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EFFECT OF BACTERIAL ISOLATE AND NUTRIENT ADDITION IN BIOGAS PRODUCTION FROM CO-DIGESTION OF MIXTURE OF ANIMAL MANURE

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Abstract

This research was carried out to ascertain the effect of bioaugmentation and the addition of nutrients during anaerobic digestion leading to biogas production. The feedstock used is a mixture of pig and poultry dung at the ratio of 4:1. *Shigella flexineri and Bacillus paramycoides* were isolated and used alongside bovine blood and meat extract. After 21 days of anaerobic digestion, the digester with S.flexineri produced gas of 10g and 95.5g for days 2 and 21 respectively. B. paramcoides gave 4.2g and 100.5g in the same manner. For the digester with bovine blood, it gave 20g and 232.2g. While the one with meat extract produced 3g and 63.9g for day 2 and day 21 respectively. From the results and findings, the following recommendations were made. The use of anaerobic bacteria to augment the activities of the indigenous methanogens will increase biogas production. The use of bovine (cow) blood or other source of nutrients will enhance gas production. The addition of meat or protein extract has no significant effect on biogas production.

Keywords: co-digestion, augmentation, methanogens, biogas, anaerobic, extract

Introduction

In the past, animal manure was only useful in farming and crop production. Today, they have become more useful not in only farming but also in biogas production. They were regarded as waste in recent years. Today they are no longer waste but useful substrates in gas production. Massive development in the cities has led to high production of waste. This has caused a big problem leading to poor management practices in developing nations (Tawoma., 2015). Biogas is a household name and has become a project many individuals, nations, and organizations would want to invest in. Biogas is produced through anaerobic digestion of organic material. Anaerobic digestion (AD) is the degradation of organic materials by microorganisms in the absence of oxygen. It is a multi-step because it involves four biological processes where the organic carbon is mainly converted to carbon (iv) oxide and methane which is a hydrocarbon.

Factors Affecting Anaerobic Digester Performance

- Effect of nutrients on bacteria
- Effect of inoculation on ad process parameters
- Effect of pH
- Effect of temperature
- Mixing or agitation
- Effect of organic loading rates
- Effect of hydraulic retention time
- Effect of chemical and physical pre-treatment

Materials and Methods

Samples and material used

Pig and poultry dung was collected from Onyewuchi Ejiaku Farms at Ubah in the Mbaoma autonomous community in Owerri North Local Government Area of Imo State. Batch culture anaerobic fermentation method was used. Also, cow dung and compost soil were collected for the isolation of cellulose-degrading bacteria which will be used to bioaugument the Indigenous bacteria in the feed sample.

Laboratory Materials/ Equipment Used

The following laboratory materials and equipment were used for the isolation of cellulase-producing bacteria:

i. Conical flask
ii. Test tubes
iii. Petri dish
iv. Bunsen burner
v. Wire loop
vi. Anaerobic jar
vii. Gas pack
viii. Pipette
ix. Capped test tube
x. Test tube rack
xi. Bijou bottles
xii. Electronic weighing balance

Laboratory Reagents Used

Similarly, the laboratory reagent used for the experiment includes the following:

i. $NaNO_3$
ii. $MgSO_4.7H_2O$
iii. NaCl
iv. $Na_2HPO_4.2H_2O$

v. $CaCl_2.6H_2O$

vi. Agar

vii. CMC (Carboxyl Methyl Cellulase) Agar

viii. Nutrient agar and distilled water

ix. Nutrient broth

Sample collection

The piggery and poultry samples were collected using 10 empty paint buckets of 20-litre capacity. The samples (cow dung and compost soil) for the isolation of cellulase-producing bacteria were collected using a clean sterile nylon bag.

Fig 1.0: Sample of cow dung and compost soil for isolation of cellulase-producing bacteria

Preparation of Media for Isolation of the cellulase producing Bacteria

According to Vimal et al., 2023, formulation of the media for isolation of cellulose-degrading bacteria was done as follows:

i. 2.5g $NaNO_3$

ii. 0.5g $MgSO_4.7H_2O$

iii. 0.23g NaCl

iv. 0.5g $Na_2HPO_4.2H_2O$

v. 0.5g $CaCl_2.6H_2O$

vi. 20g Agar

vii. 10g CMC (carboxyl methyl cellulase) Agar

viii. All in 1000ml of distilled water

The formulated media in 200 ml of distilled water was heated to melt. Later, 800 ml of distilled water was added to make it up to 1000 ml before autoclaving. Also, According to Dirya et al., 2020, 1g of cow dung and compost soil from the sterilized nylon bag were weighed into 9 ml of sterile distilled water. Serial dilution was done up to the seventh diluent. From the serial dilution, inoculation from 10^{-2}, $10^{-3,}$ and 10^{-4} diluents were done in quadruplicates and labeled appropriately.

Figure 2.0 Plates of the formulated media and plates after inoculation

Isolation and Screening of Cellulase-Producing Bacteria

After the incubation period, the plates were stained with 1% Congo red solution at room temperature for 15 min and de-stained for 20 min using 1M of NaCl. Cellulose-degrading bacterial isolates were selected by the formation of clear zones around colonies through the Congo red overlay method (Vimal et al., 2023). The colony with the highest zone clearing for plates in the incubator and anaerobic jar was selected. The bacterial isolates

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with a high zone of clearing which represents a high ability to produce cellulase were sub-cultured, purified on nutrient agar, and stored on a slant at 4°C.

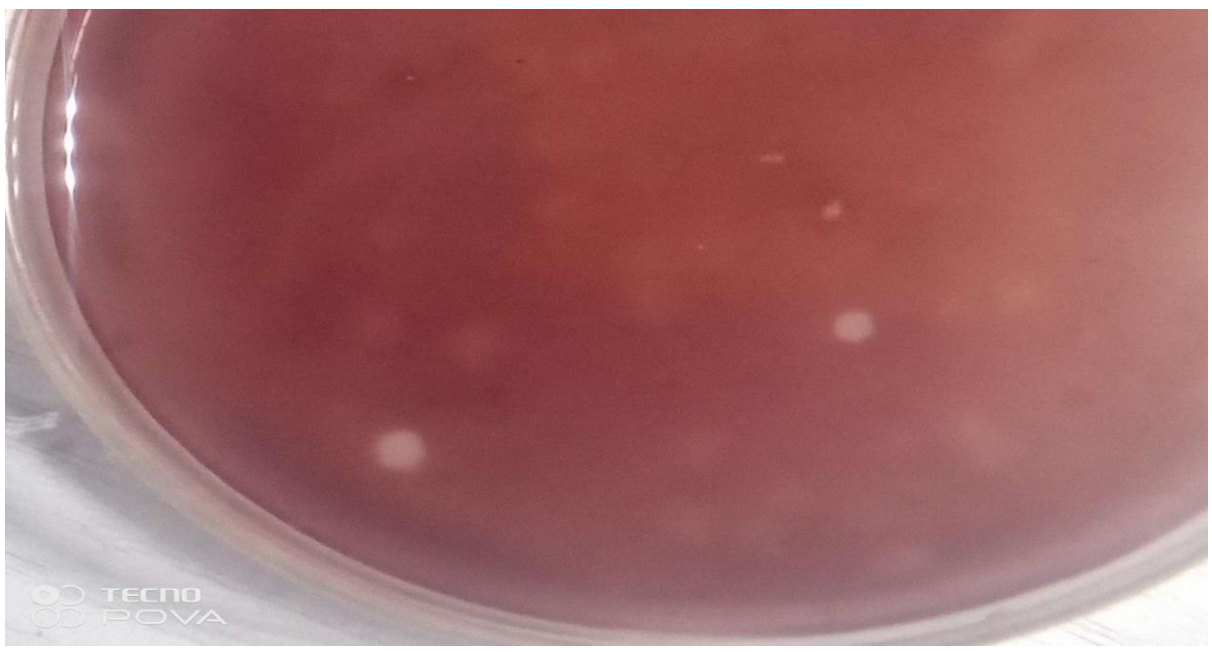

Fig 3.0: Plate showing different zones of clearing for cellulase production ability

The two organisms were labeled as follows

- Iso 1 inch (this represents the one from the incubator)
- Iso 2 Jar (this represents the one from Anaerobic jar)

MOLECULAR IDENTIFICATION

The following were done on the isolates to identify them to species level.

i. **Bacterial genomic DNA extraction**
ii. **DNA quantification**
iii. **16S rRNA Amplification**
iv. **Phylogenetic Analysis**

The two bacterial isolates were identified as (*Shigella flexineri and Bacillus paramycoides*)

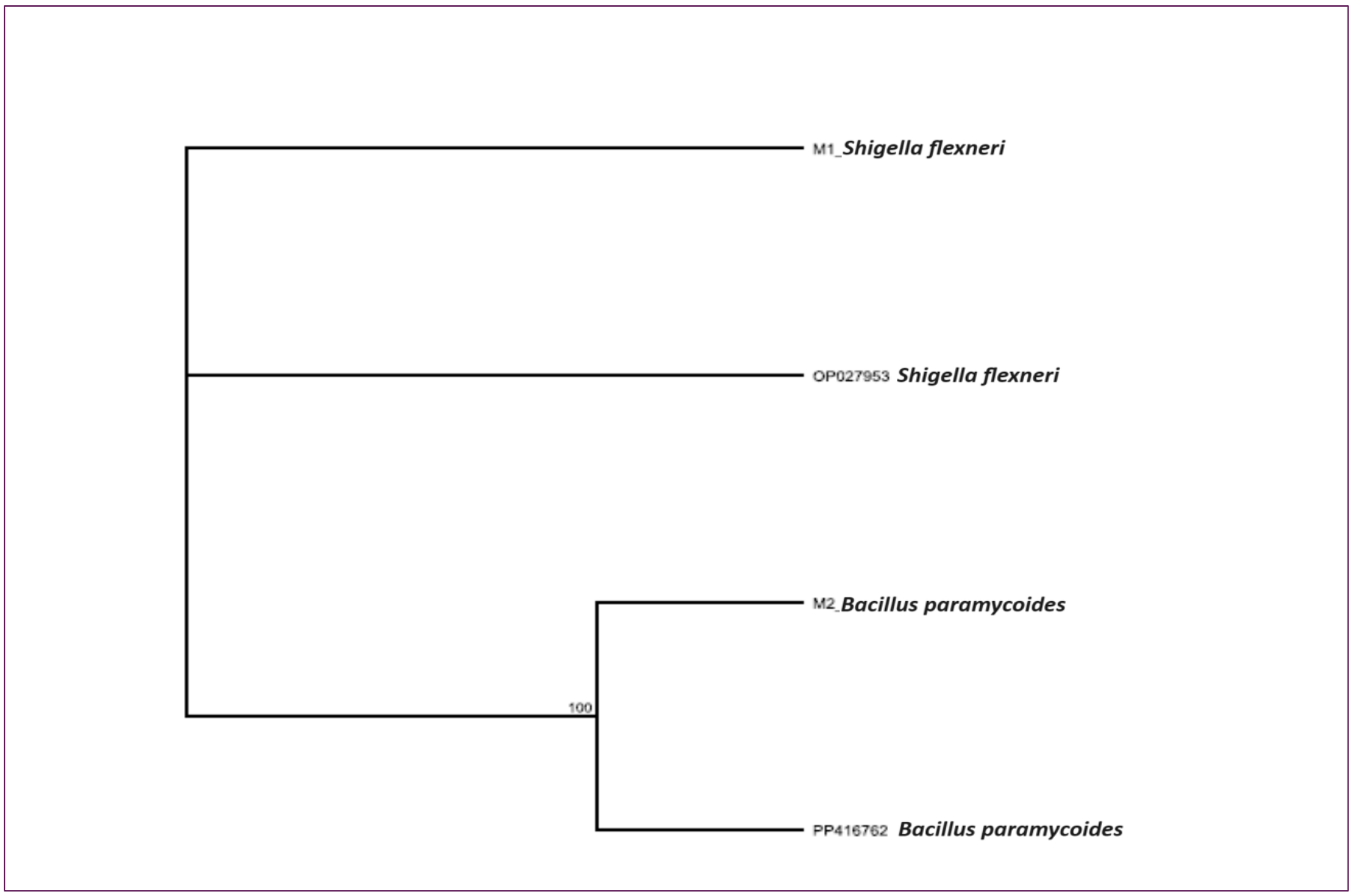

Fig 4.0: Phylogenetic tree showing the evolutionary relationship between the bacterial isolates

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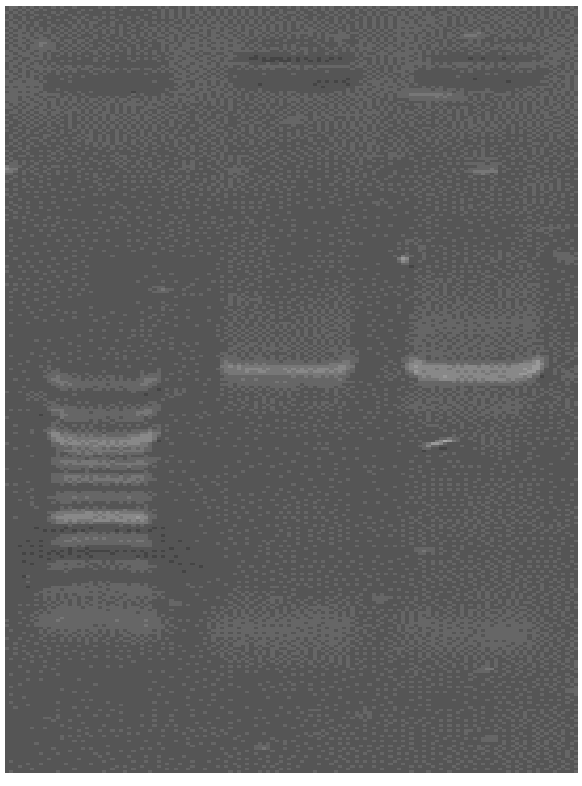

Fig 5.0: Agarose gel electrophoresis showing the amplified 16srRNA. Lanes 1-2 represent the amplified 16srRNA at 1500bp while lane L represents the 100bp DNA ladder.

Anaerobic digestion using the isolated bacteria and the addition of nutrients

The two bacterial isolates (*Shigella flexineri and Bacillus paramycoides*) were used to augment the activities of the indigenous bacteria in the substrates**.** These were done in a locally fabricated digester. The digester was allowed to stay until it stopped producing gas (21 days).

Fig 6: Ten fabricated digesters with the isolates and nutrients added.

Result

Table 1: Raw data (Mass of Tubes and gas in 21 days)

Day	Substrate mixed with shigella flexneri	Substrate mixed with bacillus paramycoides	Substrate mixed with bovine blood	Substrate mixed with protein or meat extract
0	420.00	420.00	670.00	420.00
1	430.00	424.20	690.00	423.00
2	434.20	428.10	702.30	429.20
3	438.20	439.20	710.50	431.50
4	447.50	448.10	721.70	447.00
5	455.70	450.10	750.40	450.80
6	478.30	455.50	757.80	455.60
7	480.50	461.70	770.60	470.40
8	487.20	476.30	778.60	473.50

9	495.00	499.00	785.70	478.00
10	498.00	510.00	795.20	479.90
11	499.70	510.70	880.10	480.00
12	499.80	510.90	890.00	480.00
13	500.30	511.30	892.80	481.10
14	503.20	512.20	891.90	483.00
15	510.20	513.20	899.10	484.00
16	515.10	515.10	901.40	484.00
17	515.20	520.20	902.90	483.80
18	515.30	520.40	902.00	483.80
19	515.50	520.50	902.20	483.90
20	515.50	520.50	902.20	483.90

Table 2: Calculated Mass of Gas Produced In 21 Days

This was done by subtracting the subsequent masses from the mass of the tube (day 0)

Day	Substrate mixed with shigella flexneri	Substrate mixed with bacillus paramycoides	Substrate mixed with bovine blood	Substrate mixed with protein or meat extract
0	0	0	0	0
1	10	4.2	20	3
2	14.2	8.1	32.3	9.2
3	18.2	19.2	40.5	11.5
4	27.5	28.1	51.7	27
5	35.7	30.1	80.4	30.8
6	58.3	35.5	87.8	35.6
7	60.5	41.7	100.6	50.4
8	67.2	56.3	108.6	53.5
19	75	79	115.7	58
10	78	90	125.2	59.9
11	79.7	90.7	210.1	60
12	79.8	90.9	220	60
13	80.3	91.3	222.8	61.1
14	83.2	92.2	221.9	63
15	90.2	93.2	229.1	64
16	95.1	95.1	231.4	64
17	95.2	100.2	232.9	63.8
18	95.3	100.4	232	63.8
19	95.5	100.5	232.2	63.9
20	95.5	100.5	232.2	63.9

Discussion

In Table 2 above, the digester with Shigella flexneri showed a significant increase in gas production from 10g on day 2 to 95.5g on day 21. The same was observed for the digester with *Bacillus paramycoides.* There was an increase in gas production from 4.2g on day 2 to 100.5g on day 21. This method of using exogenic bacteria to help the activities of the indigenous bacteria is called bioaugmentation. It supports the work done by Mazzurco et al 2023 and Tsapekos et al 2018. Also from Table 2, there was an enhanced increase in gas production for the digester with bovine blood. On day 2, gas production was 20g and it increased to 232.2g on day 21. For the digester with meat extract, there was not enough gas production when compared with the ones with isolates and blood.

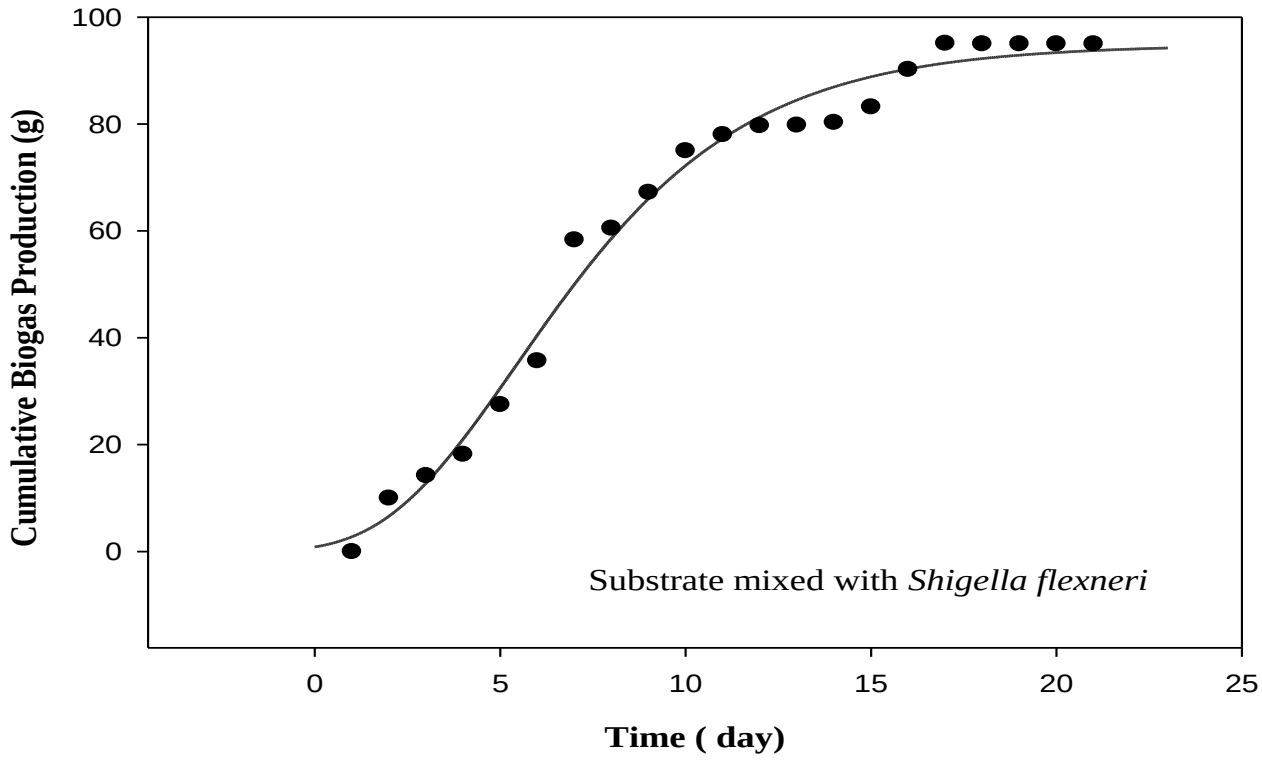

Figure 7: Graph of Substrate mixed with *Shigella flexneri*

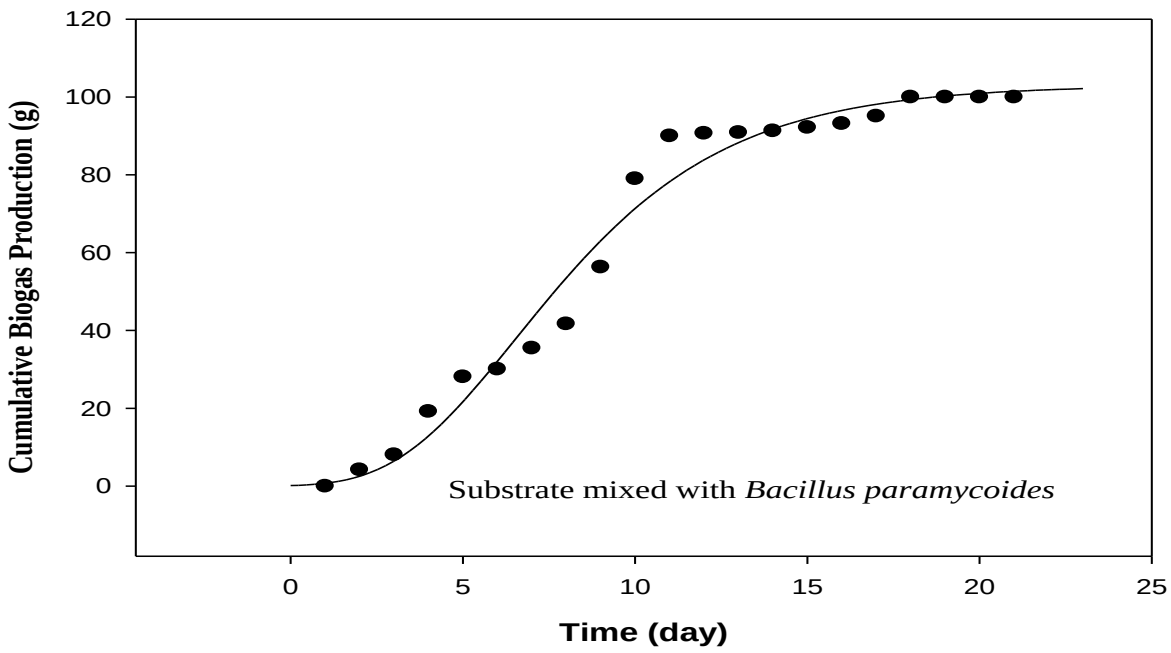

Figure 8: Graph of Substrate mixed with *Bacillus paramycoides*

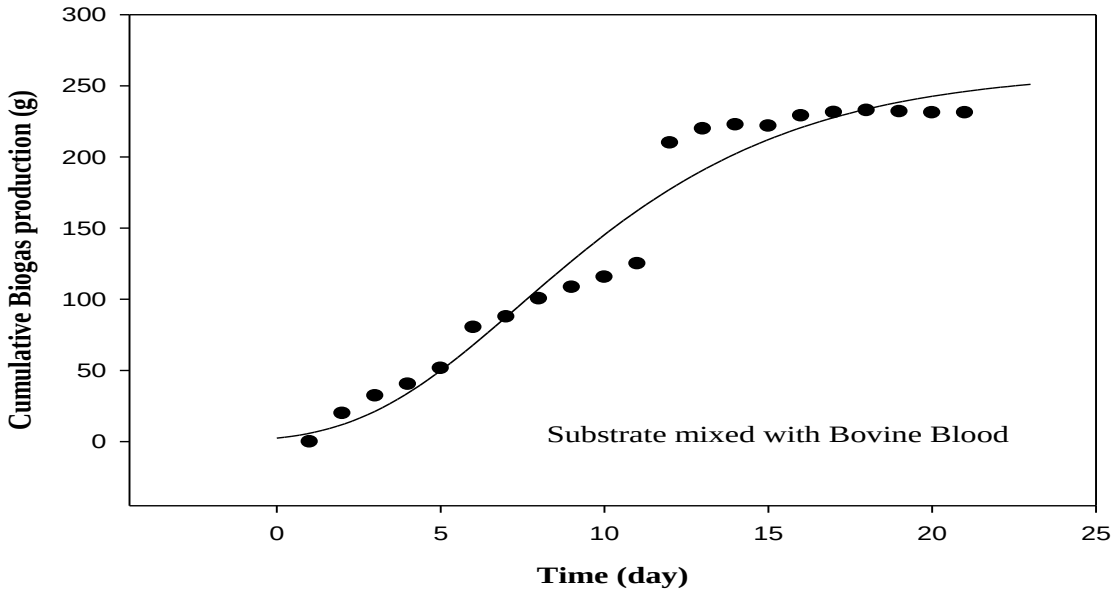

Figure 9: Graph of Substrate mixed with bovine blood

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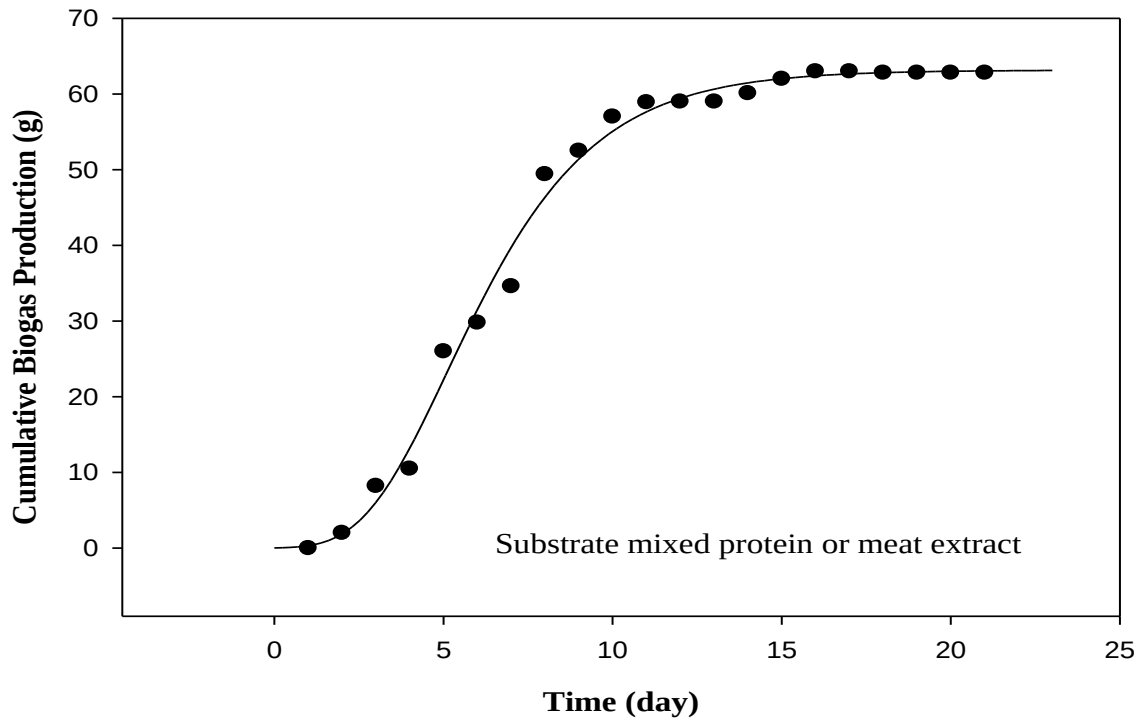

Figure 10: Graph of Substrate mixed with protein or meat extract

Conclusion

Per the work done by Aguiar et al 2010, it was reported that bioaugmentation and the addition of nutrients for microbial consumption have an enormous positive impact on biogas production**.** This work agreed with the work of other authors on the addition of nutrients into the digester.

Recommendation

From the results, analysis, and discussions, the following recommendations are made

- **i.** That the use of anaerobic bacteria to augment the activities of the indigenous methanogens will increase biogas production.
- **ii.** Used of blood or other sources of nutrients will enhance gas production.
- **iii.** The addition of meat or protein extract has no significant effect on biogas production.

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