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Influence of EM4 Inoculum on Biogas Yield and pH Dynamics in Lettuce and Cabbage Waste Fermentation

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ABSTRACT

Biogas production from vegetable waste offers a practical pathway for reducing organic residues and supporting small-scale renewable energy systems. This study investigates the influence of EM4 inoculum on gas accumulation and pH dynamics during an 8-day batch fermentation of lettuce and cabbage waste. Four reactors were prepared containing 200 g vegetable waste and 200 mL water with or without 30 g EM4. Gas accumulation was quantified by balloon circumference at day 2, day 4, day 6 and day 8 and converted to volume using geometric calculations. Lettuce generated the highest peak volumes with maximum values exceeding 330 cm³ while cabbage produced less than 60 cm³ under comparable conditions. pH trajectories showed that cabbage plus water decreased from pH 7 to pH 6 whereas lettuce plus water increased from pH 7 to pH 8. Reactors containing EM4 stabilised at pH 6 throughout the fermentation indicating inoculum-supported buffering. These results demonstrate that substrate characteristics strongly influence early-stage gas formation and that EM4 moderates pH fluctuations even under uncontrolled ambient conditions. The findings provide initial evidence that lettuce is a more degradable feedstock than cabbage and emphasise pH regulation as a key requirement for improving methane-oriented biogas performance in simple batch systems.

Keywords: biogas; vegetable waste; lettuce; cabbage; anaerobic fermentation

1. INTRODUCTION

Biogas is a renewable fuel generated by the anaerobic microbial conversion of organic matter into methane and carbon dioxide. Typical raw biogas contains roughly 50 to 70 % methane and 30 to 40 % carbon dioxide with trace contaminants that depend on substrate and process conditions.[1, 2] Anaerobic digestion offers dual environmental benefits because it decreases organic waste accumulation and reduces greenhouse gas emissions while producing a usable local energy source.[3]

Vegetable wastes are abundant in markets and food processing chains and are readily biodegradable which makes them attractive feedstocks for small scale anaerobic digestion. Previous studies demonstrate variable yields across vegetable types and stress the importance of inoculum selection substrate composition and process control to achieve stable methane production.[4–7] The present work compares lettuce and cabbage as substrates and evaluates the effect of EM4 inoculum on gas accumulation and pH stability under simple batch conditions.

Although EM4 is widely used as a microbial starter in community scale fermentation systems, scientific evaluations of EM4 performance in vegetable waste digestion remain limited. Existing reports seldom compare the behavior of different vegetable substrates when inoculated with EM4 and many studies do not integrate pH dynamics with gas development to describe overall fermentation stability.[8, 9] This gap limits understanding of how EM4 influences the balance between acidogenic bacteria and methanogenic archaea under simple batch conditions.

This study aims to evaluate the influence of EM4 inoculum on gas accumulation and pH evolution during batch fermentation of lettuce and cabbage wastes. The work also aims to compare the suitability of both substrates for biogas generation under identical conditions. The novelty lies in the simultaneous assessment of gas production and pH trajectories for two distinct leafy vegetable substrates using EM4 as inoculum which provides an integrated view of inoculum driven fermentation stability that has not been elucidated clearly in previous studies.

2. EXPERIMENTAL

2.1. Materials and reactor setup

Materials consisted of fresh lettuce waste, fresh cabbage waste, tap water, EM4 microbial inoculum, plastic bottles with capacity 1.5 liter, transparent tubing, balloons used as gas reservoirs, and a ruler for circumference measurement. All materials were prepared under standard laboratory cleanliness and used as received. Each bottle functioned as a batch anaerobic reactor equipped with a single outlet tube connected to a balloon for gas collection.

2.2. Sample preparation and experimental design

Vegetable waste was chopped into pieces with approximate size between 1 and 2 cm to increase the available surface area for microbial degradation. The experiment consisted of 4 batch reactors designed to evaluate the influence of substrate type and EM4 inoculum. The reactors contained cabbage with water, cabbage with water and EM4, lettuce with water, and lettuce with water and EM4. Each reactor received 200 gr vegetable waste and 200 mL water while EM4 was added at 30 g for reactors that incorporated inoculum. All reactors were sealed to maintain anaerobic conditions and connected to balloons that served as gas reservoirs. The reactors were then incubated at ambient laboratory temperature for 8 days to allow fermentation and gas accumulation.

2.3. Monitoring and measurements

Gas accumulation was recorded on day 2 day 4 day 6 and day 8 by measuring balloon circumference with a ruler. Circumference values were converted to gas volume by assuming near spherical geometry. Radius was

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calculated as circumference divided by 2 pi. Volume was calculated as 4 divided by 3 multiplied by pi multiplied by radius cubed. These estimates are commonly used in small scale biogas experiments where direct gas meters are not available.

pH of each reactor slurry was measured at the start of fermentation and at the end of day 8 to evaluate acidification and buffering effects associated with microbial activity. Gas composition was not measured because this experiment focused on relative gas accumulation and pH dynamics.

2.4. Data processing

Circumference values were converted to radius and then to volume using the formulas described above. Volume values represent uncorrected accumulated gas at ambient pressure and room temperature and were used to compare relative gas output across treatments.

3. RESULTS AND DISCUSSION

3.1. Gas accumulation patterns

Gas accumulation differed among treatments and showed clear separation between lettuce and cabbage substrates. The raw circumference data and the derived balloon volumes are presented in Table 1 and Table 2. Lettuce consistently produced higher peak volumes than cabbage across the 8 day digestion period. The highest volumes were observed in lettuce without EM4 followed by lettuce with EM4 then cabbage with EM4 and finally cabbage without EM4.

Table 1. Circumference of balloon gas collectors during fermentation

Sample	Day 2 (cm)	Day 4 (cm)	Day 6 (cm)	Day 8 (cm)
Lettuce	21.7	20.2	27.9	23.0
Lettuce plus EM4	20.3	18.9	25.0	19.5
Cabbage plus EM4	12.9	11.5	15.0	12.0
Cabbage	4.0	3.0	8.0	7.6

Table 2. Balloon gas volume converted from circumference measurements

Sample	Day 2 (cm^3)	Day 4 (cm^3)	Day 6 (cm^3)	Day 8 (cm^3)
Lettuce	173.4	139.7	336.4	205.2
Lettuce plus EM4	141.0	114.1	265.9	132.1
Cabbage plus EM4	36.0	25.6	57.1	29.1
Cabbage	1.1	0.46	8.58	7.41

Temporal trajectories showed irregular increases and decreases. Lettuce with water increased from day 2 to day 6 followed by a decline on day 8. Lettuce with EM4 showed a similar pattern with increases on day 2 and day 6 but reductions on day 4 and day 8. Cabbage with EM4 produced only small increases across all sampling days while cabbage without EM4 generated negligible gas during the entire experiment.

These fluctuations are characteristic of short term batch digestion at ambient temperatures because microbial activity responds rapidly to changes in substrate solubilization and transient accumulation of intermediate acids. Without temperature control or continuous mixing, the balance between hydrolysis

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acidogenesis and methanogenesis becomes unstable.[10, 11] Consequently gas volumes provide comparative indicators rather than quantitative methane yield. Figure 1 illustrates the gas accumulation pattern for the four treatments.

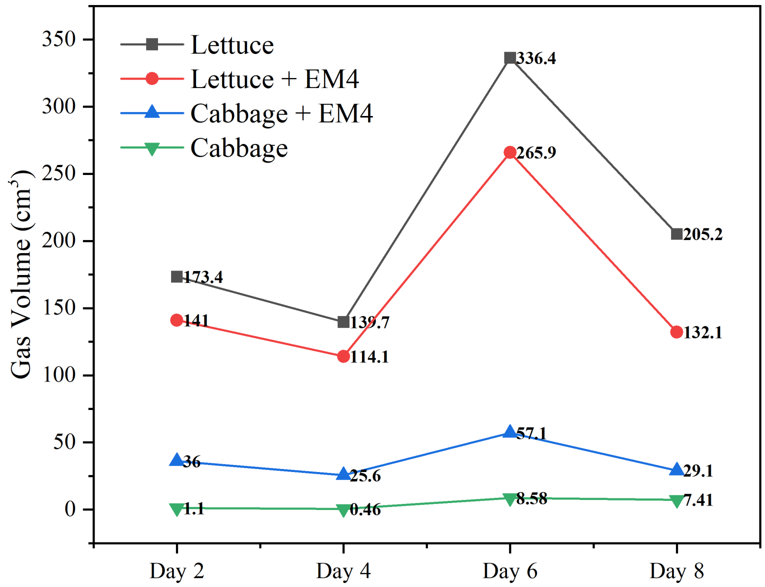

Figure 1. Gas accumulation in lettuce and cabbage reactors with and without EM4 over eight days.

3.2. Influence of substrate characteristics and EM4 inoculum

Substrate characteristics strongly affected the rate of hydrolysis and subsequent gas formation. Lettuce has higher moisture content lower lignocellulosic structure and faster biodegradability than cabbage. These features support rapid solubilization of organic matter and can explain the higher short term gas volumes observed in lettuce reactors.[10, 12]

The addition of EM4 influenced the fermentation response. EM4 contains consortia of lactic acid bacteria photosynthetic bacteria yeasts and actinomycetes which can accelerate early stages of degradation.[13, 14] In this study EM4 improved gas formation in cabbage reactors although the volumes remained lower than all lettuce treatments. In lettuce reactors EM4 did not increase peak gas yield compared with the uninoculated lettuce reactor but it modified the temporal pattern, suggesting that inoculum activity altered early hydrolysis and acid formation.

Performance of commercial inocula such as EM4 depends on viability compatibility with substrate and environmental conditions. Previous studies report that EM4 often accelerates initial breakdown but its effect on methane stage depends on inoculum to substrate ratio pH trajectories and retention time. Because the present study lasted only eight days the observed gas mainly corresponds to early acidogenic activity rather than complete methanogenesis.

3.3. pH dynamics and implications for methanogenesis

pH trajectories reflect the extent of acidogenesis and the ability of each reactor to maintain conditions favorable for methanogenesis. The cabbage plus water reactor declined from pH 7 to pH 6 which indicates acid accumulation during early fermentation and limited intrinsic buffering capacity. Lettuce plus water

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increased from pH 7 to pH 8 suggesting a mild alkalizing response that may originate from endogenous plant constituents capable of consuming or neutralizing acidity.

Reactors containing EM4 exhibited stable end-point values of pH 6 for both cabbage and lettuce. This stability implies that EM4 influenced the acid–base balance by moderating acid formation or generating metabolites that reduced pH drift. Such stabilization is favorable because methanogenic archaea operate most efficiently when the environment remains close to neutral within roughly pH 6.8 to pH 7.4. Reactors that decline toward pH 6 are less suitable for sustained methane formation because increasing acidity suppresses methanogenic activity[14, 15].

The pH results (Figure 2) therefore indicate that EM4 contributed to more stable fermentation conditions although the short eight-day retention time was insufficient for establishing a fully developed methanogenic phase. The observed pH behavior is consistent with early stage acidogenesis rathez than mature anaerobic digestion.

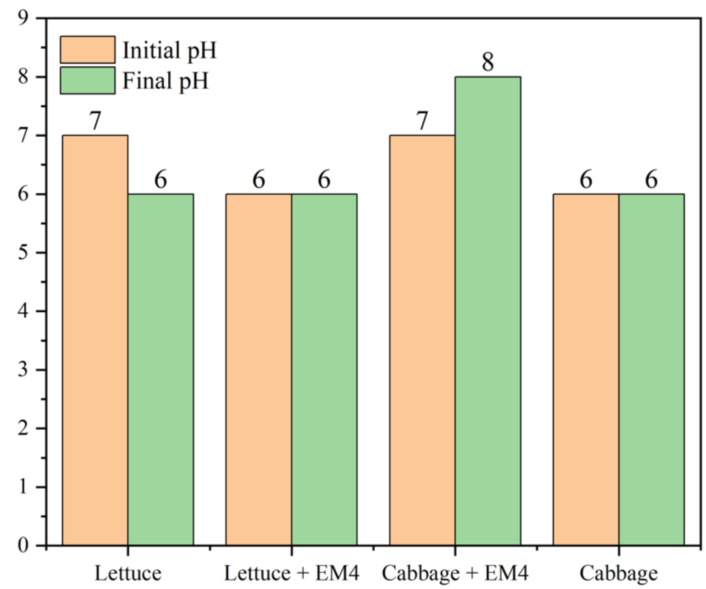

Figure 2. Initial and final pH values in lettuce and cabbage reactors with and without EM4 after eight days of fermentation.

3.4. Limitations and critical considerations

The experimental system used small household scale reactors plastic bottles short retention time and simple balloon collectors. These constraints limit the accuracy of gas measurements and restrict interpretation of absolute methane yield. The lack of temperature control and mixing introduced variability in microbial response. Gas volumes were not corrected for temperature or pressure nor analyzed for methane concentration therefore values represent uncorrected total biogas rather than methane yield.

EM4 composition is proprietary and may contain organisms that influence pH through metabolic products unrelated to methanogenic pathways. The eight day retention time was insufficient for complete anaerobic degradation of complex vegetable materials particularly cabbage which has higher lignocellulosic content.

Future studies should incorporate replicated reactors controlled mesophilic temperatures extended retention times and direct gas composition analysis. Adjustment of C to N ratio and co digestion with manure or other substrates would enhance process stability and methane output.

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Results demonstrate that lettuce and cabbage wastes can generate measurable biogas even under simple uncontrolled batch conditions. Suitability of lettuce as a more rapidly degradable substrate highlights its potential for small scale digestion systems used in household or community settings. Application of EM4 can contribute to pH stability which is important for maintaining methanogenic activity in longer digestion cycles.

Improved performance can be achieved through controlled temperature, adequate buffering, and co digestion with substrates that balance nutrient composition. These findings contribute preliminary evidence that vegetable wastes offer a viable feedstock for decentralized biogas generation when supported with appropriate inoculum and operational management.

4. CONCLUSION

This study demonstrates that lettuce produced higher short term gas volumes than cabbage and that EM4 inoculum contributed to more stable pH conditions during early fermentation. The short retention time resulted in gas formation dominated by hydrolysis and acidogenesis rather than mature methanogenesis. pH trajectories indicate that EM4 moderated acidification although values remained below the optimal range required for sustained methane formation. These outcomes show that vegetable waste can support biogas formation at small scale yet highlight the need for controlled temperature longer retention times and balanced inoculum–substrate ratios to achieve stable methanogenic activity. The findings provide an initial indication of how substrate type and EM4 inoculum influence early stage digestion and can guide future efforts to improve biogas yield from market vegetable residues.

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