A B S T R A C T

This study investigates the kinetic parameters of biogas formation during the thermophilic anaerobic digestion of farm poultry dung (FPD). In the control variant, the cumulative biogas output recorded over a 29-day fermentation period was limited to 84.5 L. Supplementation of the substrate with 10% and 20% balanced thermophilic methanogenic microbial association (BTMMA) significantly intensified methanogenesis, increasing total gas production to 151 L and 179 L, respectively corresponding to 1.7-fold and 2.1-fold enhancements compared to the control. Analysis of gas composition revealed that the treated variants achieved maximum methane concentrations of 69.7% and 64.7%, indicating a stable and efficient methanogenic activity throughout the process. Furthermore, microbiological assessment demonstrated that thermophilic fermentation effectively suppressed sanitary-indicator microorganisms, with no detectable members of the *Enterobacteriaceae* family or other pathogenic taxa. These findings highlight the potential of BTMMA-supplemented thermophilic digestion as a reliable and safe method for converting poultry manure into high quality biogas.

Introduction

Poultry farms function not only as large-scale producers of meat and eggs but also as significant generators of organic waste, primarily in the form of poultry dung. As reported by Lysenko V.P. (7), a commercial poultry facility with a stock of approximately 400,000 laying hens can annually accumulate nearly 30,000 tons of solid poultry dung, in addition to more than 400,000 m³ of wastewater enriched with high levels of organic pollutants. Under uncontrolled natural decomposition, such massive quantities of dung can release up to 700 tons of biogas, including roughly 450 tons of methane, 208 tons of carbon dioxide, and about 35 tons of hydrogen, alongside notable amounts of hydrogen sulfide and ammonia. These emissions not only represent environmental hazards but also indicate a substantial untapped potential for renewable energy production. Long-term and unregulated deposition of poultry dung around large industrial poultry farms has resulted in noticeable ecological disruptions in adjacent territories. Soils in such zones often exhibit elevated concentrations of mobile phosphates, severe nutrient imbalance, and excessive accumulation of heavy metals. Furthermore, poultry dung serves as a biological carrier of numerous undesirable components, including viable weed seeds, eggs and larvae of helminths and flies, as well as a wide spectrum of pathogenic bacteria and viruses (12). The persistence of these contaminants poses risks both to agricultural productivity and public health, accelerating

the degradation of soil ecosystems and contributing to broader environmental pollution. In light of these challenges, the development and implementation of technologies for the efficient utilization of poultry waste as a valuable resource, while simultaneously minimizing its negative ecological impact, is of particular scientific and practical significance. Among the available waste-treatment strategies, thermophilic anaerobic digestion has emerged as one of the most ecologically stable approaches. This method enables the transformation of poultry dung into biogas a renewable energy source and a stabilized organic fertilizer suitable for agricultural use. The high temperatures characteristic of thermophilic fermentation also contribute to the inactivation of pathogenic microorganisms, thereby enhancing the sanitary safety of the final digestate. Considering these advantages, the present study focuses on evaluating the efficiency of thermophilic digestion of farm-derived poultry dung employing a balanced thermophilic methanogenic microbial association (BTMMA) prepared from cattle manure. The application of such microbial consortia is expected to accelerate methanogenesis, improve the stability of the fermentation process, increase biogas yield, and enhance the hygienic quality of the final product. Understanding these effects will contribute to the development of optimized technologies for the environmentally safe and energy efficient utilization of poultry waste.

Materials and Methods

The experimental studies were conducted using a 10-L laboratory-scale fermenter. Fresh farm poultry dung (FPD) with an initial pH ranging from 7.4 to 7.6 and a moisture content of 89% served as the primary substrate. The amount of water required to adjust the moisture level of the substrate was calculated according to the formula proposed in (16):

K*water* = M*subs.* x ((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 substarte.

After moisture adjustment, BTMMA inoculum produced from cattle manure through stepwise enrichment under thermophilic conditions (55±1°C) was introduced into the substrate at concentrations of 10% and 20%. The working volume inside the fermenter was maintained at 7 L. A natural microbial consortium contained in untreated FPD served as the control variant. Throughout the fermentation process, the temperature inside the reactor was strictly maintained at 55 ± 1 °C. The total biogas yield was measured using a gas-metering device manufactured by PLAZMA GROUP LTD (Qmax = 2.5 m^3/s, Qmin = 0.016 m^3/s, Pmax = 3 vPa). The qualitative composition of the produced gas—specifically methane, carbon dioxide, and nitrogen—was analyzed using a "Chrom-5" gas chromatograph (CZ, Prague) equipped with a thermal conductivity detector (detector current: 80 mA). Chromatographic separation was carried out on a glass column (1200 × 3 mm) packed with Polysorb-1 sorbent (0.1–0.3 mm), with helium as the carrier gas at a flow rate of 70 ml/min at 20°C. Quantitative determination of microbial populations was performed using selective culture media and standard microbiological techniques as described by Egorov N.S., Zvyagintsev D.G., and Galchenko V.F. (3, 4, 18). Anaerobic microorganisms were cultivated in an anaerostat under a gas mixture of N_2 and CO_2 in a ratio of 90:10 to ensure strictly anaerobic conditions.

Results and Discussion

The analysis of the kinetics of biogas formation from farm poultry dung (FPD) under thermophilic conditions demonstrated that both the native microbial consortium and the substrates enriched with BTMMA exhibited a relatively short lag-phase. This indicates that the indigenous gas-forming microorganisms present in raw poultry dung possess an inherent capacity for rapid adaptation to thermophilic anaerobic conditions. Nevertheless, the variants supplemented with 10% and 20% BTMMA inoculum showed a substantially more intensive biogas-forming activity compared with the untreated control. The introduction of 10% BTMMA resulted in a 1.36-fold increase in the maximum gas-production rate, whereas the addition of 20% inoculum enhanced this parameter by 1.14-fold. The peak biogas volumes in these treatments reached 24.5 dm^3 and 20.5 dm^3, respectively, whereas the control variant demonstrated noticeably lower values (Figure 1 A, B, C). These results confirm that the inoculation with thermophilic methanogenic microbial associations accelerates the transition to the active gas-forming phase and enhances the metabolic efficiency of the fermentation process. The observed intensification can be attributed to the introduction of specialized thermophilic methanogens that rapidly establish dominance within the

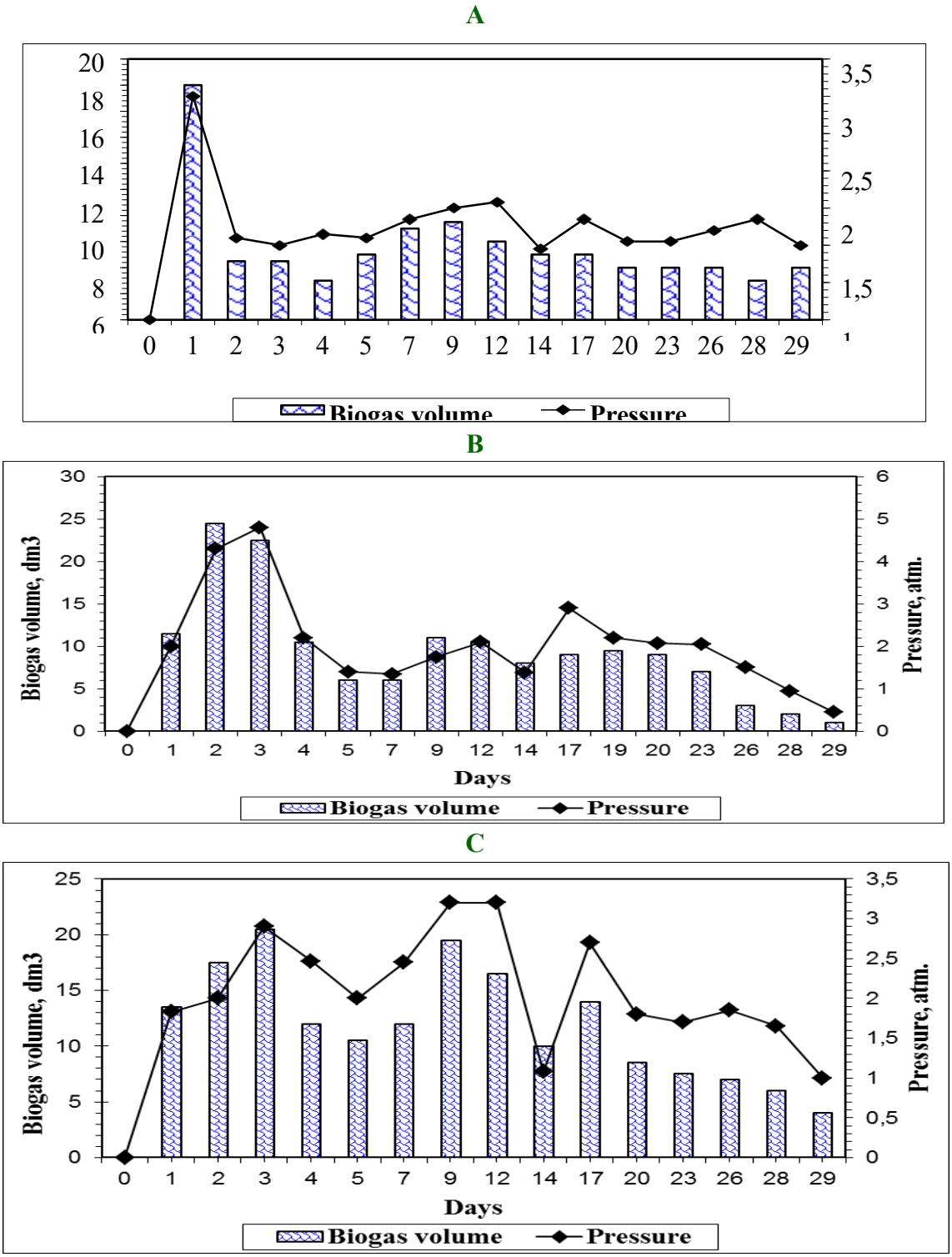
microbial community, thereby increasing methane-forming pathways and reducing the time required to reach the maximal rate of gas evolution

During the thermophilic fermentation of FPD, it was established that the introduction of 10% BTMMA noticeably altered the dynamics of biogas formation compared to the control. In this variant, two distinct peaks of intensive gas production were recorded, occurring on the 2nd and 9th days of fermentation, with biogas volumes of 24.5 and 11 dm^3, respectively (Figure 1B). When the inoculum concentration was increased to 20%, the fermentation system exhibited three pronounced peaks, observed on the 3rd, 9th, and 17th days, yielding 20.5, 19.5, and 14 dm^3 of biogas, respectively (Figure 1C). These results indicate that higher inoculum concentrations promote more complex and prolonged metabolic activity within the methanogenic community. A comparative assessment of fermentation duration across all three experimental variants (samples №1, №2, and №3) showed that the gasification phase in the inoculated systems lasted 23–26 days. In contrast, the control variant (sample №1) displayed a decline in active gas production after the 9th day, suggesting exhaustion of the native microbial potential or inhibition under thermophilic conditions. The cumulative biogas yield over the 29-day period further confirmed the strong stimulating effect of BTMMA. Total gas volume in the control did not exceed 84.5 dm^3, whereas the addition of 10% and 20% inoculum increased biogas output by 1.7-fold and 2.1-fold, resulting in 151 dm^3 and 179 dm^3, respectively. Measurement of biogas pressure also showed marked differences: in the control system, pressure reached a maximum of 3 atm, whereas in the 10% BTMMA treatment it increased up to 4.8 atm, further supporting the intensified bioconversion activity in treated variants.

The introduction of BTMMA, obtained through enrichment of cattle manure under thermophilic conditions, not only increased total gas output but also enhanced methane formation within the biogas mixture. Analysis of methane production kinetics demonstrated that methanogenesis commenced on the first day of fermentation in all variants, irrespective of inoculum presence (Figures 2, 3, and 4). However, within the first 24 hours, methane concentration in the treated variants (samples №2 and №3) was approximately 2.9 times higher than in the control (sample №1), suggesting a rapid establishment of active methanogenic pathways. In the natural microbial community of FPD (sample №1), the maximum methane concentration of 54.8% was recorded on the 29th day of fermentation (Figure 2). By contrast, in the variants supplemented with 10% and 20% BTMMA (samples №2 and №3), methane levels reached comparable values 54.1% and 55.5% by the 4th day of fermentation (Figures 3 and 4), demonstrating a substantially accelerated onset of intensive methanogenesis. Moreover, the highest methane outputs in the experimental treatments were achieved considerably earlier than in the control. In the 10% BTMMA variant, the maximum methane concentration of 69.7% was observed on the 20th day, while in the 20% inoculated sample the peak concentration of 64.7% occurred on the 10th day of fermentation. These findings clearly indicate that BTMMA inoculation enhances both the rate and the efficiency of methane formation, making the thermophilic digestion process more productive and energetically favorable.

Analysis of methane-formation patterns revealed that in trial variant №2, enriched with a 10% BTMMA inoculum, two distinct peaks of intensive methanogenesis were observed—unlike the control sample. These peaks occurred on the 4th and 20th days of fermentation, reaching methane concentrations of 54.1% and 69.7%, respectively (Figure 3). In sample №3, supplemented with 20% inoculum, three pronounced methane-formation peaks were detected, appearing on the 4th, 10th, and 29th days of the process. Methane contents during these peaks amounted to 55.5%, 64.7%, and 58.7%, respectively (Figure 4). It was also determined that at the onset of fermentation, both in the control and inoculated variants, CO_2 and N_2 were present at relatively high concentrations. An important observation is that periods of intensive total biogas production did not coincide with the phases of maximum methane formation. This indicates that methane generation follows a metabolic sequence distinct from the formation of other gaseous intermediates. As reported in earlier studies (6, 9, 16), during the development of primary and acetogenic microflora, degradation of organic intermediates such as formate, acetate, lactate, butyrate, methanol, and ethanol results in the production of several gaseous compounds including CO_2, CO, H_2, and N_2. Many of these serve as essential precursors for methanogenesis. Since methane formation represents the terminal stage of anaerobic digestion, the rate and magnitude of methane production depend not only on the activity of methanogenic archaea themselves but also on the preceding microbial groups that supply the necessary substrates (2).

Fig.1 Gas-formation dynamics of the microbial association in farm poultry dung (FPD) under thermophilic conditions (55 °C).



(A) Control variant (Sample №1) containing natural microbial flora of FPD;
(B) Experimental variant (Sample №2) supplemented with 10% BTMMA inoculum;
(C) Experimental variant (Sample №3) supplemented with 20% BTMMA inoculum.

Fig.2 Composition of biogas (CH_4, CO_2, and N_2) produced during thermophilic fermentation of farm poultry dung (FPD) in the control variant (sample №1).

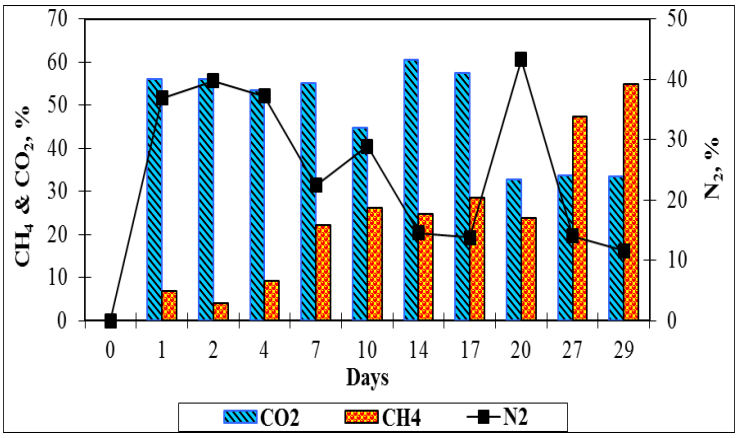


Fig.3 Contents of CH_4, CO_2, and N_2 in the biogas produced during thermophilic fermentation of farm poultry dung (FPD) supplemented with 10% BTMMA inoculum (sample №2).

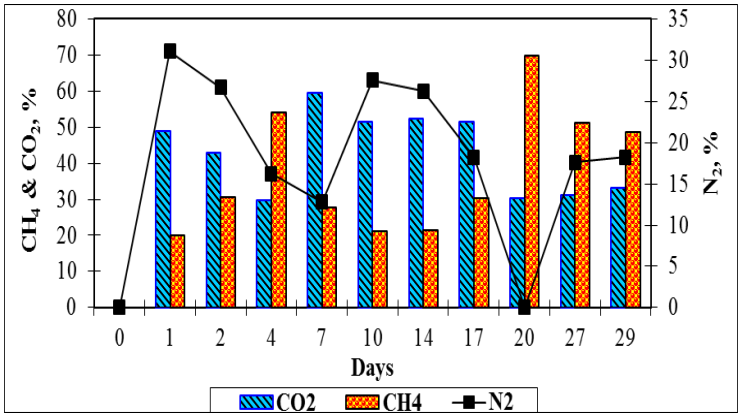


Fig.4 Contents of CH_4, CO_2, and N_2 in the biogas produced during thermophilic fermentation of farm poultry dung (FPD) supplemented with 20% BTMMA inoculum (sample №3).

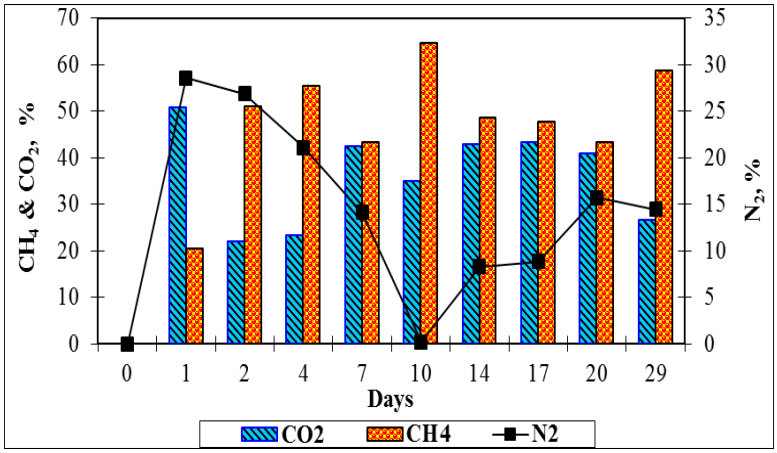


Table.1 Abundance of major physiological groups of microorganisms in initial and fermented farm poultry dung (FPD).

Physiological groups of microorganisms	Sample	Cell count, thousands/ml		
		initial	2nd day	19th 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
Ammonifing 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

The elevated CO_2 levels observed during the initial stage of FPD fermentation, followed by subsequent increases in methane, confirm that CH_4 generation constitutes the final step in the breakdown of organic matter (Figures 2, 3, and 4). A clear correlation was observed between decreasing CO_2 concentrations and increasing methane levels during thermophilic fermentation. This suggests that CO_2 may serve as one of the key precursors for methane formation under these conditions. The progressive reduction of CO_2 concurrent with rising CH_4 production reflects the transition from acetogenic to methanogenic stages of the process. Our results further demonstrated that nitrogen transformations within the biogas system were linked to methanogenic activity. In the inoculated variants containing 10% and 20% BTMMA, the highest levels of nitrogen release in the gas phase coincided with the periods of maximum methane formation—on the 20th and 10th days of fermentation, respectively (Figures 3 and 4). It is well established that methanogens utilize ammonia nitrogen and certain amino acids as nitrogen sources, and nitrogen-fixing capabilities have been described for several methanogenic species (5). Therefore, it is plausible that under thermophilic conditions, molecular nitrogen may become a more accessible nitrogen source due to the intensified activity of primary and acetogenic microbial groups.

Earlier studies conducted under mesophilic conditions demonstrated that methane production in FPD proceeds with a considerably prolonged lag-phase, both with and without the addition of 20% inoculum of a balanced methanogenic microbial association (BMMA) (13). This underscores the advantages of thermophilic digestion, which provides not only faster microbial activation but also more efficient methane generation.

Studies conducted under mesophilic conditions demonstrated that during the fermentation of FPD by the native microbial consortium, the maximum methane concentration did not exceed 31.5%. When 20% BMMA inoculum was added, methane production improved, with a peak of 42.5% recorded on the 15th day of fermentation. In contrast, thermophilic fermentation displayed markedly different behavior. With the addition of 20% BTMMA inoculum, intensive methane formation began almost immediately, and by the second day of the process, methane content already exceeded 56%. This rapid onset highlights the strong physiological adaptability and activity of thermophilic methanogenic consortia compared with mesophilic ones.

It is well known that large commercial poultry farms routinely apply broad-spectrum antibiotics for disease prevention and growth regulation (8, 11). In addition, various chemical disinfectants are used to sanitize facilities, and residues of these compounds inevitably enter the waste stream, including poultry dung. Evidently, in mesophilic fermentation of FPD unlike the fermentation of housekeeping chicken manure (HChM) the observed delay in gas production by both uninoculated and BMMA-inoculated microbial communities is likely associated with the inhibitory effects of antibiotics and disinfectants. These substances negatively influence hydrolytic microorganisms that initiate the first stage of anaerobic degradation of suspended and solid organic matter. According to Vavilina *et al.,* (15), hydrolysis often represents the rate-limiting stage of anaerobic digestion.

Therefore, suppressed activity of hydrolytic and primary microflora leads to delayed formation of substrates required by methanogenic archaea, ultimately reducing methane yield. Previous studies have shown that in mesophilic fermentation of HChM, methane production begins only after a prolonged lag-phase, and even with the addition of BMMA, significant methane levels (55.2%) were not achieved until the 11th day of fermentation. Comparative analysis of FPD and HChM fermentation kinetics demonstrated that while the addition of 20% BMMA stimulated methane formation in HChM, the same inoculum had a considerably weaker effect on FPD. This discrepancy is most likely explained by the fact that industrial poultry waste contains higher concentrations of antibiotic and disinfectant residues compared with manure from small household farms. Chlorinated disinfectants, for example, are known to disrupt bacterial cell membranes, cause leakage of essential intracellular components, alter the structural integrity of spores, and impair enzyme systems. Antibiotic residues further inhibit the growth and metabolic activity of primary and acidogenic microorganisms that supply crucial intermediates for methanogenesis. During anaerobic digestion, primary microflora produce essential metabolites such as H_2, CO_2, CO, acetate, formate, butyrate, propionate, ethanol, and methanol (4, 6, 9, 16). Under mesophilic conditions (≈30°C), low methane accumulation in FPD both with and without BMMA inoculation—has been attributed to delayed development of these initial microbial groups (1). Thermophilic fermentation at 55 °C, however, changes this dynamic. The metabolic activity within the fermentation system generates additional heat, which accelerates the degradation of antibiotic molecules. It is well established that many antibiotics lose biological activity when exposed to elevated temperatures. Therefore, under thermophilic conditions, the inhibitory impact of antibiotic residues on hydrolytic and acetogenic microflora becomes minimal. This facilitates a more rapid formation of substrates necessary for the growth and function of methane-producing archaea.

Thus, our results show that the delays observed in methane production during mesophilic fermentation of FPD both with natural microflora and with the addition of BMMA are primarily caused by the inhibitory effects of antibiotics and disinfectants on hydrolytic and acetogenic microorganisms. Under thermophilic conditions, which accelerate antibiotic degradation, these inhibitory effects are greatly reduced, enabling faster and more efficient methane formation when using BTMMA.

Author Contributions

Khusanov Saydulloxon Ilyoshkon Ugli: Investigation, formal analysis, writing—original draft.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

References

1. Bonch-Osmolovskaya, E. A. (1979). Methane formation by microbial communities. In *Progress in Microbiology* (Issue 14, pp. 106–123). Nauka.
2. Egorov, N. S. (1995). *Guide to practical studies in microbiology* (3rd ed., pp. 125–128).
3. Galchenko, V. F., Dulov, L. E., Kramer, B., & co-authors. (2001). Biogeochemical processes of the methane cycle in soils, marshes and lakes of Western Siberia. *Microbiology*, 70(2), 215–225.
4. Gusev, M. V., & Mineeva, L. A. (2003). *Microbiology* (4th ed.). Academia Publishing Center.
5. Ilarionov, S. A., & Bonch-Osmolovskaya, E. A. (1986). Methane formation from methanol by bacterial associations. *Microbiology*, 55(2), 282–288.
6. Lysenko, V. P. (2007). Recycling of waste in poultry farming – the way to a comprehensive solution to avian influenza. *The Poultry Farm*, (6), 32.
7. Maslovsky, K. S., Maksimova, V. D., Aristov, B. M., & co-authors. (1989). The effectiveness of antibiotics in broilers' growing. In *Microorganisms—Stimulators and Inhibitors of Plant and Animal Growth: Proceedings of the All-Union Conference* (Part 2, pp. 127–128). Tashkent.
8. Namsaraev, B. B., Zhilina, T. N., Kudyrova, A. V., & co-authors. (1999). Bacterial formation of methane in soda lakes of the South-Eastern Transbaikalia. *Microbiology*, 68(5), 671–676.
9. Pantskhava, E. S., Sachkova, E. G., Gorbunova, N. V., & co-authors. (1991). Effect of ammonium ions on the conversion of organic matter to methane. *Applied Biochemistry and Microbiology*, 27(3), 442–453.
10. Pathak, S. H. C. (n.d.). *Antibiotics in manure and soil – A grave threat to human and animal health* (Policy Paper No. 43).
11. Pomazanova, Yu. N., & Popok, L. B. (2009). Use of chicken manure to improve soil fertility. In *Problems of Reclamation of Household, Industrial and Agricultural Waste* (pp. 112–118). Krasnodar.
12. Varel, V. H., Wells, J. E., Shelver, W. L., & co-authors. (2012). Effect of anaerobic digestion temperature on odour, coliforms and chlortetracycline in swine manure or monensin in cattle manure. *Journal of Applied Microbiology*, 112(4), 705–715. https://doi.org/10.1111/j.1365-2672.2012.05261.x
13. Vavilin, V. A., Rytov, S. V., & Lokshina, L. Ya. (1997). The balance between hydrolysis and methanogenesis in the anaerobic decomposition of organic matter. *Microbiology*, 66(6), 846–851.
14. Vedenov, A. G., & Vedenova, T. A. (2006). Biogas technology in the Kyrgyz Republic. *Fluid*, 18.
15. Zavarzin, G. A. (1986). Trophic connections in the methanogenic community. *Proceedings of the USSR Academy of Sciences, Biology Series*, (3), 341–360.
16. Zvyagintsev, D. G. (1991). *Methods of soil microbiology and biochemistry*. Moscow.

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