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ENHANCING THE BIOGAS PRODUCTION POTENTIAL OF MAIZE GREEN MASS ENSILED WITH A LACTIC ACID BACTERIAL COMPOSITION

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Aim. To determine the effect of an inoculant composed of *Lentilactobacillus buchneri*, *Levilactobacillus brevis*, and *Lactiplantibacillus plantarum* strains on the fermentation characteristics of maize silage, as well as to assess its methane potential under standard anaerobic digestion conditions.

Materials and Methods. The fermentation properties of the silage were evaluated using standard analytical methods for determining pH, dry matter, and key organic acids. Microbiological analysis was performed using quantitative plating on selective media to determine the counts of lactic acid bacteria, yeasts, molds, and clostridial microorganisms. The biogas potential was assessed after anaerobic digestion using activated sludge as the inoculum; the biogas volume and methane content were measured with a Bosean K-600 gas analyzer.

Results. The use of the inoculant promoted more intensive lactic fermentation, as evidenced by a lower pH (3.78 vs. 4.15 in the control), a higher proportion of lactic acid (79.6% vs. 61.6%), and a lower proportion of acetic acid (24.2% vs. 36.9%). Butyric acid was not detected in either variant. The number of lactic acid bacteria exceeded spoilage microflora by almost fourfold, while no butyric acid-producing bacteria were registered. The application of *L. buchneri* and *L. brevis* contributed to the formation of acetic and propionic acids, thereby improving the aerobic stability of the silage after ensiling and during transfer of the ensiled biomass to anaerobic digestion. The experimental sample showed a reduction in yeast and mould counts (7.2×10^3 CFU/g vs. 3.5×10^4 CFU/g in the control). Methane fermentation demonstrated increased methane yield due to the higher lactic acid content in the biomass ensiled with the proposed starter composition, the improved buffering capacity of the mixture, and reduced dry matter losses.

Conclusions. Inoculating maize silage with *Lentilactobacillus buchneri, Levilactobacillus brevis,* and *Lactiplantibacillus plantarum* strains provides improved conditions for lactic fermentation of the ensiled maize mixture, enhances its organic acid profile, increases aerobic stability, and promotes the formation of a substrate with higher biogas potential. The obtained results demonstrate the feasibility of using such inoculants in silage production technologies for bioenergy purposes.

Keywords: maize silage, bacterial starter composition, lactic fermentation, lactic-to-acetic acid ratio, aerobic stability, methanogenesis, biogas, methane yield.

Biogas is a flammable gas produced through the decomposition of organic raw materials by microorganisms (fungi, bacteria, archaea) in the absence of oxygen [1, 2]. affecting the achievement of Sustainable Development Goal (SDGThis mixture consists primarily of methane (CH_4) and carbon dioxide (CO_2). However, iit also contains intermediate and secondary compounds such as water, molecular nitrogen (N_2), ammonia (NH_3), residual traces of oxygen (O_2) and carbon monoxide (CO), sulfur compounds (H_2S, methyl-SH, ethyl-SH), volatile organic compounds (terpenes (α-pinene, 3-carene, β-pinene, limonene, γ-terpinene), aromatic compounds, alcohols, ketones, alkanes, alkenes, etc.), siloxanes,

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and more [3, 4].one in France and the other in Lebanon. Biogas was produced from the organic component of household municipal waste (i.e., food/kitchen waste and green waste The CH_4 content in biogas may vary from 30 to 80%, while CO_2 may range from 15 to 60%, depending on the substrate and process conditions [4], but typically they make up 50-75% and 25–45%, respectively. It should be noted that natural gas consists of 80–90% CH_4, which makes it more energy-dense [5].

Biogas formation occurs as a result of anaerobic digestion (AD), a process accompanied by the breakdown of organic material [6]. dependence on fossil fuels contributes to environmental damage. Biogas generation from biodegradable organic materials is a potential and sustainable substitute for addressing global energy supply inadequacy and curbing the environmental challenges associated with fossil fuels. Biotechnologies particularly anaerobic digestion technology are important process for the recovery of energy from organic materials. Biogas comes from bio-decomposition of various organic substrates and trash. Human excreta, agricultural wastes, industrial food residues, municipal wastes, food wastes and residues, fishery wastes, aquatic plants and forest residues are among the common organic wastes from which biogas is produced today. Properly designed biogas systems play a crucial role in renewable energy production, providing electricity, heating, and lighting from organic waste materials that would otherwise go to landfill. These systems convert agricultural residues, food waste, livestock manure, and even energy crops into biogas, which can be used to power generators, provide heat for cooking, or supply light in homes. In urban and remote areas, biogas digesters offer clean, alternative energy solutions that not only meet local energy demands but also enhance living conditions by reducing the reliance on expensive or polluting energy sources. For instance, households can save on energy costs and improve air quality by using biogas for cooking instead of traditional fuels. Besides, the implementation of biogas technology can significantly mitigate environmental impact by lowering greenhouse gas emissions, reducing waste, and promoting sustainable agricultural practices and supporting circular economy. This review explores a diverse range of potential substrates for biogas production, highlighting their viability as alternatives to fossil fuel-based energy sources and emphasizing the multifaceted benefits they provide to communities. Four phases of AD are distinguished, each involving specific biochemical transformations necessary for the successful degradation of the substrate (Table 1).

Phase I — hydrolysis of organic polymers. At this stage, complex high-molecular-weight compounds such as proteins, lipids, and carbohydrates are broken down into simpler molecules — amino acids, fatty acids, and sugars — by hydrolytic microorganisms using their extracellular enzymes, including proteases, lipases, cellulases, amylases, and others. The duration and efficiency of this phase depend on many factors, and the presence of hemicellulose and lignin in the organic feedstock may limit the overall rate of AD, as these compounds are poorly susceptible to hydrolysis [7].

Phase II — acidogenesis. The hydrolysis products from the previous stage can cross the cell membranes of the microorganisms involved, enabling the progression to subsequent steps [17]. Approximately 70% of these products (the ratio may differ for proteins and lipids) are further converted into acetic acid (acetate), water, and carbon dioxide. The remaining portion is transformed into intermediate compounds, such as other short-chain organic acids (propionic acid and keto acids) and alcohols. This phase is considered the shortest in the AD process and is also accompanied by the release of ammonia and hydrogen sulfide, recognizable by their characteristic unpleasant odor [4, 7, 18].

Phase III — acetogenesis. At this stage, the products of acidogenesis (short-chain organic acids, alcohols) are transformed into acetic acid, which is then converted into acetate through electron loss in a more alkaline environment. Small amounts of methylated compounds may also form during this phase. These reactions release hydrogen, which — at high partial pressure — can negatively affect the biochemical processes of this stage; however, the excess hydrogen is consumed by specific groups of bacteria (homoacetogens), converting it into acetic acid/acetate [6, 7, 18].

Phase IV — methanogenesis. This is the final stage of AD, during which methane is actually formed. Most biogenic methane (approximately two-thirds) is produced via acetoclastic methanogenesis by acetophilic (acetoclastic) methanogenic archaea from acetate. A smaller portion (about one-third) is generated from carbon dioxide and hydrogen by hydrophilic (hydrogenotrophic) methanogenic archaea and, to a lesser extent,

Table 1. **Phases of anaerobic methane fermentation** [4, 6, 8–15]

Anaerobic fermentation phase	Substrate	Genera of microorganisms involved in the process	Key chemical reactions
Hydrolysis	Proteins, lipids, carbohydrates	*Acetoanaerobium*, *Acetovibrio*, *Acinetobacter*, *Actinomyces*, *Bacillus*, *Bacteroides*, *Butyrivibrio*, *Caldicellulosiruptor*, *Chryseobacterium*, *Clostridium*, *Corynebacterium*, *Enterococcus*, *Halocella*, *Herbinix*, *Klebsiella*, *Micromonospora*, *Paenibacillus*, *Proteus*, *Ruminococcus*, *Saccharomyces*, *Stenotrophomonas*, *Streptococcus*, *Thermobifida*	polypeptide + $nH_2O \rightarrow \rightarrow$ ×amino acids lipid (TG*) + $3H_2O \rightarrow$ glycerol + 3 LCFA** $(C_6H_{10}O_5)_n + nH_2O \rightarrow \rightarrow nC_6H_{12}O_6$ *TG — triglyceride **LCFA — long-chain fatty acids
Acidogenesis	Amino acids, fatty acids, sugars	*Acetobacter*, *Bacillus*, *Clostridium*, *Escherichia*, *Fusobacterium*, *Lactobacillus**, *Porphyromonas*, *Pseudomonas*, *Salmonella*, *Staphylococcus*, *Streptococcus*	amino acids → volatile fatty acids + keto acids/ alcohols+ $NH_3 + CO_2 + + H_2$ LCFA+ $H_2O \rightarrow$ acetate + + $H_2 + CO_2$ Sugars + $H_2O \rightarrow$ acetate + + butyrate + propionate + + ethanol+ $CO_2 + H_2$ $C_6H_{12}O_6 \rightarrow 3CH_3COOH$ (acetate)
Acetogenesis	Short-chain fatty acids (acetic, lactic, propionic), keto acids (pyruvate), alcohols	*Acetobacterium*, *Clostridium*, *Eubacterium*, *Pelotomaculum*, *Propionibacterium*, *Ruminococcus*, *Sporomusa*, *Syntrophobacter*, *Syntrophomonas*, *Thermoanaerobacter*, *Thermosyntropha*	volatile fatty acids → → acetate + $H_2 + CO_2$ keto acids → → acetate + $H_2 + CO_2$ alcohols → acetate + H_2
Methanogenesis	Acetate (acetoclastic pathway), carbon dioxide and hydrogen (hydrogenotrophic pathway), methanol, methanethiol, methylamines (methylotrophic pathway)	*Methanobacterium*, *Methanobrevibacter*, *Methanocaldococcus*, *Methanococcoides*, *Methanococcus*, *Methanocorpusculum*, *Methanoculleus*, *Methanofollis*, *Methanogenium*, *Methanohalophilus*, *Methanolinea*, *Methanolobus*, *Methanomassiliicoccus*, *Methanomethylovorans*, *Methanoplanus*, *Methanoregula*, *Methanosarcina*, *Methanosphaera*, *Methanosphaerula*, *Methanospirillum*, *Methanothermobacter*, *Methanothrix*	Acetoclastic reactions: $CH_3COOH \rightarrow CH_4 + CO_2$ Hydrogenotrophic reactions: $CO_2 + 3H_2 \rightarrow CH_4 + H_2O$ Methylotrophic reactions: $4CH_3OH \rightarrow 3CH_4 + CO_2 + 2H_2O$ $CH_3SH + H_2 \rightarrow CH_4 + H_2S$ $4CH_3NH_2 + 2H_2O \rightarrow 3CH_4 + CO_2 + 4NH_3$ Syntrophic interaction: $2CH_3CH_2OH + CO_2 \rightarrow CH_4 + 2CH_3COOH$

**Note:* the genus name *Lactobacillus* is presented according to [8]i.e., upstream, mainstream, and downstream approaches. In the first part of this review, upstream strategies, including pretreatments by fungal, microbial consortium, and enzymatic as well as some other biological methods including microaeration, composting, ensiling, and genetic and metabolic engineering are discussed in detail. The impacts of upstream strategies on biogas production as well as their potentials in further improving the biogas industry are comprehensively scrutinized. Despite their promising impacts on biogas production, such biological innovations are time-consuming and require extra equipment and facilities that should be addressed in future studies. Overall, most information on biogas production has been generated through lab-scale investigations and not by commercial plants, undermining the commercial value of these data for the right decision-making. Pilot data would be necessary for techno-economic analyses with acceptable accuracies. Therefore, the future efforts should be directed toward providing the missing data for re-engineering designs, calculations, and life cycle assessment (LCA. However, according to the current classification [16]ecological and genotypic levels. This study evaluated the taxonomy of Lactobacillaceae and Leuconostocaceae on the basis of whole genome sequences. Parameters that were evaluated included core genome phylogeny, (conserved, this genus has been divided into 23 new ones (*Lactiplantibacillus*, *Lacticaseibacillus*, etc.), its name is used here in a broad sense.

from methylated compounds (methanol, methanethiol, methylamines, etc.) by methylotrophic methanogenic archaea. This phase is the longest stage of the AD process [4, 7, 19].

The pH value directly influences microbial activity and the progression of biochemical reactions, thereby affecting biogas yield. Biogas formation can occur only within a pH range of 6.8-7.5 [20], with the optimal range being 7.0-7.2 [7].

Despite the fact that methane — produced by archaea — is the main component of biogas, the proportion of archaea in biogas reactors may not exceed 1.5%. The remaining microbiota consists primarily of bacteria from the class *Clostridia* (phylum *Firmicutes*) and the phylum *Bacteroidota*, which play an important role in preventing reactor acidification [11]. Moreover, the number of clostridia may be twice that of bacteroidota [21].

A wide range of substrates with different origins, characteristics, and methane yields are used for biogas production. A general classification divides them into lignocellulosic and non-lignocellulosic substrates. The former include fresh, dried, and ensiled plant biomass, as well as wood-processing residues, husks, and similar materials; the latter include animal-derived wastes, food-industry residues [22], wastewater, macro- and microalgae, and so on [7]. The chosen substrate has a major impact on biogas yield, as its structure and nutrient composition provide the compounds required for methane formation and are directly linked to the activity, reproduction, and development of the microorganisms involved in AD [23]. Therefore, to ensure efficient biogas generation, the substrate must meet several criteria: it should be biodegradable, have an optimal balance of macro- and micronutrients, and preferably contain no lignocellulose [7].

However, lignocellulose-containing substrates are the most common and readily available materials for biogas production, with an estimated potential of 37.2 billion cubic meters of biogas per year [24]. Since lignocellulose consists of cellulose, hemicellulose, and lignin interconnected by strong bonds, this poses challenges for the practical use of plant-derived substrates, limiting their applicability for industrial biogas production [25–27]. One of the effective approaches to facilitate AD is the pretreatment of feedstock, which enhances biomass degradation and includes physical and mechanical methods (milling, ultrasonication, steam explosion, etc.), chemical methods (acids, alkalis, organic solvents, etc.), and biological methods (fungal, microbial, enzymatic) [28]. Physical and chemical pretreatment methods may inhibit subsequent AD stages and, consequently, biogas generation, whereas biological methods lack such drawbacks — although they are characterized by longer processing times [29].

Although using enzymes can increase biogas yield by more than 30%, this approach remains rather costly. Even employing cheaper fungal enzymes does not resolve the issue of economic feasibility for industrial implementation [30]. The use of bacteria (*Bacillus subtilis*, *Acinetobacter johnsonii*) and fungi (*Trichoderma viride*, *Aspergillus niger*), which can enhance biogas yield by up to 40% [31], is being actively explored, as well as ensiling, which can increase methane production by 30% in monosubstrate AD of biomass and by nearly 50% when co-digesting biomass with livestock and poultry waste [32].

The effectiveness of preliminary ensiling of plant biomass for biogas production has been confirmed for numerous substrates: maize (the most common substrate), legumes (white sweet clover, hairy vetch, white lupine) [33], sugar beet [34], elephant grass (*Cenchrus purpureus*) [35], switchgrass (*Panicum virgatum*) [36], and even willow [37]. Due to the accumulation of organic acids during ensiling, mild lignocellulose hydrolysis occurs, which, in turn, provides enzymes with access to cellulose and its gradual decomposition into glucose [38]. As a result, the AD process — particularly its first and rate-limiting phase — proceeds significantly faster [39].

A concern associated with this method of substrate preparation is the loss of organic material and, consequently, the potential for inaccuracies in determining the amount of methane produced per unit of total dry matter of the plant biomass [40]. However, these concerns are offset by studies that use alternative approaches to evaluating process efficiency, such as comparing methane yields per hectare of fresh versus ensiled crops [32]. Moreover, for a stable biogas production process, it is necessary to store biomass year-round, making it important to develop conditions and technological practices that ensure effective preservation of the substrate's nutrients for AD. One such approach is the use of appropriate starter cultures when the dry matter content is high (around 30%).

The study aimed to determine the effect of an inoculant formulated from strains of

Lentilactobacillus buchneri, *Levilactobacillus brevis*, and *Lactiplantibacillus plantarum* on the fermentation properties of maize silage, and to evaluate its methane potential under standard anaerobic digestion conditions.

Materials and Methods

Preparation of raw material for ensiling

Fresh green maize biomass intended for silage was chopped to a particle size of 1–2 cm using a knife-type shredder. For ensiling the samples under study, a developed bacterial inoculant composition was used, consisting of *Lentilactobacillus buchneri*, *Levilactobacillus brevis*, and *Lactiplantibacillus plantarum* in a 0.75:0.25:1 ratio. The application of this inoculant promotes the formation of a wide range of antimicrobial metabolites with pronounced fungicidal and bactericidal activity.

The inoculant was suspended in sterile water and added to the green biomass in an amount that ensured a concentration of 1×10^5-10^6 CFU/g of substrate. The control sample of chopped maize intended for silage was treated with an equivalent amount of sterile water.

The prepared biomass was tightly packed into laboratory polyethylene bags, from which air was removed by vacuuming, and the bags were then hermetically sealed using a heat sealer.

The hermetically sealed samples were stored at 20–25 °C for 60 days. Analyses were performed after the completion of the 60-day cycle.

Substrate preparation

The silage was chopped into 10–20 mm particles to increase the fermentation surface area and stabilize mass transfer. If necessary, the dry matter content was adjusted to 25–35% by adding water.

Anaerobic digestion

The prepared ensiled material was loaded into an airtight digester-type bioreactor and fermented under mesophilic conditions (35–38 °C). If necessary, the medium was alkalized to pH 6.5 before the start of anaerobic digestion. The hydraulic retention time was 40 days. During the methanogenesis stage, fermented activated sludge was used as an inoculum to convert acetate, hydrogen, and carbon dioxide into methane.

The dry matter content was determined by drying the samples in a drying oven at 105 °C to constant weight, and the ash content was measured in a muffle furnace at 650 °C. The pH of the sample solutions was determined potentiometrically using a pH meter MP 512 "Ulab" (China).

The content of key organic acids in the ensiled mass was also determined by gas chromatography using an Agilent 1260 Infinity II LC System (Agilent Technologies, USA).

The amount of biogas produced was calculated from the reduction in substrate volume, and the methane content of the biogas was measured using a Bosean K-600 gas analyzer.

The number of yeasts and molds was determined according to DSTU ISO 7954:2006 Microbiology of food and animal feeding stuffs. General guidelines for the enumeration of yeasts and microscopic fungi. Technique for counting colonies cultivated at 25 °C (ISO 7954:1997, IDT)

Each sample type (control and experimental) was examined in triplicate from the ensiling stage onward.

Results and Discussion

As noted above, plant-based raw materials have high potential for biogas production, particularly given their renewability. Among these, ensiled maize is considered the most promising plant feedstock in terms of yield; it is traditionally stored in clamps for feeding livestock. However, this application also has limitations owing to its low protein content, loss of dry matter and vitamins, and the acidity of the feed resulting from lactic acid fermentation, which is necessary to prevent spoilage during storage. Therefore, the use of maize silage for biofuel production through AD represents a promising approach.

One obstacle to using plant-based raw materials is the presence of cellulose and lignin, which in maize silage can reach 22% and 4%, respectively. One approach to improving silage properties for biogas production is the use of specific lactic acid bacterial inoculants. Both homofermentative and heterofermentative lactobacilli can modify the ratio of organic acids, suppress the growth of undesirable microbiota, reduce dry matter losses, and increase silage stability, thereby enhancing substrate accessibility for anaerobic degradation.

In our study, we proposed a starter culture composition consisting of *Lactiplantibacillus plantarum*, which ensures rapid acidification of the chopped material; *Lentilactobacillus*

buchneri, which promotes active synthesis of acetic acid and 1,2-propanediol to enhance aerobic stability after opening the silage clamp; and *Levilactobacillus brevis*, which provides an additional contribution to heterofermentative metabolism and acetic acid production. The ratio of *Lentilactobacillus buchneri*: *Levilactobacillus brevis*: *Lactiplantibacillus plantarum* was modified from 0.75: 0.25: 2, typical for silage intended as animal feed, to 1: 1: 1 for silage aimed at subsequent anaerobic digestion in biogas production. The selected ratio of cultures helped reduce the loss of sugars and the energy potential of the silage by suppressing yeast and fungal growth during storage and transfer to the anaerobic bioreactor.

The proposed inoculation dose of 1×10^5–10^6 CFU/g of fresh biomass ensured that the number of lactic acid bacteria in the silage exceeded the spontaneous microbiota by nearly fourfold. Butyric acid-producing microorganisms were not detected in the inoculated silage, consistent with the complete absence of butyric acid in the samples. The acetic acid produced during fermentation exhibits higher antimicrobial activity. The number of yeasts and molds in the control sample reached 3.3×10^4 CFU/g, whereas in the silage with the added starter culture composition it amounted to 7.2×10^3 CFU/g.

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Table 2 presents the parameters of maize silage produced with a lactic acid bacteria inoculant compared with the control. The data show notable shifts toward more intensive lactic acid fermentation in the experimental variant, accompanied by a reduction in the proportion of acetic acid.

In the experimental sample, a lower pH was recorded (3.78 vs. 4.15), indicating more effective acidification of the ensiled material. This is confirmed by a substantial increase in the proportion of lactic acid — 79.6% of the total organic acids, whereas in the control it accounted for only 61.6%. Accordingly, the lactic-to-acetic acid ratio in the experimental variant nearly doubled (3.29 vs. 1.67), which is a characteristic indicator of the dominance of homofermentative processes and optimal silage stabilization during the early stages of anaerobic fermentation.

The proportion of acetic acid decreased to 24.2%, compared to 36.9% in the control. This indicates reduced heterofermentative microorganism activity, thereby improving the controllability of the fermentation process. In addition, if part of such silage were used as feed for farm animals, it would be more appealing due to its less sharp taste.

Butyric acid was not detected in either sample, confirming the absence of clostridial fermentation and an acceptable sanitary quality of the silage.

The dry matter content did not differ significantly between the two samples (34.2% and 35.1%).

Overall, the data show that the use of the combined inoculant promoted a more efficient course of lactic acid fermentation, a faster decrease in pH, a reduction in proteolytic processes, and improved silage stability.

Overall, the data show that the combined inoculant promoted a more efficient course of lactic acid fermentation, a faster decrease in pH, reduced proteolysis, and improved silage stability.

Since biogas is produced under conditions similar to those required for silage fermentation — namely, in both cases biomass is decomposed by microorganisms under anaerobic conditions [41] — biogas production has repeatedly been viewed as a logical continuation of the silage process [42]. The key issue in combining these two processes is balancing the dry matter (DM) content of the silage with the amount of methane that can be produced from the ensiled plant biomass. This is because, during year-long storage of green biomass after harvest, its DM content inevitably decreases, largely due to the conversion of organic compounds into volatile products such as acids and alcohols.

However, our studies (Table 3) demonstrated that biogas and methane production after AD in the ensiled samples treated with the proposed LAB starter culture composition was higher: approximately 556 L/kg DM in the control samples and 622 L/kg DM in the experimental ones.

Known approaches to improving the efficiency and cost-effectiveness of biogas production include co-ensiling several types

Table 2. **Physicochemical indicators of silage**

Parameter	Control	Treatment
pH	4.15 ± 0.10	3.78 ± 0.10
Lactic acid, % of total % from the sum of organic acids	61.6 ± 4.9	79.6 ± 6.4
Acetic acid, % of total % from the sum of organic acids	36.9 ± 1.9	24.2 ± 1.5
Lactic-to-acetic acid ratio	1.67 ± 0.04	3.29 ± 0.05
Butyric acid	Not detected	Not detected
Dry matter, %	34.2 ± 0.1	35.1 ± 0.1

Table 3. **Biogas Potential of Maize Silage**

Parameter	Control	Treatment
Biogas (L/kg DM)	556 ± 10	622 ± 10
Methane, %	51.8 ± 2.5	57.3 ± 2.5
Methane potential (L CH4/kg DM)	279 ± 5	343 ± 5

of plant raw materials, modifying the buffer capacity of the moist substrate, adjusting the carbon-to-nitrogen ratio, and using additives of various origins [43].

In our studies, the advantage observed in the experimental samples in terms of both biogas yield and its methane content was, in our view, specifically due to the increased buffer capacity of the mixture resulting from a substantial (almost twofold) increase in lactic acid concentration in the ensiled maize biomass.

It should also be noted that a potential methodological error may occur when determining DM, as losses can occur both during drying to constant weight and during opening the ensiled biomass and transferring it to the anaerobic bioreactor. Nevertheless, DM losses are undoubtedly present. However, in the experimental samples, evaporation is evidently lower, since the mixture contains more lactic acid (the boiling point of lactic acid is 200 °C, whereas that of acetic acid is 118 °C; at pH 3.78, a smaller portion of acetic acid is present in its undissociated form).

Thus, it can be asserted that the increased biogas and methane yield in the ensiled maize samples in our study was specifically due to the application of the developed starter culture composition, which ensured a higher lactic acid content and the preservation of DM in the substrate that is converted into biogas during AD. The results are consistent with the literature, which reports silage as one of the most efficient substrates for biogas production [44, 45] and demonstrates improvements in the fermentation profile of ensiled mixtures [46], whose partially hydrolyzed composition is readily converted into methane by activated sludge [47–50].

Thus, the obtained results demonstrate that maize silage is a highly efficient substrate for anaerobic conversion into biogas, provides a stable methane yield, and can be recommended as a primary feedstock for biogas plants of various capacities.

Conclusions

1. Inoculating the maize silage mixture with a 1:1:1 composition of *Lentilactobacillus buchneri*, *Levilactobacillus brevis*, and *Lactiplantibacillus plantarum* prior to anaerobic digestion in a methanogenic-type bioreactor provided an improved fermentation profile of the substrate compared with the control. The experimental samples showed a lower pH (3.8 vs. 4.2 in the control) and a higher lactic-to-acetic acid ratio (3.3 vs. 1.7 in the control), resulting in better substrate buffering capacity for anaerobic digestion and improved preservation of dry matter. No butyric acid was detected in the samples, which can inhibit methanogenesis under certain conditions and indicate clostridial fermentation.

2. When the proposed bacterial composition was used as an inoculant for ensiling maize green biomass, the biogas yield after anaerobic digestion increased by 12%, and the methane potential by 22%.

The use of a lactic acid bacteria inoculant during the ensiling of plant biomass for year-round storage is a promising tool for

improving the efficiency of biogas technologies based on maize silage.

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Author contributions

Alla Lukianets — conducted the study, collected data, and wrote the manuscript; Svitlana Danylenko — developed the concept and design of the study.

REFERENCES

1. Issahaku, M., Derkyi, N.S.A., Kemausuor, F. (2024). A systematic review of the design considerations for the operation and maintenance of small-scale biogas digesters. *Heliyon*, 10(1), e24019. https://doi.org/10.1016/j.heliyon.2024.e24019
2. Nahwani, A., Soeprijanto, S., Widodo, E. (2024). Strategic model for integrating biogas a framework for sustainable energy integration in agro-industries. *Sci. Rep.*, 14(1), 1–15.
3. Tanios, C., Billet, S., Méausoone, C., Landkocz, Y., Gennequin, C., Labaki, M., Abi-Aad, E. (2025). Impact of waste origin and post-treatment techniques on the composition and toxicity of biogas. *Sci. Total Environ.*, 966, 178688. https://doi.org/10.1016/j.scitotenv.2025.178688
4. Ostos, I., Flórez-Pardo, L.M., Camargo, C. (2024)A metagenomic approach to demystify the anaerobic digestion black box and achieve higher biogas yield: a review. *Front. Microbiol.,*. 15(11), 1437098. https://doi.org/10.3389/fmicb.2024.1437098.
5. Aravani, V. P., Sun, H., Yang, Z., Liu, G., Wang, W., Anagnostopoulos, ..., Papadakis V. G. (2022). Agricultural and livestock sector's residues in Greece & China: Comparative qualitative and quantitative characterization for assessing their potential for biogas production. *Renewable and Sustainable Energy Reviews.*, 154, 111821. https://doi.org/10.1016/j.rser.2021.111821
6. Ngabala, F. J., Emmanuel, J. K. (2024). Potential substrates for biogas production through anaerobic digestion-an alternative energy source. *Heliyon. Elsevier Ltd*,. 10(23). e40632. https://doi.org/10.1016/j.heliyon.2024.e40632
7. Dhull, P., Kumar, S., Yadav, N., Lohchab, R. K. (2024). A comprehensive review on anaerobic digestion with focus on potential feedstocks, limitations associated and recent advances for biogas production. *Environmental Science and Pollution Research. Springer Berlin Heidelberg*,. *Birol*, 32(32), 19129–19164. https://doi.org/10.1007/s11356-024-33736-6
8. Tabatabaei, M., Aghbashlo, M., Valijanian, E., Kazemi Shariat Panahi, H., Abdul-Sattar Nizami, Ghanavati, H., Sulaiman, A., Mirmohamadsadeghi, S., Karimi, K., (2020). A comprehensive review on recent biological innovations to improve biogas production, Part 1: Upstream strategies. *Renew. Energy. Pergamon*, 146, 1204–1220. https://doi.org/10.1016/j.renene.2019.07.037Get rights and content
9. Bueno de Mesquita, C.P., Wu, D., Tringe, S. G. (2023).Methyl-Based Methanogenesis: an Ecological and Genomic Review. *Microbiol. Mol. Biol. Rev.*, 87(1). https://doi.org/10.1128/mmbr.00024-22
10. Ottoni, J. R., Bernal, S. P. F., Marteres, T. J., Luiz, F. N., Dos Santos, V. P., Mari, Â. G., ..., Passarini, M. R. Z. (2022). Cultured and uncultured microbial community associated with biogas production in anaerobic digestion processes. *Arch Microbiol.,* **204**, 340. https://doi.org/10.1007/s00203-022-02819-8
11. Kabaivanova, L., Petrova, P., Hubenov, V., Simeonov, I. (2022). Biogas Production Potential of Thermophilic Anaerobic Biodegradation of Organic Waste by a Microbial Consortium Identified with Metagenomics. *Life,* 12(5), 702. https://doi.org/10.3390/life12050702
12. Liang, Y., Zhao, L., Zhao, Y., Li, Z., Feng, J., Yao, Z., ..., Yu, J. (2022). Novel insights from lignocellulosic waste to biogas through regulated dry-wet combined anaerobic digestion: Focusing on mining key microbes. *Bioresource technology*, 348, 126778. https://doi.org/10.1016/j.biortech.2022.126778
13. Banu, J. R., Kumar, G.,& Chattopadhyay, I. (2021). Management of microbial enzymes for biofuels and biogas production by using metagenomic and genome editing approaches. *3 Biotech*, 11(10), 429. https://doi.org/10.1007/s13205-021-02962-x.
14. Vítězová, M., Lochman, J., Zapletalová, M., Ratering, S., Schnell, S., Vítěz, T. (2021). Archaeal community dynamics in biogas fermentation at various temperatures assessed by mcrA amplicon sequencing

using different primer pairs. *World journal of microbiology & biotechnology*, 37(11), 188. https://doi.org/10.1007/s11274-021-03152-w

15. Pyzik, A., Ciezkowska, M., Krawczyk, P. S., Sobczak, A., Drewniak, L., Dziembowski, A., Lipinski, L. (2018). Comparative analysis of deep sequenced methanogenic communities: identification of microorganisms responsible for methane production. *Microbial cell factories*, 17(1), 197. https://doi.org/10.1186/s12934-018-1043-3
16. Zheng, J., Wittouck, S., Salvetti, E., Franz, C. M. A. P., Harris, H. M. B., Mattarelli, P., ..., Lebeer, S. (2020). A taxonomic note on the genus *Lactobacillus*: Description of 23 novel genera, emended description of the genus *Lactobacillus* Beijerinck 1901, and union d *Leuconostocaceae*. *International journal of systematic and evolutionary microbiology*, 70(4), 2782–2858. https://doi.org/10.1099/ijsem.0.004107
17. Swinbourn, R., Li, C., Wang, F. (2024). A Comprehensive Review on Biomethane Production from Biogas Separation and its Techno-Economic Assessments. *ChemSusChem*, 17(19), e202400779. https://doi.org/10.1002/cssc.202400779.
18. Hashemi, S., Hashemi, S. E., Lien, K. M., Lamb, J. J. (2021). Molecular Microbial Community Analysis as an Analysis Tool for Optimal Biogas Production. *Microorganisms*, 9(6), 1162. https://doi.org/10.3390/microorganisms9061162
19. Vanwonterghem, I., Evans, P. N., Parks, D. H., Jensen, P. D., Woodcroft, B. J., Hugenholtz, P., Tyson, G. W. (2016). Methylotrophic methanogenesis discovered in the archaeal phylum Verstraetearchaeota. *Nature microbiology*, 1, 16170. https://doi.org/10.1038/nmicrobiol.2016.170
20. Dussadee, N., Reansuwan, K., Ramaraj, R. (2014). Potential development of compressed bio-methane gas production from pig farms and elephant grass silage for transportation in Thailand. *Bioresource technology*, 155, 438–441. https://doi.org/10.1016/j.biortech.2013.12.126
21. Fliegerová, K., Mrázek, J., Kajan, M., Podmirseg, S. M., Insam, H. (2012). The effect of maize silage as co-substrate for swine manure on the bacterial community structure in biogas plants. *Folia microbiologica*, 57(4), 281–284. https://doi.org/10.1007/s12223-012-0125-6
22. Chettri, D., Verma, A. K., Ghosh, S., Verma, A. K. (2024). Biogas from lignocellulosic feedstock: current status and challenges. *Environmental science and pollution research international*, 31(27), 1–26. https://doi.org/10.1007/s11356-023-29805-x
23. Nwokolo, N., Mukumba, P., Obileke, K., Enebe, M. (2020). Waste to Energy: A Focus on the Impact of Substrate Type in Biogas Production. *Processes*, 8(10), 1224. https://doi.org/10.3390/pr8101224
24. Tamilselvan, R., Immanuel Selwynraj, A. (2024). Enhancing biogas generation from lignocellulosic biomass through biological pretreatment: Exploring the role of ruminant microbes and anaerobic fungi. *Anaerobe*, 85, 102815. https://doi.org/10.1016/j.anaerobe.2023.102815
25. Saini, J. K., Saini, R., Tewari, L. (2015). Lignocellulosic agriculture wastes as biomass feedstocks for second-generation bioethanol production: concepts and recent developments. *3 Biotech*, 5(4), 337–353. https://doi.org/10.1007/s13205-014-0246-5
26. Patinvoh, R. J., Osadolor, O. A., Chandolias, K., Sárvári Horváth, I., Taherzadeh, M. J.. (2017). Innovative pretreatment strategies for biogas production. *Bioresource technology*, 224, 13–24. https://doi.org/10.1016/j.biortech.2016.11.083
27. Sawatdeenarunat, C., Surendra, K. C., Takara, D., Oechsner, H., Khanal, S. K. (2015). Anaerobic digestion of lignocellulosic biomass: challenges and opportunities. *Bioresource technology*, 178, 178–186. https://doi.org/10.1016/j.biortech.2014.09.103
28. Xu, N., Liu, S., Xin, F., Zhou, J., Jia, H., Xu, J., Jiang, M., Dong, W. (2019). Biomethane Production From Lignocellulose: Biomass Recalcitrance and Its Impacts on Anaerobic Digestion. *Frontiers in bioengineering and biotechnology*, 7, 191. https://doi.org/10.3389/fbioe.2019.00191
29. Olatunji, K. O., Ahmed, N. A., Ogunkunle, O. (2021). Optimization of biogas yield from lignocellulosic materials with different pretreatment methods: a review. *Biotechnology for biofuels*, 14(1), 159. https://doi.org/10.1186/s13068-021-02012-x
30. Zhao, X., Zheng, Z., Cai, Y., Zhao, Y., Zhang, Y., Gao, Y., Cui, Z., Wang, X. (2020). Accelerated biomethane production from lignocellulosic biomass: Pretreated by mixed enzymes secreted by Trichoderma viride and Aspergillus sp. *Bioresource technology*, 309, 123378. https://doi.org/10.1016/j.biortech.2020.123378
31. Chen, J., Cai, Y., Wang, Z., Wang, S., Li, J., Song, C., ..., Ying, H. (2024). Construction

of a Synthetic Microbial Community for Enzymatic Pretreatment of Wheat Straw for Biogas Production via Anaerobic Digestion. *Environmental science & technology*, 58(21), 9446–9455. https://doi.org/10.1021/acs.est.4c02789

32. Riau, V., Burgos, L., Camps, F., Domingo, F., Torrellas, M., Antón, A., Bonmatí, A. (2021). Closing nutrient loops in a maize rotation. Catch crops to reduce nutrient leaching and increase biogas production by anaerobic co-digestion with dairy manure. *Waste management (New York, N.Y.)*, 126, 719–727. https://doi.org/10.1016/j.wasman.2021.04.006
33. Kintl, A., Huňady, I., Sobotková, J., Vítěz, T., Brtnický, M., Vejražka, K., Elbl, J. (2024). Data on the effect of co-fermentation of maize and leguminous crops on biogas production, methane production and methane content in biogas. *Data Br.*. 56, 110842. https://doi.org/10.1016/j.dib.2024.110842
34. Ahmed, S., Einfalt, D., Kazda, M. (2016). Co-Digestion of Sugar Beet Silage Increases Biogas Yield from Fibrous Substrates. *BioMed research international*, 2016, 2147513. https://doi.org/10.1155/2016/2147513
35. Pomdaeng, P., Chu, C. Y., Sripraphaa, K., Sintuya, H. (2022). An accelerated approach of biogas production through a two-stage $BioH_2/CH_4$ continuous anaerobic digestion system from Napier grass. *Bioresource technology*, 361, 127709. https://doi.org/10.1016/j.biortech.2022.127709
36. Zhao, X., Liu, J., Liu, J., Yang, F., Zhu, W., Yuan, X., ..., Wang, X., (2017). Effect of ensiling and silage additives on biogas production and microbial community dynamics during anaerobic digestion of switchgrass. *Bioresour. Technol.*, 241, 349–359 https://doi.org/10.1016/j.biortech.2017.03.183
37. Nyári, J., Kakuk, B., Bagi, Z., Rákhely, G., Kovács, K. L. (2021). Use of ensiled green willow biomass in biogas fermentation. *Biologia futura*, 72(2), 263–271. https://doi.org/10.1007/s42977-021-00067-3.
38. Ambye-Jensen, M., Johansen, K.S., Didion, T., Kádár, Z., Schmidt, J. E., Meyer, A.S. (2013). Ensiling as biological pretreatment of grass (Festulolium Hykor): The effect of composition, dry matter, and inocula on cellulose convertibility. *Biomass and Bioenergy*, 58. https://doi.org/10.1016/j.biombioe.2013.08.015
39 Mishra, S., Singh, P. K., Dash, S., Pattnaik, R. (2018). Microbial pretreatment of lignocellulosic biomass for enhanced biomethanation and waste management. *3 Biotech*, 8(11), 458. https://doi.org/10.1007/s13205-018-1480-z
40. Kreuger, E., Nges, I. A., Björnsson, L. (2011). Ensiling of crops for biogas production: effects on methane yield and total solids determination. *Biotechnology for biofuels*, 4, 44. https://doi.org/10.1186/1754-6834-4-44
41. Jameel, M. K., Mustafa, M. A., Ahmed, H. S., Mohammed, A. J., Ghazy, H., Shakir, M. N., ..., Kianfar E., (2024). Biogas: Production, properties, applications, economic and challenges: A review. *Results Chem. Elsevier*, 7, 101549. https://doi.org/10.1016/j.rechem.2024.101549
42. Teixeira, F. R., Buffière, P., Bayard, R. (2016).Ensiling for biogas production: Critical parameters. A review. *Biomass and Bioenergy,* 94, 94–104. https://doi.org/10.1016/j.biombioe.2016.08.014
43. Sun, H., Cui, X., Li, R., Guo, J., Dong, R. (2021). Ensiling process for efficient biogas production from lignocellulosic substrates: Methods, mechanisms, and measures. *Bioresource technology*, 342, 125928. https://doi.org/10.1016/j.biortech.2021.125928.
44. Amon, T., Amon, B., Kryvoruchko, V., Zollitsch, W., Mayer, K., Gruber, L. (2007). Biogas production from maize and dairy cattle manure—Influence of biomass composition on the methane yield. *Agriculture, Ecosystems & Environment,* 118, 1–4, 173–182 https://doi.org/10.1016/j.agee.2006.05.007
45 Chynoweth, D. P., Turick, C. E., Owens, J. M., Jerger, D. E., Peck, M. W. (1993). Biochemical methane potential of biomass and waste feedstocks. *United Kingdom,* 5, 1, 95–111 https://doi.org/10.1016/0961-9534(93)90010-2
46. Liu, M., Wang, Y., Wang, Z., Bao, J., Zhao, M., Ge, G., Jia, Y., Du, S. (2022). Effects of Isolated LAB on Chemical Composition, Fermentation Quality and Bacterial Community of *Stipa grandis* Silage. *Microorganisms*, 10(12), 2463. https://doi.org/10.3390/microorganisms10122463
47. Kung, L., Jr, Shaver, R. D., Grant, R. J., Schmidt, R. J. (2018). Silage review: Interpretation of chemical, microbial, and organoleptic components of silages. *Journal of dairy science*, 101(5), 4020–4033. https://doi.org/10.3168/jds.2017-13909
48. Filya, I., Sucu, E. (2010). The effects of lactic acid bacteria on the fermentation, aerobic stability and nutritive value of

maize silage. *Grass and Forage Science*, 65, 446–455 https://doi.org/10.1111/j.1365-2494.2010.00763.x
49. Filya, I., Sucu, E. (2007) The Effect of Bacterial Inoculants and a Chemical Preservative on the Fermentation and Aerobic Stability of Whole-crop Cereal Silages Asian-Aust. *J. Anim. Sci.,* 20(3), 378–384 https://doi.org/10.5713/ajas.2007.378
50. Sucu, E., Filya, I. (2006). Effects of Homofermentative Lactic Acid Bacterial Inoculants on the Fermentation and Aerobic Stability Characteristics of Low Dry Matter Corn Silages. *Turkish Journal of Veterinary & Animal Sciences,* 30(1), 83–88.

ПІДВИЩЕННЯ ПОТЕНЦІАЛУ УТВОРЕННЯ БІОГАЗУ ІЗ ЗЕЛЕНОЇ МАСИ КУКУРУДЗИ, СИЛОСОВАНОЇ КОМПОЗИЦІЄЮ МОЛОЧНОКИСЛИХ БАКТЕРІЙ

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Мета. Визначити вплив використання інокулянта, сформованого на основі штамів *Lentilactobacillus buchneri*, *Levilactobacillus brevis* та *Lactiplantibacillus plantarum*, на ферментаційні властивості кукурудзяного силосу, а також оцінити його метановий потенціал за стандартних умов анаеробного зброджування.

Методи. Ферментаційні властивості силосу досліджували за стандартними аналітичними методиками визначення: pH, сухих речовин, вмісту ключових органічних кислот. Мікробіологічний аналіз проводили з використанням кількісного висіву на селективні середовища з визначенням чисельності молочнокислих бактерій, дріжджів, пліснявих грибів та маслянокислих мікроорганізмів. Біогазовий потенціал оцінювали після анаеробного зброджування з активним мулом як інокулятом, кількість біогазу та вміст у ньому метану визначали за допомогою газоаналізатора Bosean K–600.

Результати. Використання інокулянта сприяло інтенсивнішому молочнокислому бродінню, що підтверджено нижчим pH (3,78 проти 4,15 у контролі), збільшенням частки молочної кислоти (79,6 % проти 61,6 %) та зменшенням частки оцтової кислоти (24,2 % проти 36,9 %). Масляну кислоту в обох варіантах не виявлено. Чисельність молочнокислих бактерій перевищувала гнильну мікрофлору майже в 4 рази, водночас як маслянокислі бактерії не реєструвалися. Застосування культур *L. buchneri* та *L. brevis* сприяло утворенню оцтової та пропіонової кислот, що забезпечило кращу аеробну стабільність силосу після закінчення силосування та передачі силосованої біомаси на анаеробне зброджування. У дослідному зразку зафіксовано зменшення кількості дріжджів і цвілі ($7{,}2\times10^3$ КУО/г проти $3{,}5\times10^4$ КУО/г у контролі). Метанове зброджування показало підвищення виходу метану внаслідок збільшення кількості молочної кислоти в силосованій за допомогою запропонованої заквашувальної композиції біомасі, підвищення буферності суміші та зменшення втрат сухих речовин.

Висновки. Інокуляція кукурудзяного силосу штамами *Lentilactobacillus buchneri, Levilactobacillus brevis* та *Lactiplantibacillus plantarum* забезпечує кращі умови для молочнокислого бродіння закладеної на силос кукурудзяної суміші, покращує в ній співвідношення органічних кислот, підвищує аеробну стабільність та сприяє формуванню субстрату з вищим біогазовим потенціалом. Отримані результати демонструють доцільність використання таких інокулянтів у технологіях силосування для біоенергетичних цілей.

Ключові слова: кукурудзяний силос, бактеріальна заквашувальна композиція, молочнокисле бродіння, співвідношення молочної та оцтової кислот, аеробна стабільність, метаногенез, біогаз, вихід метану.