Co-digestion of cow dung with organic kitchen waste to produce biogas using *Pseudomonas aeruginosa*

M. E. Ojewumi1, P. C. Ogele1, D.T. Oyekunle1, J.A. Omoleye1, S. O. Taiwo2, Y. D. Obafemi2

1*Chemical Engineering Department, 2Biological Sciences, Microbiology Unit, Covenant University, P.M.B 1023, Canaan Land, Sango, Ogun State, Nigeria.

* Corresponding author’s e-mail: modupe.ojewumi@covenantuniversity.edu.ng

**Abstract:** The anaerobic digestion process for biogas production was investigated on food waste (FW) and cow dung (CD) using *Pseudomonas aeruginosa* as inoculum. The food waste and cow dung were co-digested as the substrate with bacteria (*P. aeruginosa*). Five digesters were prepared to observe the maximum gas production potential, methane production rate and duration for biogas production. Food waste (FW) was co-digested with bacteria in two proportions (1:5ml, 1:10ml) and co-digested with Cow dung (CD) in two proportions (1:1, 1:0.5). The digesters were all operated in batch process under mesophilic condition (35ºC). The daily weights of the tubes were measure and some physical characteristics of the substrate were determined before and after the process. Production of gas started 3-4 days after commencement for the digesters with cow dung, 4-5days for the digesters with bacteria and 3 days with only food waste. Food waste with 1kg, cow dung produced the most biogas with a cumulative volume of 88.5g/kg. The highest concentration of biogas was found in the 1st digester consisting of 2kg CD and 2kg FW with a methane content of 52% and 48% CO2. The availability and renewable nature of food waste, ease of management of biogas produced and development of energy makes biogas a better option than the use of fossil fuel to the much-awaited solution to the energy crisis in Nigeria and developing countries.

**Keyword:** Co-digestion, *Pseudomonas aeruginosa*, inoculum, food waste, methane, cow dung

1. **Introduction**

The increase in kitchen and agricultural waste have resulted in endangering the life of plants and animals. Researchers have found out ways that this environmental pollution can be of positive relevance by recycling the waste to be re-used or reduce to ensure a cleaner and healthier environment [1].

The uncontrolled discharge of large amounts of food waste (FW) causes severe environmental pollution in many countries. Within different possible treatment routes, anaerobic digestion (AD) of FW into biogas, is a proven and effective solution for FW treatment and valorisation [2, 3]. The food waste in Nigeria has hit an estimate of $750 billion yearly. For a ton of food waste generated; 1.9 tonnes CO2 eq/t is emitted. With the increase of urban populations in Nigeria, more than 30 million tons of food wastes are produced every year [4]. The increasing amount of food waste and other materials will become a big problem to the environment if there is no reasonable solution such as the conversion into biodiesel or other useful materials[5-7]. Compared to methods like incineration and landfill, anaerobic digestion is more efficient because it can produce clean energy (biogas) and slurry that can be used as organic fertilizers. Also, anaerobic digestion has a significant benefit of reducing greenhouse gas since methane and carbon dioxide are both produced in a closed reactor,
avoiding its uncontrolled production and release to the atmosphere [8]. Co-digestion of kitchen wastes and other types of wastes, like waste water from organic municipal wastes, sewage sludge and, animal manures has been widely studied [9-14]. However, limited reports can be found on positive performance of single-stage anaerobic digestion system treating kitchen waste [15, 16]. This food waste is burned or dumped in open region may cause serious health and natural issues. Burning of food waste comprising of high moisture content results in the release of dioxins which may result in several environmental problems. Also, burning reduces the economic value of the substrate. Hence, suitable methods are required for management of food waste.

*P. aeruginosa* is a bacteria of the order Pseudomonadales, family Pseudomonadaceae and genus *Pseudomonas*. *P. aeruginosa* and other bacteria can be used for various experimental activities such as Bioremediation [17-20].

The process of decomposition of both FW and CD can be compared with fermentation which can either be carried out with or without air. When air is used in the fermentation vessel, the type of fermentation which occurs is called aerobic fermentation where free oxygen acts as the hydrogen acceptor. Another type of fermentation pathway occurs when little or no air is introduced into the fermentation vessel, this is known as anaerobic fermentation [21-23].

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**Fig. 1.** Block diagram of material handling from collection stage to the analysis of gas produced

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2. Materials and Method

2.1. Sources of Waste

The food waste was collected from Covenant University Cafeteria. The food waste composed of different leftovers of cooked foods. Bones, containers and plastic bags were removed before the food waste were dehydrated by a screw extruder and crushed to a mean particle size of < 2mm by an electrical grinder.

Cow dung was obtained from a cow rearing farm near the University. It was obtained in solid form and then homogenized. Both FW and CD were stored separately in the freezer until needed.
2.2. Inoculum

*P. aeruginosa* with methanogenic ability was cultured in the Microbiology laboratory of Covenant University, Ota using [19, 24, 25] method. It was mixed with sterile distilled water till it reached the Mcfalan standard (to standardize the bacteria). The bacteria used in the study was from a water area around the farm which contains all required microbes (hydrolysing, acidogenic, acetogenic and methanogenic bacterial group) crucial for the anaerobic digestion process. The bacteria were kept at 4°C until needed.

Table 1 Parameters of Different Feed Materials

| S/N | Material | Kind    | Initial pH | Carbon-nitrogen ratio (C : N) |
|-----|----------|---------|------------|-------------------------------|
| 1   | Rice     | Crop    | 6          | 25:1                          |
| 2   | Fish     | Proteins| 7          | 15:1                          |
| 3   | Cabbage  | Vegetable| 7          | 11:1                          |
| 4   | Moinmoin | Proteins| 7          | 15:1                          |

(Reference for C: N value; www.norganics.com)

2.3. Bioreactor Set up

2kg of the wastes were obtained and blended with distilled water to make a uniform slurry (homogenous mixture). Five (5) 50L plastic kegs were used as bioreactor with five bicycle tubes to collect the produced gas. An inlet pipe was connected to the bioreactor to collect the gas and pass through the outlets hose connected to the rubber tyre tube, Figure 1.

Fig. 2. Equipment Set up
2.4. **Blending**
2kg of waste was blended in a 60:40 ratio with water. Distilled water was used to bring the volume up to 2L. This was done using a grinder to create a homogenous mixture thereby leading to a faster process due to smaller particle size.

2.5. **Waste Analysis**
Waste and 50ml water were mixed till a uniform solution was obtained. A dissolved oxygen probe was inserted into the continuously mixed solution until a constant value was achieved.

2.6. **Moisture content determination**
10g of each sample was weighed and heated at 115°C till a constant weight was achieved. This shows that certain amount of water has been removed. The new weight was then measured and moisture content calculated. A laboratory oven (Vision Scientific (Japan)) model-LDO-201-E was used.

2.7. **Total solid**
30ml of homogenous waste were weighed into tared, cleaned and dried porcelain dish. The majority of the water was evaporated for about an hour. The evaporation was completed by oven drying at 103°C. Samples were cooled, put inside desiccator and weighed. The different weight from the tared weight were recorded as the total solids.

2.8. **pH determination**
1g of air-dried waste sample were weighed into a 100 ml beaker. 20 ml of distilled water was added to it and it was carefully stirred for 10 seconds using a rod. pH electrode was immersed into the suspension and reading was taking when it was steady. pH was measured before and after the digestion process.

2.9. **Waste Loading**
The reactor was loaded with 2kg of food waste each to obtain an organic loading of 1.0g VS/L (Volatile solid/Liquid). The slurry and the inoculum were loaded into the digester in the ratio shown in table 2. The bacteria were measured using a syringe (0-15ml) into the five digesters. Batch digestion experiments were conducted at mesophilic temperature for all the reactors. The reactors were manually mixed once a day for 30 seconds before measuring volume of biogas produced.
Table 2 Experimental set up for Degradation of FW and CD Waste

| Digester | Food waste (kg) | Bacteria (ml) | Cow Dung (kg) | Ratios |
|----------|----------------|---------------|---------------|--------|
| 1        | 2              | --            | 2             | 1:1    |
| 2        | 2              | --            | 1             | 1:0.5  |
| 3        | 2              | 5             | --            | 1:5ml  |
| 4        | 2              | 10            | --            | 1:10ml |
| 5        | 2              | --            | --            | 1      |

2.10. Gas Collection & Testing

The daily gas produced was noted and cumulative gas produced was measured by downward displacement of the gas with water. A flame test was also conducted on the gas produced.

2.11. Measuring Methods

The following methods were used to measure different parameters such as pH, gas composition and volume of gas produced.

2.11.1. Volume

The measurement of the volume was carried out by downward displacement of water. The volume of the gas gets measured at the top of the measuring cylinder. The measuring cylinder was filled with water and then mounted on top of the trough with water. The gas outlet pipe from the digester was connected to the cylinder. The valve of at the gas outlet pipe was opened. The gas passed into the measuring cylinder. The gas displaced the water downward and occupied the space at the top. The volume displaced was noted from the scale of the measuring cylinder. If the gas coming out was found to exceed the capacity of the measuring cylinder scale, the valve was closed at the appropriate position up to where the gas volume could be recorded. The gas collected inside measuring cylinder was allowed to escape. The water was again filled in measuring cylinder and mounted. The cycle was repeated until the gas was evolved.

2.11.2. Gas Composition

The composition of the gas produced was checked using a syringe method. A sample of the gas was drawn up from the tube with a 20ml syringe. 10ml of the gas was pumped into a dilute NaOH solution. 10mL of NaOH was also drawn into the syringe. The syringe was shaken for 30 seconds. The remaining gas left in the tube was then recorded. This test shows the amount of methane in a sample of gas since the CO₂ from the gas dissolves in the solution.
2.11.3. Biogas withdrawing and weighting
The tube was connected to the digester; the biogas flows into the tube as a result of the pressure inside the digester and pressure inside the tube. The tube was weighed daily using an electronic weighing scale. The digester was also weighed daily. The process was repeated till the end of the lagging period.

3. Presentation of Results

3.1. Composition of Waste Analysis

Table 3: Food Waste Analysis before the process

| Analysis                  | Value |
|---------------------------|-------|
| pH                        | 6.84  |
| TS (%)                    | 18    |
| Moisture Content (%)      | 80.05 |
| Dissolved Oxygen          | 5.8 ppm |

Table 4: pH

| S/N  | Digesters                  | Before the process | After the process |
|------|----------------------------|--------------------|-------------------|
| 1    | 2 kg FW + 2 kg CD          | 6.84               | 4.02              |
| 2    | 2 kg FW + 1 kg CD          | 6.84               | 4.04              |
| 3    | 2 kg FW + 5 ml P. aeruginosa | 6.84         | 3.94              |
| 4    | 2 kg FW + 10 ml P. aeruginosa | 6.84         | 3.92              |
| 5    | 2 kg FW                    | 6.84               | 4.10              |
3.2. Biogas Produced

Fig. 3. Daily weight of Biogas produced, CD ratios (Digester 1 & 2)

Fig. 4. Cumulative yield of Biogas produced, CD ratios (Digester 1 & 2)
Fig. 5. Daily weight of biogas produced, Bacteria (BA) ratios (digester 3 & 4)

Fig. 6. Cumulative yield of Biogas, Bacteria (BA) ratios (Digester 3 & 4)
Fig. 7. Daily weight of biogas produced (dwp) on food waste (Digester 5)

Fig. 8. Cumulative yield of Biogas produced (cmw) on food waste (Digester 5)
Fig. 9. Daily weight of gas produced for all digesters

Fig. 10. Cumulative yield of gas produced for all digesters
4. Discussion of Results

4.1. Food waste with Cow Dung

From figures 3 and 4, it was shown that digester 2 (1 kg CD + 2 kg FW) produced more biogas than digester 1 (2 kg CD + 2 kg FW). The cumulative yield of digester 2 was 88.5 g of biogas produced while digester 1 was 58.5 g of biogas produced. Production of gas began between the 4\textsuperscript{th} and 5\textsuperscript{th} day for digester 1 and between the 3\textsuperscript{rd} and 4\textsuperscript{th} day for digester 2.

In the 1\textsuperscript{st} digester, the highest biogas produced for digester was 7 g/kg which was produced on the 17\textsuperscript{th} day. It seems that from the 20\textsuperscript{th} day there was a steady decrease of biogas produced daily. This is justified by the pH of the waste after the process which is 4.02 to show that it was not conducive for more production of biogas, since \textit{P. aeruginosa} cannot strive in an acidic medium. The lowest yield was on the 16\textsuperscript{th} day with a weight of 0.1 g/kg. This can be due to various factors not analysed in this research.

In the 2\textsuperscript{nd} digester, the highest yield of biogas was produced on the 15\textsuperscript{th} day, two days before digester 1. The weight of the gas was 9 g/kg and on the 20\textsuperscript{th} day, 7 g/kg was produced. A steady decrease of biogas produced began on the 23\textsuperscript{rd} day till the 30\textsuperscript{th} day. This could also be justified by the pH of the waste at 4.04 which was too acidic for methanogenesis to occur. The production in the 2\textsuperscript{nd} digester might be more due to lesser competition of food for the bacteria in the waste or faster digestibility.

4.2. Food waste with Bacteria

From figures 5 and 6, it was shown that digester 4 (10 ml BA + 2 kg FW) produced more biogas than digester 3 (5 ml BA + 2 kg FW). The cumulative yield of digester 4 is 61.2 g of biogas produced while digester 3 is 51 g of biogas produced. Production of gas began between the 5\textsuperscript{th} and 6\textsuperscript{th} day for digester 3 and between the 3\textsuperscript{rd} and 4\textsuperscript{th} day for digester 4.

In the 3\textsuperscript{rd} digester, the highest biogas produced for digester is 4 g/kg which was produced on the 14\textsuperscript{th} day. It was seen here that from the 19\textsuperscript{th} day there was a steady decrease of biogas produced daily. This is justified by the pH of the waste after the process which is 3.94 to show that it was not conducive for more production of biogas since \textit{P. aeruginosa} cannot strive in an acidic medium. The lowest yield was on the 30\textsuperscript{th} day with a weight of 0.2 g/kg. This can be due to various factors not analysed in this project.

In the 4\textsuperscript{th} digester, the highest yield of biogas was produced on the 15\textsuperscript{th} day. The weight of the gas is 5.5 g/kg and on the 18\textsuperscript{th} day, 4.4 g/kg was produced. A steady decrease of biogas produced began on the 23\textsuperscript{rd} day till the 30\textsuperscript{th} day. This could also be justified by the pH of the waste at 3.92 which is too acidic for methanogenesis to occur. The high production in the 4\textsuperscript{th} digester might be due to the larger
amount of *P. aeruginosa* which breaks down the complex compounds in the waste into low molecular weight fatty acids and increases bioavailability.

### 4.3. Food waste

From figures 7 and 8, it was shown that in the 5th digester, the highest yield of biogas was produced on the 15th day. The weight of the gas produced is 4.8 g/kg and on the 20th day, 3.8 g/kg was produced. A steady decrease of biogas produced began on the 23rd day till the 30th day. This could also be justified by the pH of the waste at 4.1 which is too acidic for methanogenesis to occur. The production in the 5th digester was with no inoculum added. The cumulative yield produced was 56.3 g/kg. Production of the gas started on the 4th day.

### 4.4. Comparison of all ratios

From figures 9 and 10, the 2nd digester (1 kg CD + 2 kg FW) produced the most biogas and had the highest yield. The least cumulative yield was from digester 1 (2 kg CD + 2 kg FW) and the least daily yield was from digester 3 (5 ml BA + 2 kg FW). This result proves that more yield can be obtained from co-digestion of cow dung than the addition of bacteria or digestion of the food waste.

### 4.5. Gas Test

Syringe method was used as a substitute to roughly calculate the methane content in the gas produced. This is calculated from methane volume left/ initial volume in the syringe. From the table 5, digester 1 have the highest methane content. This could be due to the co-digestion of the waste with cow dung, as cow dung has a high C/N ratio. The lowest methane yield was observed in digester 4 containing 10 ml of *P. aeruginosa*, this could be from the acidogenesis phase being the rate limiting phase of the reaction thereby reducing the amount of methane produced. Between digester 1 & 2, the methane content is directly proportional to the amount of cow dung added in the digester. Between digester 3 & 4, the methane content is inversely proportional to the amount of bacteria added. Food waste on its own had a percentage of 44% which is within the ratio of methane content produced from it [26].

| S/N  | Digester            | CH₄: NaOH (ml) | Total volume (ml) | Methane Content |
|------|---------------------|----------------|-------------------|----------------|
| 1    | 2 kg FW + 2 kg CD   | 2.6: 7.4       | 10                | 52%            |
| 2    | 2 kg FW + 1 kg CD   | 2.4: 7.6       | 10                | 48%            |
| 3    | 2 kg FW + 5 ml BA   | 2.1: 7.9       | 10                | 42%            |
| 4    | 2 kg FW + 10 ml BA  | 2: 8           | 10                | 40%            |
| 5    | 2 kg FW             | 2.2: 7.8       | 10                | 44%            |
4.5.1 Flame Test
Flame test was conducted on the gas produced. A blue flame ignited as the gas was passed through a lighter. This proves that the gas contained in the tube is biogas.

![Flame test on the biogas produced](image)

5. Conclusions
Anaerobic co-digestion of FW and CD enhanced the production of biogas and methane yield. The results show that digestion of only food waste will produce biogas to an extent. The further addition of cow dung would increase the methane content and biogas yield because cow dung can produce biogas naturally. The use of *P. aeruginosa* to break down the lipids was effective, but the methane content and biogas yield was low compared to digestion of only food waste. CD ratio with FW for digester 1 (2 kg CD + 2 kg FW) and digester 2 (1 kg CD + 2 kg FW) with the lesser cow dung produced more biogas. The ratio with more cow dung has a higher methane content.

Conflicts of Interest
The authors declare no conflicts of interest regarding the publication of this paper.

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Nomenclature
FW – Food Waste
CD – Cow Dung
C/N – Carbon/Nitrogen
VS – Volatile Solids
*P. aeruginosa* - *Pseudomonas aeruginosa*
BA – Bacteria
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