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Research Article

Biogas Production from Bioaugmented and Biostimulated Animal Dungs

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Abstract

This research is aimed at biogas production from bioaugmented and biostimulated animal dungs using custom-built bioreactor of 20 liter capacity in the anaerobic digestion process. Piggery and poultry dung were used as feedstock while sodium carbonate solution, *Shigella flexneri, Bacillus paramycoides*, bovine blood, charcoal water, magnesium sulphate solution, zinc nitrate solution, protein extract, pH 8 solution and natural water which serve as control were used as treatments. After 21 days of batch anaerobic digestion, bioreactors with $NaCO_3$, *Shigella sp, Bacillus sp*, bovine blood, protein extract, charcoal water, zinc nitrate, natural water, $MgSO_4$ and pH of 8 gave gas production of 80.6g, 95.5g, 100.3g, 232.2g, 63.9g, 58.3g, 7.4g, 90.0g, 139.2g and 100.0g respectively. Bioreactors with bovine blood and magnesium sulphate gave the highest gas production because the blood was nutrient-rich and the magnesium sulphate which turned the water hard encouraged wide range of bacterial growth and sustenance of pH of the reaction environment. Zinc nitrate reacted with water in the slurry to form nitric acid which turned the internal reactor environment to be acidic which doesn't favour methanogens. Also, the gas from the feedstock amended with Magnesium sulphate, bovine blood and charcoal water showed a significant increase in methane production. The gas from the feedstock amended with charcoal gave the lowest percentage composition of carbon dioxide, which showed that the charcoal was responsible for adsorption of the carbon dioxide. This research work recommends that measured amount of bovine blood should be used to supply nutrient to the indigenous bacteria. Calculated amount of Magnesium sulphate should be used to buffer the pH. Finally, charcoal water should be used in slurry preparation to remove the ammonia and carbon dioxide by adsorption as gas production commences.

Keywords: *Biodigester; Modelling; Isolation; Fermentation; Slurry*

Introduction

Biogas is a renewable and an environmental friendly form of energy which can be used to replace other forms of energy resource. The applications and use of biogas can bring down the rising costs of petroleum products and falling of trees for energy production. Urbanisation has led to rapid production of "wastes" leading to poor management practices in developing nations [1]. Since human must generate waste always and the same human is not even able to manage it well, this biogas generation has become a way of waste management. Biogas is a household name that is at the lips of everyone now and has become a profit-making venture many individuals, and countries would

want to invest into. Due to serious problems in energy sector of many countries of the world; it has become important that a suitable a replacement of the already used hydrocarbon-generated energy The high standards of living has increased the release of pollutants and greenhouse gases (GHGs) into the environment. This has led to a global crisis as the use of fossil fuel are still being consumed at a high rate [2]. Even though biogas production is eco-friendly, it has negative implications following it. These implications can come when the processes that will bring about its production are not followed. Also, because the gas is generated from household waste, and other biodegradable there are bounds to be problems in its managements. If the wastes are not properly handled, it will become a threat to the environment.

Materials and Methods

Materials/equipments used

The following laboratory materials and equipment were used for the isolation of cellulose-producing bacteria: Conical flask, test tubes, petri dish, Bunsen burner, wire loop, anaerobic jar, gas pack, pipette, capped test tube, test tube rack, bijou bottles, electronic weighing balance.

Reagents used

Similarly, laboratory reagent used for the experiment includes the following: $NaNO_3$, $MgSO_4.7H_2O$, NaCl, $Na_2HPO_4.2H_2O$, $CaCl_2.6H_2O$, Agar, CMC (carboxyl methyl cellulase) Agar, Nutrient agar and distilled water, Nutrient broth.

Sample collection

Fresh piggery and poultry dungs which serve as feed stock were collected from a privately owned farm at Ubah in Mbaoma autonomous community in Owerri North Local Government Area of Imo State. Batch culture anaerobic fermentation method was used. Also, fresh cow dungs and compost soil were collected for isolation of cellulose degrading bacteria which will be used to bioaugment the indigenous bacteria in the feed sample [3,4].

Preparation of CMC media for isolation of cellulase-producing bacteria

The CMC agar medium components as shown in appendix (Table) were dissolved in 1litre of distilled water contained in 2 litre flask. The medium was sterilized by autoclaving at 121°C at 15psi for 15 minutes. The medium was then used to isolate cellulase producing bacteria [4].

Sample processing, serial dilution and isolation and screening of cellulase-producing bacteria

The cow dung and compost soil were processed by dissolving 1g of each in 9 ml of sterile physiological saline solution contained in 15 ml beaker. They were thoroughly stirred for 2 minutes to detach the organisms from the particles. The mixture was allowed to stay for 20 minutes to allow for proper sedimentation. The supernatant represents the entire bacteria community. Ten-fold serial dilution was done. A 0.1 ml of 10^{-2}, and 10^{-4} were aseptically inoculated onto the CMC agar medium. They were labelled appropriately and incubated for 5 - 7 days.

After incubation period, pure culture of the isolates were obtained by streaking method. The pure culture isolate plates were stained with 1% Congo red solution at room temperature for 15 minutes and de-stained for 20 minutes using 1M NaCl. Cellulose degrading bacterial isolates were selected by the formation of clear zones around colonies through the Congo red overlay method [4]. The colony with the highest zone clearing for plates in incubator and anaerobic jar were selected. The most cellulolytic bacterial colonies were sub-cultured and purified on Lysogenic medium. The bacterial strains were stored on CMC supplemented agar slant at 4°C. The two organisms isolated were identified as *Shigella flexneri* and *Bacillus paramycoides* using molecular identification method.

Batch culture anaerobic digestion

Total of 10-20 liter containers were used to fabricate digester. The method for anaerobic fermentation is called batch culture because, after feeding/pouring the feedstock into the digesters, there was no provision for further opening and adding more substrate till the end of reaction (retention time). It is also called anaerobic because after closing the digester, no provision for oxygen to enter.

Bioaugmented and biostimulated animal dungs during anaerobic digestion

Out of the ten (10) buckets used for slurry preparations, the following were used to treat the slurry before feeding them into the already fabricated digester:

1. Digester (1): Raw sodium carbonate (200g) was added
2. Digester (2): *Shigella flexneri* was added after inoculum development to break the lignin network to allow the indigenous methanogen to ferment.
3. Digester (3): *Bacillus paramycoides* was added after inoculum development to break the lignin network to allow the indigenous methanogen to ferment.
4. Digester (4): Bovine blood was added.
5. Digester (5): Protein/Meat extract was added (200g) (a commercially extracted and dried meat extract was used).
6. Digester (6): Charcoal water (500g of crushed charcoal in 1000 dm^3 of water) was added.
7. Digester (7): Zinc Nitrate (200g) was added.
8. Digester (8): Nothing was added (as control).
9. Digester (9): Magnesium sulphate added (200 g/dm^3 of water) was added.
10. Digester (10): water of pH 8 was added.

Figure 1: *Anaerobic digestion of bioaugmented and biostimulated animal dungs.*

Results

After 21 days of anaerobic digestion, the following results were obtained.

Day	Substrate mixed with raw Na_2CO_3 (g)	Substrate mixed with *shigella flexneri* (g)	Substrate mixed with *bacillus paramycoides* (g)	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)	Cum gas production (g)
0	0	0	0	0	0	0	0	0	0	0	0
1	6.1	10	4.2	20	3	7.1	1.3	4.2	24	8.4	88.3
2	8.1	14.2	8.1	32.3	9.2	10.3	2.1	9	41.1	11.5	145.9
3	12.5	18.2	19.2	40.5	11.5	13.4	4	12.4	50.5	16.7	198.9
4	40.3	27.5	28.1	51.7	27	20.1	4.5	32.1	68	25.1	324.4
5	60.5	35.7	30.1	80.4	30.8	29.1	4.5	41.5	70.8	32.8	416.2
6	66.9	58.3	35.5	87.8	35.6	33.8	4.6	52	77.2	39.4	491.1
7	73.6	60.5	41.7	100.6	50.4	40.2	4.7	61.1	90.1	45.6	568.5
8	74.1	67.2	56.3	108.6	53.5	45.3	4.5	67.9	97	48.5	622.9
19	75	75	79	115.7	58	46	4.5	69	100.7	74.1	697
10	78	78	90	125.2	59.9	47.1	4.5	75	103.4	75	736.1
11	78.7	79.7	90.7	210.1	60	48	4.5	76	120.1	78	845.8
12	79.1	79.8	90.9	220	60	48.4	4.5	77.1	129	78.7	867.5
13	79.3	80.3	91.3	222.8	61.1	48.9	4	78.2	130	79.1	875
14	80.2	83.2	92.2	221.9	63	49	3.5	79	130.4	79.3	881.7
15	80.2	90.2	93.2	229.1	64	50	3.4	80	136.9	80.2	907.2
16	80.3	95.1	95.1	231.4	64	57.2	4.4	80	136.9	80.2	924.6
17	80.4	95.2	100.2	232.9	64.8	58	5.4	80.2	137	80.1	934.2
18	80.5	95.3	100.4	232	64.8	58.2	6.4	80.2	138.1	90	945.9
19	80.6	95.5	100.5	232.2	64.9	58.3	7.4	90	139.2	100	968.6
20	80.6	95.5	100.5	232.2	64.9	58.3	7.4	90	139.2	100	968.6
Cum. gas prod.	1295	1334.4	1347.2	3027.4	970.4	826.7	90.1	1234.9	2059.6	1222.7	

Table 1: *Gas produced after 21 days of anaerobic digestion.*

Results of the biogas analysis

The gas collectors from the digester that gave the highest gas productions were further analyzed. The analysis was done using gas chromatography (GC). The results were represented below.

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	

Table 2: *Sample A [Amended with water of pH 8].*

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	23.19	

Table 3: *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	23.19	

Table 3: *Sample B [Amended with $MgSO_4$].*

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	

Table 4: *Sample C [Amended with charcoal water].*

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	

Table 5: *Sample D [Amended with bovine blood].*

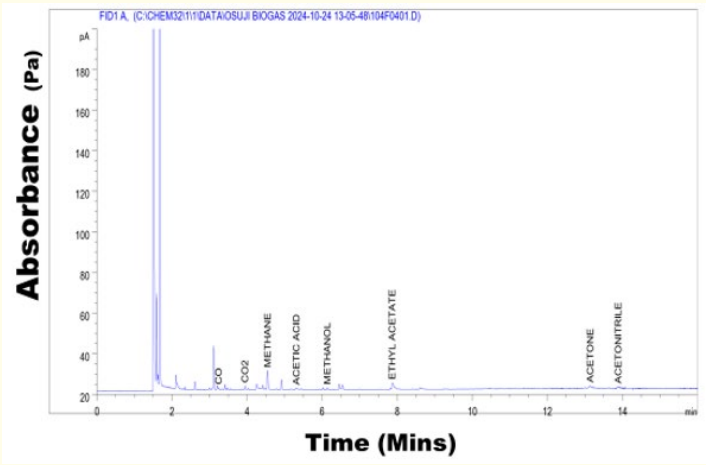


Figure 2: *Results gas analysis from digester [Amended with water of pH 8].*

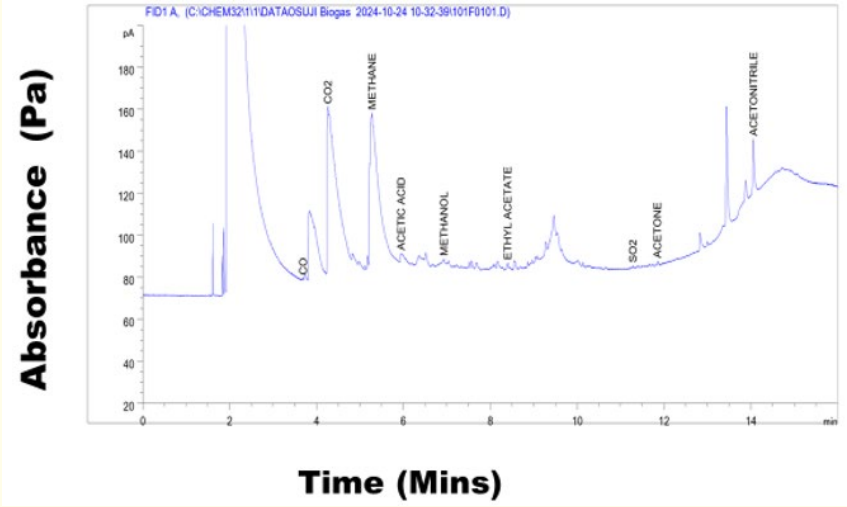


Figure 3: *Results of gas analysis from digester [Amended with $MgSO_4$].*

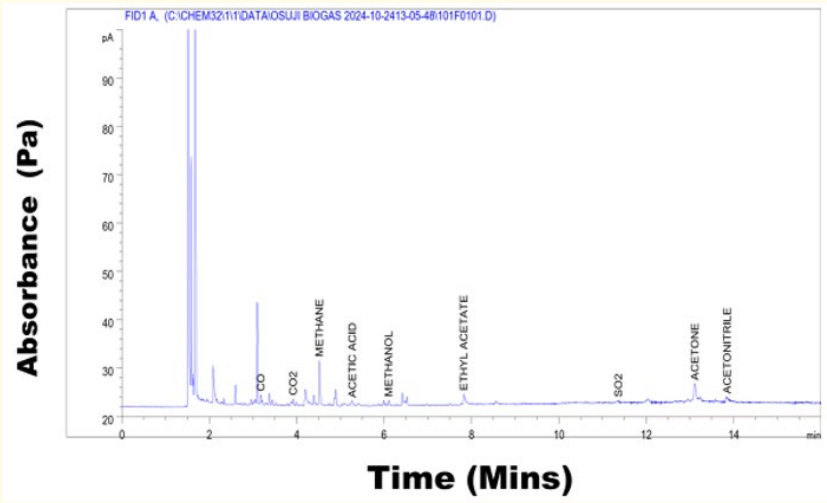


Figure 4: *Results of gas analysis from digester [Amended with charcoal water].*

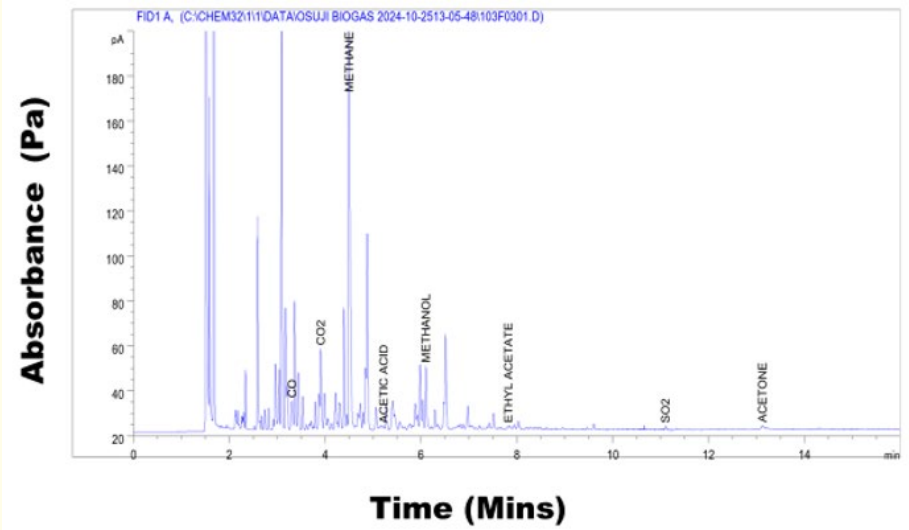


Figure 5: *Results of gas analysis from digester [Amended with bovine blood].*

Discussion and Conclusion

In feedstock amended with water of pH 8, it was discovered that the total gas concentration is 18.38. In this gas concentration, methane which was the main gas been sort for in biogas has the concentration of 10.15. This concentration is equivalent to the percentage composition of 55.24. This showed that the methane gas was the highest gas component of the biogas in this sample. This was followed by carbon dioxide with concentration of 2.40 which corresponded to percentage composition of 13.01.

In the feedstock amended with magnesium sulphate, it was discovered that the total gas concentration is 23.19. In this gas concentration, methane which was the main gas been sort for in biogas has the concentration of 18.08. This concentration is equivalent to the percentage composition of 77.98. This showed that the methane gas was the highest gas component of the biogas in this sample. This was followed by methanol with concentration of 9.90 which corresponded to percentage composition of 42.66. Carbon dioxide has the concentration and percentage composition of 3.06 and 13.20 respectively.

In the feedstock amended with charcoal water, it was discovered that the total gas concentration is 220.42. In this gas concentration, methane which was the main gas been sort for in biogas has the concentration of 171.76. This concentration is equivalent to the percentage composition of 77.93. This showed that the methane gas was the highest gas component of the biogas in this sample. This was followed by ethyl acetate with concentration of 17.06 which corresponded to percentage composition of 7.74. Carbon dioxide and carbon monoxide had the least concentration and percentage composition. This means that the charcoal has a significant effect on the carbon dioxide content of biogas.

For the feedstock amended with *Shigella flexneri.* This isolate is not a methanogenic bacterium. It functioned very well in the acidogenesis and acetogenesis stages of fermentation. It is involved in the biological pretreatment method of the feedstock. For the feedstock amended with *Bacillus paramycoides,* it played the same role *Shigella flexneri* played. It functioned as biological pretreatment bacterium and bioaugmentation. The advantages of biological pretreatment include low energy requirements and mild environmental conditions.

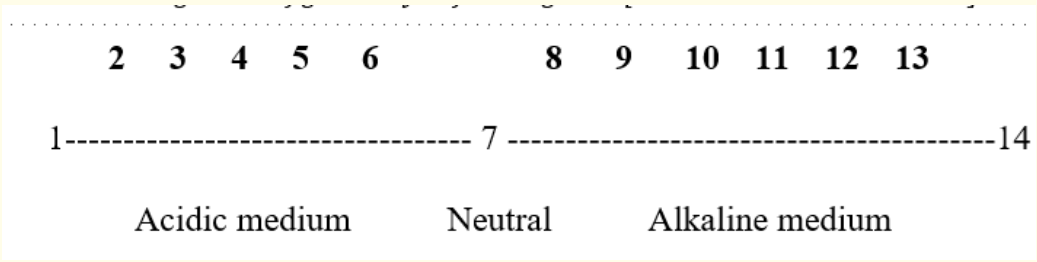
As explained above, ammonia is toxic to the methanogens. Addition of nitrate compound led to the formation of ammonium. When zinc nitrate reacted with water, a strong acid was formed. In this reaction, zinc nitrate dissociates in water to form zinc hydroxide and nitric acid. The hydroxide is not soluble in water, it will precipitate out of the solution. Nitric acid formed will turn the slurry to be acidic. This acidic solution will affect the entire reaction. Also, the formation of ammonium is toxic to the methanogens.

$Zn(NO_3)_2 + 2H_2O$ ---------- $Zn(OH)_2 + 2HNO_3$

$NO_3 + 3H_2O$ ---------- $NH_4(OH)_2 + 2O_2$

For the feedstock amended with pH 8. The pH is the degree of measure of acidity or alkalinity of a medium. It can also be defined as the negative hydrogen ion concentration. Methanogenetic activities is strongly affected by pH. As such, methanogenic activity will decrease when pH in the digester deviates from the optimum value. The pH value of anaerobic digester determines the performance and stability of the system to produce methane. Therefore, adjusting the pH towards right will enhance gas production. That was why the digester with water of pH 8 gas reasonable gas production.

For the feedstock amended with sodium carbonate, sodium carbonate is an alkali. It works very well in chemical pretreatment of the feedstock. It functions well in the breaking of the lignin of the lignocellulose.



Figure

Recommendation

From the above detailed discussions and conclusions, this study is recommending as follows:

- That the volume of blood should not be more than the water used in the slurry formation as this could affect proper mixing.
- That biochar content of fermentation of the lignocellulose should be increased using charcoal water in slurry preparation to ensure ammonia reduction and carbon dioxide by adsorption.
- That measured amount of bovine blood, Magnesium Sulphate and Charcoal can be used as additive in biogas production.
- That metallic nitrate compound should not be used in biogas production. Also, nitrate compounds should not be added as nutrient to avoid nitric acid and ammonium formation.

Contribution to Knowledge

At the end of this research work, the following were discovered:

1. Bovine blood has proved to be a better treatment in biogas production. But it's volume should be commensurate with the amount of feed stock to avoid uncontrollable gas production that can bust the gas collector.
2. Hard water also proved to be the best liquid for slurry preparation. This can be achieved by using magnesium sulphate which gives permanent hardness instead of looking for natural occurring hard water.
3. Also, use of charcoal water in slurry preparation will reduce the amount of carbon dioxide and ammonia by adsorption which will in turn increase the amount of methane (biogas). This was shown in gas analysis.
4. In the case of biostimulation of indigenous bacteria, any compound of metallic nitrate should not be added as the nitrate will react with water to form Nitric acid and ammonium hydroxide which will slow down methanogenesis.

Conflict of Interest

In this research work, there is no conflict of interest.

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