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Use of biological methods and addition of organic substances in anaerobic fermentation of biomass to increase methane production and reduce carbon dioxide level

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ABSTRACT

This research work was done to ascertain the percentage composition of methane produced and the level of carbon dioxide when some organic substances were added to the fermentation tank during biogas production. The following: bovine blood, magnesium sulphate, charcoal water, and water of pH 8 were used as additives. After 21 days of batch culture fermentation, the individual gases were analyzed using gas chromatography. For gas from slurry amended with water of pH 8, the total gas concentration was found to be 18.38. In this gas concentration, methane, which is the primary gas sought in biogas production, had a concentration of 10.15. This was followed by carbon dioxide, which had a concentration of 2.39, corresponding to a percentage composition of 13.01%. For the slurry with $MgSO_4$, it was found that the total gas concentration in the feedstock amended with magnesium sulphate was 23.19. Carbon dioxide, on the other hand, had a concentration of 3.06 and a percentage composition of 13.20%. The analysis of the gas produced from the feedstock treated with charcoal water revealed a total gas concentration of 220.42. In this gas concentration, methane, the primary gas sought after in biogas production, had a concentration of 171.76. This concentration corresponds to a percentage composition of 77.92%. Carbon dioxide and carbon monoxide exhibited the lowest concentrations and percentage compositions. The four gases analyzed showed that the levels of methane and carbon dioxide have a significant effect on their levels.

KEY WORDS: *Biodigester*; *Modeling*; *Isolation*; *Fermentation*; *Slurry*

1. Introduction

Biogas is a familiar concept that has become an attractive project for individuals, countries, and organizations eager to invest in it. Due to issues in the energy sector and a global shortage, there is a significant demand for alternatives to the fossil fuels currently in use (Fagerstrom *et al*., 2018). Biogas is a renewable and eco-friendly energy source that can substitute for wood and other fuels in various applications. It helps lower the rising costs of oil products and decreases the need to cut down trees for energy.

Bioaugmentation involves the addition of specialized microbial cultures, which are usually cultivated separately under controlled conditions,

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to carry out a specific remediation task in a particular environment, whether *in situ* or within a bioreactor (Alvarez and Illman, 2006). This method has been used in agriculture since the 1800s, such as the incorporation of nitrogen-fixing *Rhizobium* species to legume roots (Gentry *et al*., 2004) and is now being increasingly utilized to enhance the biodegradation of persistent organic pollutants in groundwater and soils. Two different bioaugmentation approaches have been developed. One approach involves injecting microorganisms that possess the desired catabolic capabilities to either complement or replace the existing population of native microorganisms (Osuji *et al*., 2022). Biostimulation, which involves the addition of nutrients and other stimulatory substrates or electron acceptors as needed, is typically used alongside bioaugmentation. This combination enhances the survival of the added cells and optimizes their metabolic capabilities, ultimately leading to more effective long-term biodegradation.

The bio-stimulation process involves adding extra nutrients to a polluted system in order to enhance the growth of indigenous microorganisms (Saini *et al*., 2015). As a result, nutrient supplementation for the degradation of hydrocarbons has typically concentrated on the addition of nitrogen and phosphorus, whether in organic or inorganic forms (Osuji *et al*., 2024).

2. Materials and Methods

2.1 Sample collection

Fresh pig and poultry manure (feedstock) was collected from Onyewuchi Ejiaku Farms, located in Ubah within the Mbaoma autonomous community of the Owerri North Local Government Area in Imo State. This was accomplished using a clean, washed 20-liter paint bucket and a trowel. The feedstock was additionally crushed by hand to decrease the particle size. In addition to using hands to crush the block-form materials, we also removed non-biodegradable items such as nylon and stones. This is a method of physical pretreatment. The batch-culture anaerobic fermentation method was employed (Osuji *et al*., 2024).

The feedstocks were collected using a clean trowel and transparent sterile nylon bag. The trowel was use used to collect the fresh cow dung from the site at Obinze cattle market and carefully put into the transparent bag. The trowel was later washed and rinsed with clean water and used the second time for compost soil (Babatope *et al*., 2012, Raid Mammod *et al*., 2020).

2.2 Proximate compositional analysis of the feedstock

The proximate and physicochemical properties of the feed sample were characterized (**Table 1**) by measuring the pH (using Sensolyt-SE electrode WTW, Germany) and other physical parameters such as ash, moisture, weight lost, calcium, potassium, phosphorus, potassium, magnesium, sulphur, Biochemical oxygen demand (BOD) and Chemical oxygen demand (COD) (Babatope *et al*., 2012).

2.3 Anaerobic fermentation in a locally fabricated digester

The batch culture anaerobic fermentation was done in locally (customed) fabricated digester as shown in the **Fig. 1**. A 20-litre container was used to fabricate the digester. Hose was also used to connect the container with the vehicle tube which serves as gas collector. A T-valve was attached which serve to regulate the inflow of gas.

Fig. 1: Anaerobic digestion set-up outside the lab

Table 1: Proximate compositional analysis of the feed stock

Parameters	Pig dungs	Poultry
Ash (%)	12.17	14.27
Moisture (%)	5.71	2.71
Weight (g)	34.85	42.85
% Weight Lost	12.60	96.10
BOD (mg/L)	6.00	6.00
COD (mg/L)	3.20	3.20
Calcium (%)	2.20	2.20
Phosphorous (%)	1.60	1.60
Potassium (%)	0.90	0.90
Magnesium (%)	0.80	0.80
Sulphur (%)	0.30	0.30

2.4 Preparation of slurry from the feedstock for anaerobic fermentation

This was accomplished by measuring 2000 grams of pig manure and 500 grams of poultry manure in a ratio of 4:1, using a digital weighing scale and an empty 4-liter plastic custard container. These were carried out in four locations based on the number of treatments to be administered. Each measurement was placed in a separate 20-liter empty paint bucket. Five liters of water were added to each of the ten buckets. A sturdy iron rod was employed to mix the pig and poultry dung with five liters of water.

2.5 Different treatments on the already prepared slurry

Of the seven treatments, the following were used to treat the slurry before feeding them into the already fabricated digester.

1. Slurry one: Bovine blood was incorporated.
2. Slurry two: A commercially extracted and dried meat extract weighing 200 grams was added.
3. Slurry three which consists of charcoal water made by mixing 500 grams of crushed charcoal in 1000 liters of water was added.
4. Slurry four: 200 grams of Zinc Nitrate were added.
5. Slurry five: No additional substances were added, serving as the control."
6. Slurry six: 200 g of magnesium sulfate per dm^3 of water was added.
7. Ten liters of water with a pH of 8 were added to the slurry seven.

2.6 Biogas analysis

Biogas analysis involves examining the composition, concentration and quality of biogas. Four gas samples were analyzed: A, B, C, and D because they gave the highest gas production out of the seven treatments.

Sample A: represented gas from feedstock with water that had a pH of 8 added to it.

Sample B: represented gas from the feedstock with added $MgSO_4$.

Sample C: represented gas produced from feedstock with added charcoal water.

Sample D: represented gas derived from feedstock with the addition of cow blood. Biogas was analyzed using a gas chromatography-flame ionization detector, as outlined by AOAC in 1995. The gas chromatograph utilized in this study was an Agilent Technologies 6890 from the USA, which was equipped with a flame ionization detector (FID) and a capillary column measuring 30 m × 0.25 mm × 0.25 µm (Elite-5). The workstation utilized for data processing was the Total Chrome Navigator, as described by Seppanen-Laakso *et al*. (2002).

3. Results and Discussion

The results of Batch A (**Table 2**) showed variation in gas production among the different substrate treatments during the 21-day fermentation period. The increase in the weight of the tubes indicated the production of biogas during anaerobic fermentation. Among the treatments, the substrate mixed with bovine blood showed the highest increase in weight, followed by $MgSO_4$ (hard water), water at pH 8, and the control. The treatments containing charcoal water, protein or meat extract, and zinc nitrate showed comparatively lower increases in weight. Based on the observed biogas production and the performance of the different treatments, four representative samples were selected for further gas composition analysis using gas chromatography. These samples were designated as Sample A (feedstock mixed with water at pH 8), Sample B (feedstock mixed with $MgSO_4$), Sample C (feedstock mixed with charcoal water), and Sample D (feedstock mixed with bovine blood).

In the gas derived from feedstock mixed with water at a pH of 8, the total gas concentration was found to be 18.38. In this gas concentration, methane, which is the primary gas sought in biogas production, had a concentration of 10.15. This concentration corresponds to a percentage composition of 55.23%. This indicated that methane was the predominant gas component of the biogas in this sample. This was followed by carbon dioxide, which had a concentration of 2.39, corresponding to a percentage composition of 13.01%.

This duration lasted for 21 days. By the end of the 21 days, the digester containing bovine blood produced the highest amount of gas. This was followed by a mixture containing magnesium sulphate, charcoal water, and water at a pH of 8. The digester containing zinc nitrate produced the least amount of gas.

3.1 Biogas analysis using gas chromatography

The analysis was done using gas chromatography (GC). The tables of the results were represented above in the tables.

Gas from the feedstock amended with water of pH 8

The total gas concentration in the feedstock, which was adjusted with water at a pH of 8 (**Table 3** and **Fig. 2**), was found to be 18.40. In this gas concentration, methane, which is the primary gas

Table 2: Batch A (Weight (g) of tubes and gas in 21 days)

Day	Substrate mixed with bovine blood (g)	Substrate mixed with protein or meat extract (g)	Substrate mixed with solution of charcoal water (g)	Substrate mixed with zinc nitrate (g)	Substrate mixed with water only (control) (g)	Substrate mixed with $MgSO_4$ (hard water) (g)	Substrate mixed with water of pH 8. (g)
0	670.00	420.00	420.00	420.00	420.00	420.00	420.00
1	690.00	423.00	427.10	421.30	424.20	444.00	428.40
2	702.30	429.20	430.30	422.10	429.00	461.10	431.50
3	710.50	431.50	433.40	424.00	432.40	470.50	436.70
4	721.70	447.00	440.10	424.50	452.10	488.00	445.10
5	750.40	450.80	449.10	424.50	461.50	490.80	452.80
6	757.80	455.60	453.80	424.60	472.00	497.20	459.40
7	770.60	470.40	460.20	424.70	481.10	510.10	465.60
8	778.60	473.50	465.30	424.50	487.90	517.00	468.50
9	785.70	478.00	466.00	424.50	489.00	520.70	494.10
10	795.20	479.90	467.10	424.50	495.00	523.40	495.00
11	880.10	480.00	468.00	424.50	496.00	540.10	498.00
12	890.00	480.00	468.40	424.50	497.10	549.00	498.70
13	892.80	481.10	468.90	424.00	498.20	550.00	499.10
14	891.90	483.00	469.00	423.50	499.00	550.40	499.30
15	899.10	484.00	470.00	423.40	500.00	556.90	500.20
16	901.40	484.00	477.20	424.40	500.00	556.90	500.20
17	902.90	483.80	478.00	425.40	500.20	557.00	500.10
18	902.00	483.80	478.20	426.40	500.20	558.10	510.00
19	902.20	483.90	478.30	427.40	510.00	559.20	520.00
20	902.20	483.90	478.30	427.40	510.00	559.20	520.00

sought in biogas, has a concentration of 10.15.

This concentration corresponds to a percentage composition of 55.22. This indicated that methane was the predominant gas component in the biogas from this sample. Next, carbon dioxide was measured at a concentration of 2.40, which corresponded to a percentage composition of 13.01.

Gas from the feedstock amended with Magnesium sulphate

It was found that the total gas concentration in the feedstock amended with magnesium sulfate (**Table 4** and **Fig. 3**) was 33.58. In this gas concentration, methane, the primary gas sought after in biogas production, had a concentration of 18.10. This concentration corresponds to a percentage composition of 77.97%. This indicated

Table 3: Sample A (Amended with water of pH 8)

Components	Concentration	% Composition
CO	0.40	2.17
CO_2	2.40	13.01
Methane	10.15	55.22
Acetic Acid	1.15	6.26
Methanol	0.18	1.01
Ethyl Acetate	1.11	6.01
SO_2	-	
Acetone	0.70	3.76
Acetonitrile	0.30	1.60
Total	18.40	

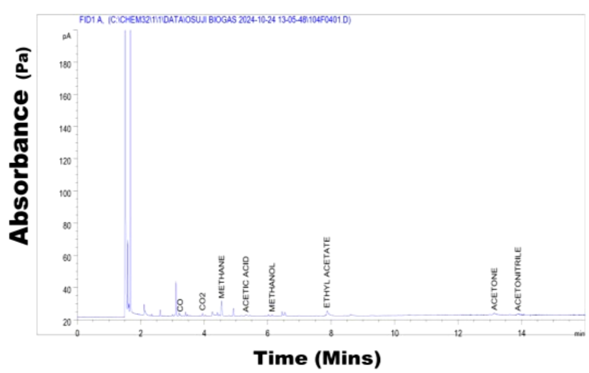

Fig. 2: Showing results of gas analysis from digester (Amended with water of pH 8)

that methane was the predominant gas component in the biogas from this sample. This was followed by methanol, which had a concentration of 9.90, corresponding to a percentage composition of 42.66%. Carbon dioxide, on the other hand, had a concentration of 3.10 and a percentage composition of 13.20%.

Table 4: Sample B (Amended with $MgSO_4$)

Components	Concentration	% Composition
CO	0.30	1.30
CO_2	3.10	13.20
Methane	18.10	77.97
Acetic Acid	0.51	2.17
Methanol	9.90	42.66
Ethyl Acetate	0.09	0.38
SO_2	0.42	1.82
Acetone	0.74	3.18
Acetonitrile	0.42	1.80
Total	33.58	

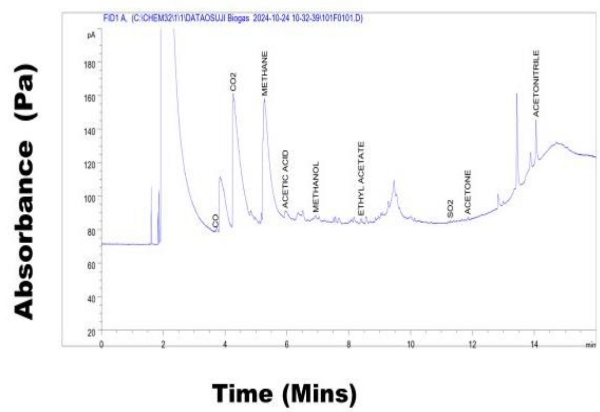

Fig. 3: Showing results of gas analysis from digester (Amended with $MgSO_4$)

Gas from the feedstock amended with charcoal water

The analysis of the gas produced from the feedstock treated with charcoal water (**Table 5**, **Fig. 4**) revealed a total gas concentration of 220.42. In this gas concentration, methane, the primary gas sought after in biogas production, had a concentration of 171.76. This concentration corresponds to a percentage composition of 77.93%. This indicates that methane was the predominant gas component in the biogas sample. This was followed by ethyl acetate, which had a concentration of 17.06 and corresponded to a percentage composition of 7.74%. Carbon dioxide and carbon monoxide exhibited the lowest concentrations and percentage compositions. This indicates that charcoal significantly influences the carbon dioxide content of biogas.

Table 5: Sample C (Amended with charcoal water)

Components	Concentration	% Composition
CO	0.29	0.13
CO_2	0.99	0.45
Methane	171.76	77.93
Acetic Acid	5.79	2.63
Methanol	10.07	4.57
Ethyl Acetate	17.06	7.74
SO_2	2.73	1.24
Acetone	5.90	2.68
Acetonitrile	6.10	2.77
Total	220.42	

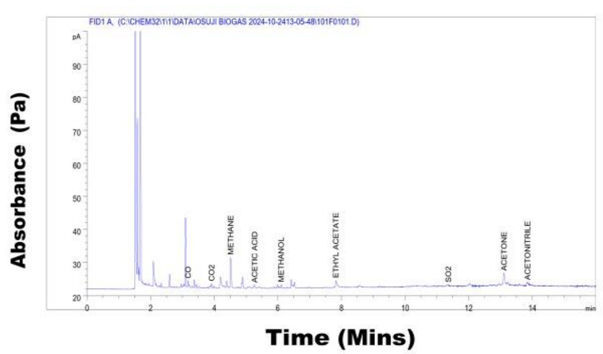

Fig. 4: Showing results of gas analysis from digester (Amended with charcoal water)

Gas from the feedstock amended with bovine blood

The gas derived from the feedstock enriched with bovine blood (**Table 6**, **Fig. 5**) was found to have a total concentration of 86.08. In this gas concentration, methane - the primary gas sought after in biogas - had a concentration of 75.13. This concentration corresponds to a percentage composition of 87.28%. This indicated that methane was the predominant gas component in the biogas from this sample. This was followed by carbon dioxide, which had a concentration of 5.09, corresponding to a percentage composition of 5.92%.

Table 6: Sample D (Amended with bovine blood)

Components	Concentration	% Composition
CO	0.80	0.93
CO_2	5.09	5.92
Methane	75.13	87.28
Acetic Acid	1.97	2.29
Methanol	2.70	3.14
Ethyl Acetate	0.20	0.24
SO_2	0.72	0.84
Acetone	0.11	0.12
Acetonitrile	-	
Total	86.08	

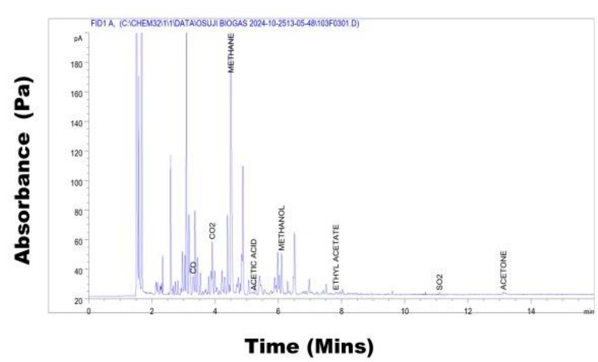

Fig. 5: Showing results of gas analysis from digester (Amended with bovine blood)

4. Conclusion

The results concerning the impact of different treatments on anaerobic fermentation are presented as follows. The digester containing sodium carbonate functions as an alkaline medium. It is very effective for the chemical pretreatment of the feedstock and can effectively break down the lignin present in lignocellulosic materials.

In this study, we investigated the effects of charcoal water, also known as biochar water, within the digester. This type of water can positively influence biogas production in an anaerobic digestion system. It can improve the digestion of substrates by adsorbing impurities while preserving nutrients and microorganisms, thereby improving substrate digestion and increasing biogas production. The effect of charcoal water on the anaerobic digestion of the feedstock was clearly reflected in the gas analysis. The gas sample from the digester treated with charcoal showed the lowest percentage of carbon dioxide.

For the treatment involving magnesium sulphate, hard water contains a high concentration of minerals, especially elevated levels of calcium and magnesium ions. These ions can influence the growth and metabolism of microorganisms. However, if their levels are not properly measured, they can lead to negative consequences. Hard water can help stabilize pH levels during fermentation, creating a more consistent environment for microbial activity. It can also support the growth of microbes, as it has been demonstrated that hard water can promote the growth of a wide variety of bacteria. Its application in biogas production may stimulate significant bacterial growth through the process of biostimulation. Hard water is characterized by high concentrations of minerals, mainly calcium and magnesium. These minerals occur naturally in water sources and can vary depending on geographical location. Furthermore, the minerals found in hard water can encourage bacterial growth and reduce the effectiveness of sanitizing agents.

In the digester with an alkaline pH of 8, the activity of methanogens is significantly affected by the pH level. As a result, methanogenic activity may decrease if the pH in the digester moves away from its ideal range. The pH level of an anaerobic digester is important for assessing the effectiveness and stability of the system in producing methane. Therefore, adjusting the pH to an appropriate level can enhance gas production. This may explain why the digester containing water at a pH of 8 generated a satisfactory amount of gas.

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