Journal of Engineering Research Innovation and Scientific Development 4 (1), (2026), 13-18 https://doi.org/10.61448/jerisd41264

Journal of Engineering Research Innovation and Scientific Development
journal homepage: www.jerisddelsu.com
Print ISSN 2971-6594 Online ISSN 2971-6608

Bio-Treatment of Liquid-Solid Waste through Anaerobic Digestion for the Mitigation of Environmental Pollution

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Article history: Received: 03-12-25, Revised: 03-02-26, Accepted: 10-02-26, Published: 20-02-26

Abstract

Globally, energy supply remains one of the inevitable challenges bedevilling numerous underdeveloped and developing countries. It is well known that a lot of companies and industries have faced closure, with some encountering difficulties to establish themselves, due to the cost and availability of energy in certain regions. This necessitates the quest for an alternate sustainable source of energy. In this study, three biogas digesters were set up for the production of biogas. Also, three fermentation slurries were prepared (pig dung, cattle dung and water alone); (pig dung, banana waste and sugar wastewater); (pig dung, corn waste and sugar wastewater) for the anaerobic digestion experiment. The process was carried out at the following operating conditions: temperature 38 ^{0}C, pH 6.5, total solid content 8% and retention time of 240 hours. Gas production was collected by means of connecting a hose to an air bag. The bacterial population of the fermentation slurries were counted at intervals of time at varying dilution rates using a colony counter. The total solid content of pig dung, cattle dung, banana waste and corn waste were 46.5%, 29.4%, 25.1% and 41.8% respectively, while the corresponding volatile, carbon, and moisture content were 76.56%, 0.425, and 41.35%; 76.87%, 0.427, and 5.5%; 78.09%, 0.434, and 9.05%; 83.9, 0.494, and 50.05% respectively. Additionally, the cumulative biogas yield was highest in the mixture of pig dung, cattle dung and water alone, which produced 3.35kg of biogas, while the mixtures of pig dung, banana waste, sugar waste water and pig dung, corn waste, sugar waste water produced 3.15kg and 2kg of biogas, respectively. Also, the kinetic parameters (specific growth rate and doubling time) for dilutions 10-2 and 10-4 were determined as hr^{-1} and respectively.

Keywords: Biogas; sugar wastewater; doubling time; corn waste; monod kinetics

1. Introduction

Conventional fuels (also fossil fuels), which serve as the major source of energy in underdeveloped and developing countries, have in addition to its high cost, caused severe climate changes and even environmental pollution. Increase in global average temperature, drought, flooding, crop failures are some effects of the use and excavation of fossil fuels (Dow and Downing, 2006; Mills, 2009; Sen, 2009). The rapid reduction in availability of these conventional fuels and its increased environmental threat has stimulated the search for alternative sources to this source of energy and hence the need for renewable energies such as biogas, biodiesel, solar is a must. (Alemayehu and Abile, 2014).

Renewable energy is energy that is derived from natural sources and is replenished by nature such as wind energy, hydropower energy, solar energy, geothermal energy and even bioenergy. In other words, renewable energy can be termed "free energy" since it is given directly from nature and "inexhaustible energy" since it is continuously replenished by nature and cannot be exhausted. Renewable energy offers among the best alternatives for stable energy supply. The kind of renewable energy in focus is biogas, which is virtually one of the best substitutes to conventional fuels. Biogas is unique among other renewable energies because it uses organic wastes which can be used to produce fertilizer for agricultural use. It also does not have any geographical hindrance nor does it require advanced technology as it operates on very simple principles (Weiland, 2003; Chynoweth, 2004; Ploj *et al.,* 2006; Navickas, 2007).

Biogas, composed of methane, carbon dioxide and other components, is a kind of renewable energy derived from the anaerobic digestion of organic materials (biomass). Biomass is any material that is composed of organic compounds and is capable of decay. When these substances decay, they release gases which are composed of majorly methane. Biogas is produced naturally in areas where organic wastes are found such as swamps, lake sediments and landfill which causes pollution when exposed to the air. Anaerobic digestion is a process used to produce biogas from biomass. It involves the conversion of complex organic compounds to simpler ones by the means of microorganisms in the absence of oxygen. When organic substances degrade, complex substances are reduced into simpler ones. This process is carried out in bioreactors (biodigester).

The mechanism of anaerobic digestion process involves four (4) stages. The first stage is hydrolysis. In this stage, bacteria break down complex molecules in the organic substance into monomers and simple substances. The second stage is the acidogenesis, where simple sugars and long-chain fatty acids are further broken to volatile fatty acids (VFAs), acetate, alcohols, carbondioxide and hydrogen. The third stage is acetogenesis, in which the VFAs and alcohols are further broken down into acetic acid and hydrogen. The final stage is methanogenesis, where carbondioxide, hydrogen, formate, methanol, and acetate are transformed into methane which is biogas (Kasinath *et al.,* 2021; Aworanti *et al.,* 2023).

A digester is a closed vessel where reaction (combination or breakdown) of material(s) occurs to produce a product(s). It is where the breaking down of organic materials occurs through the action of some bacteria (methanogenic bacteria) and Achaea species in a biological reactor (bio-digester) to produce biogas (Aworanti *et al.,* 2023). The anaerobic digestion process provides rural areas with clean cooking biomethane and biomethane electricity which improves health conditions, reduces dependence on traditional biomass fuels such as wood, and fostering economic growth through job creation (Kammen *et al.*, 2020). Biogas is a combination of gases and it has methane content of about 55-65%, 35 – 45% of carbon dioxide, 0 – 1% of hydrogen sulphide, 0 – 1% nitrogen, 0 – 1% hydrogen, 0 – 3% carbon monoxide, 0 – 2% oxygen and traces of ammonia and water vapor (Zhang, 1999; Kalia *et al*., 2000; Keefe, 2000). It is a colorless, odorless and highly flammable gas which has a calorific value of 4500–5000 kcal/m^3 when its methane content is about 60 to 70% (Madu and Sodeinde, 2001; Igoni *et al.,* 2008). Biogas holds high potential to be a sustainable solution for energy generation and waste management. It produces energy that is free and renewable (replenished by nature) and hence has tendency for lasting and in exhaustible energy. In addition, to sustainable energy generation, biogas systems reduce greenhouse gas emissions and the need for landfilling and incineration thereby minimizing environmental pollution (Kumar *et al.,* 2021).

Biochemical kinetics is the study of the growth rate of microorganism in a substrate with respect to time using Monod kinetics equation. Monod kinetics equation is used to determine the specific growth rate (μ), maximum specific growth rate (μ_{max}) and the half saturation constant (k_s). The biomasses which can be used for producing biogas are animal dung (pig, cattle dung, and poultry droppings), fruit waste (banana, mango, orange) and industrial and sludge waste (sugar waste water, sludge waste) are all organic materials. Pig dung contains high amount of ammoniacal nitrogen which produces biogas. Pig and cattle dung were selected as main substrates due to their high potential for biogas production. Pig dung is found to contain various nutrients, such as potassium, phosphorous calcium and a high buffering capacity that prevents accumulation of volatile fatty acids (VFAs) and thereby facilitate biogas production. Pig and cattle dung also contains high lignocellulose materials which helps to produce methane and the enzymes present in their four chambered stomach help in the breaking down of the complex plant fibers present in their cud which can be converted to methane (Werner *et al.*, 1989; Willkie, 2005). However, animal dung contains high nitrogen and alkalinity which can inhibit the growth of methane producing bacteria and biogas yield. Hence, for this study, animal waste is co-digested with fruit waste and wastewater. It is however not advisable to use only pig manure alone due to its low carbon content hence the use of fruit waste as co-substrates.

Fruit or agricultural waste contain high carbon which increases the carbon to nitrogen ratio of the mixture and dilute ammonia, an inhibitory component to biogas production. Co-digestion with plant (or fruit) will help increase the carbon content that is deficient in the dung and thereby increasing the yield of the biogas. Each of the fruits waste (banana and corn) contains lignocellulose biomass which makes them suitable for producing biogas too. This increases the quality and quantity of the anaerobic digestion process of the animal waste. The use of animal wastes, together with fruit waste and wastewater has potential to reduce lag phase, extend the exponential phase and enhance the quality and quantity of produced biogas.

Animal and fruit wastes are biodegradable (decomposable) wastes, and if not properly disposed contribute to various kinds of environmental pollutions by breeding flies, insects, rodents and generates unpleasant odors from the decomposition of the wastes. The utilization of these wastes for biogas production will not only produce energy but eliminate the consequences of liquid-solid pollution to the environment. This can serve as an effective waste management system as well as sustainable energy production. In general, biogas production from wastes is not only possible but advantageous, and implementation would be of great impact on both economic development and environmental health. Environmental pollution generated from waste production, improper waste disposal and inadequate waste treatment facility contribute to the degradation of the environment particularly in developing nations. These environmental pollutions can be mitigated by employing suitable waste management practice, such as biotreatment of liquid-solid waste through the use of anaerobic digesters (Han *et al.,* 2019). The anaerobic digester not only serves as a waste management method but also contributes to the reduction of both waste and greenhouse gas emission, and generate cleaner alternative renewable energy. Therefore, the optimal method for treating the organic waste by converting the waste to biogas was investigated to improve environmental degradation. The aim of this research work is to determine the kinetic parameters and the quantity of biogas produced by the anaerobic digestion of animal waste mixed with co-substrate and sugar waste water.

2. Materials and Methods

2.1 Collection and Preparation of Samples

2.1.1 Sample Collection

Animal dung (pig and cattle dung) which served as the main substrates were collected from Sam Ade, in Ogbomoso, while the co-substrates (banana and corn waste) were collected from Sabo market, also in Ogbomoso. Sugar cane was collected from Wazo market, Ogbomosho, Oyo State, Nigeria. The substrates are shown in Figures 1 to 5


Figure 1: Pig dung


Figure 2: Cattle dung


Figure 3: Banana waste


Figure 4: Corn waste


Figure 5: Sugar cane

2.1.2 Sample Preparation

The unwanted parts of the corn waste were removed, the silage was ground using an electric blender, then sieved to a particle size below 10 µm. while the spoilt banana and peels were pounded together in a mortar to form a paste. This was done to increase the surface area of the substrates, biodegradability and enhance the nutrient availability for micro- organisms in the AD process (Palmowski and Mller, 2000; Sun and Cheng, 2002). Finally, sugar waste water was simulated by debarking sugar cane, extracting its juice using mortar and pestle, and diluting with water to obtain a 15% concentration. The buffer solution was prepared according to Wang *et al.* (2014). A 0.5M concentration of the solution was produced by dissolving 28g of KOH in 1000ml of distilled water. The buffer solution was stored in a covered bottle till the time of use.

2.2 Proximate and Ultimate Analysis of Animal, Fruit and Sugar Wastewater

The proximate and ultimate analysis of the sample (total solids, volatile solids, and moisture content and carbon content) were performed according to the standard procedure presented in AOAC (2019). Total solids (TS) were determined by measuring the amount of solid residue left after the sample had been oven dried. Each substrate was weighed (10 g) and heated in an electric hot oven at 105 °Cfor 24 hrs. The samples were then re-weighed and TS was calculated using Equation 1. Volatile solids of the samples were determined by igniting the oven dried substrate in a muffle furnace at temperature of 550°C for 3hrs. The percentage of volatile solids was obtained by Equation 2. Moisture content was evaluated by oven drying 20 g of each fresh substrates for 7 days at 103°C and measuring the weight of the samples at regular intervals, until constant weight was achieved. The moisture content was calculated by Equation 3 and the carbon content was calculated using Equation 4.

$$\%TS = \frac{mDS}{mFS} \times 100 \quad (1)$$

$$\%VS = \frac{mDS - m(ash)}{mDS} \times 100 \quad (2)$$

$$Moisture\ content = \frac{(initial\ mass - final\ mass)}{100} \quad (3)$$

$$\%Carbon = \frac{\%VS}{1.8} \quad (4)$$

where;
%TS= Percentage of total solids mDS = Mass of dry sample (final weight)
mFS= Mass of fresh sample %VS= percentage of volatile solids, mDS= mass of dry solids, m(ash)= remaining mass after ignition

2.3 Isolation and Identification of *Bacteria* Isolates in Animal, Fruit, and Sugar wastewater

Bacterial isolates present in the animal, fruit and sugar wastes water samples were identified using biochemical tests, following the methods described by Sharma and Singh 2015; Dihman *et al* 2022.Serial dilution was carried out on a homogenous mixture of all substrates in order to vary the concentration of organisms in the substrate. Peptone water of 9ml was put into 11 test tubes and sterilized at 121 ^{0}C for 15 minutes then 1 g of homogenous mixture of the substrates was dropped in the first test tube, mixed and labeled as 'stock'. The remaining test tubes were labeled 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, etc. 2 g of the stock was poured into test tube 10^{-1} and mixed well. Similarly, 2ml of the 10^{-1} test tube was put into 10^{-2} test tube and mixed thoroughly. The process was repeated until all 10 test tubes were exhausted. The test tubes were then incubated anaerobically for 3 days. Microorganisms were grown using streak plate method. For this method, MRS agar was sterilized, poured into 5 petri dishes, and allowed to solidify. The odd numbered test tubes were selected (i.e. test tube 10^{-1}, 10^{-3}, 10^{-5}, 10^{-7},and 10^{-9}), and streaked on the solidified agar using a micropipette of 0.2 micrometer. The process was carried out aseptically. The petri dishes were then wrapped in foil paper before placing in an anaerobic jar, and incubated anaerobically for another 24 hrs. After the 24 hours completion, each petri dish was checked for distinct colonies. The petri dishes with distinct colonies were isolated to get pure culture by plating one colony on fresh agar. It was incubated anaerobically again, to obtain purer colonies. The pure culture obtained was spread in slant bottles containing sterilized peptone water. It was incubated anaerobically for another 24 hours. Thereafter, microbial characterization and counting was done on the incubated sample.

2.4 Description of Digester

An automated 20 L jacketed cylindrical vessel bio-digester was used in this study which serves as the main bio-reactor, the cylindrical shape is made to have enough space to accommodate the substrates and the lower part at the bottom is conical shaped to enable easy discharge of the slurry by gravity. It consists of two segments, the stainless steel vessel (digester) and a heating element jacket . The digester is surrounded by a heating element which serves as a means of heat transfer to the digester. It is enclosed in a jacket made of stainless-steel material to prevent loss and has a thickness of 1.6 cm. The digester is fitted with inlet and outlet valves of 2.5 cm diameter with two flexible pipes connected to the valves. A control panel was incorporated with the digester. A stirrer which comprises of a shaft with eight baffles powered by an electric motor of 0.0375Kw was fitted at the top of the digester. The temperature and the agitation inside the reactor was controlled by proportional integral derivative panel. The inlet valve is used to introduce feed slurry into the digester while the outlet valve is connected to an air bag for gas collection (Agbede et al., 2020).

2.5 Experimental Apparatus Setup and Procedure

The experimental apparatus set-up consists of a automated 20 L jacketed cylindrical vessel bio-digester. Flexible pipe was fitted into the outlet valve of the biodigester. The inlet valve was used to introduce feed slurry into the digester while the outlet flexible pipe was connected to an air bag to collect the produced gas. The outlet of the air bag was then connected to the inlet of the biogas pump by flexible pipe, the biogas generated was pumped through the outlet flexible pipe of the biogas-pump to the biogas burner. Three (3) automated 20 L jacketed cylindrical vessel bio-digesters were loaded with prepared fermentation slurry shown in Table 1. The working capacity of the automated biodigesters was maintained at 16L. It operated at a steady reaction operating conditions: agitation of 20rpm, retention time of 30 days, temperature of 40 °C, total solid content of 8 % and organic loading rate of 16L. The organic total solids (OTS) in each of the samples ($E_1 – E_3$) was set at 229.68 g as shown in Table 1. Biogas produced was convey from the reactor through the outlet valve connected to an air bag . The outlet of the air bag was then connected to the inlet of the biogas pump and the outlet of the pump was connected to a biogas burner. The process was monitored daily and the volume (kg) of biogas produced was measured and recorded every 24 hours. Also, the methane content of the biogas was determined using a gas analyzer.

Table 1: Composition of Substrate Fed into the Digester

Experiment	Pig dung (g)	Cattle dung (g)	Banana waste (g)	Corn waste (g)	Water (g)	Sugar wastewater (g)	OTS (g)
E_1 (control)	200	338.76	0	0	421.38	0	229.68
E_2	200	0	390.61	0	0	834.93	229.68
E_3	200	0	0	205.27	0	867.26	229.68

E_1: Pig dung, cattle dung and water; E_2: Pig dung, banana waste and sugar wastewater; E_3: Pig dung, corn waste and sugar wastewater

2.6 Preparation of the Fermentation Slurry and Determination of Water Content

The fermentation slurry was prepared according to Adebayo et al. (2015); Edunjobi *et al*. (2023). The water content in the slurry was determined using 8% total solid content and was calculated using Equation 5. The mass of each substrates(C_s), concentration of substrate ($M_s$mass of each substrate (m_{s1} and m_{s2}) and goTS were calculated using Equation 6, 7, 8, and 9

$$Main\ amount\ of\ water\ required = \frac{TS \times 10 \times \%WC}{\%TSC} \quad (5)$$

$$M_s = \frac{M_i C_i}{2C_s}\ ;\ m_s = \frac{100 \times goTS - m_i c_i}{c_s} \quad (6)$$

$$C_s = \frac{oDM(\%DM) \times DM(\%FM)}{100} \times n_{s1} + \frac{oDM(\%DM) \times DM(\%FM)}{100} \times n_{s2} \quad (7)$$

$$m_{s1} = \frac{m_s}{f_{s1} + f_{s2}} \times f_{s1} \quad (8)$$

$$m_{s2} = \frac{m_s}{f_{s1} + f_{s2}} \times f_{s2} \quad (9)$$

where for the ratio of fraction and fraction of the substrate can be calculated using Equations 10 and 11.

$$r_s = \frac{n_s}{oTS(\%FM)} \quad (10)$$

$$f_{s1} = \frac{r_s}{smallest\ r} \quad (11)$$

The total amount of water content in each experiment was determined by Equation 12.

Total amount of water content in each experiment = (mass of the substrate A x *Actual amount of water required per gram of fresh substrate) + (mass of substrate B + Actaual amount of water required per gram of fresh substrate B)* (12)

2.7 Kinetic Studies of Biogas Production

As proposed by Monod (1942), Monod model was adopted to describe the specific growth rate (μ) and cell growth (r_x) of the micro-organism present in the samples in the reactor. The specific growth rate (μ), cell growth (r_x) were calculated using 13 and the time interval (t_d) required to double the population was calculated by Equation 14

$$\mu = \frac{1}{X} \cdot \frac{dx}{dt} = \frac{1}{X}\left[\frac{x_1 - x_0}{t_1 - t_0}\right] \quad (13)$$

$$\text{Doubling time } (t_d) = \frac{\ln 2}{\mu} \quad (14)$$

cell concentration mg/l (x), specific growth rate (μ),and retention time(t)

From the Monod kinetics equation;

$$\frac{1}{X} \cdot \frac{dx}{dt} = \frac{\mu_{max} S}{k_s + S}$$

Finding the inverse,

$$\frac{1}{\mu} = \frac{k_s}{\mu_{max}} \frac{1}{S} + \frac{1}{\mu_{max}} \quad (15)$$

3. Results and Discussion

3.1 Proximate and Ultimate Analysis of Substrates

The results of the proximate and ultimate analysis of pig dung, cattle dung, banana waste and corn waste are presented in Table 2.

3.2 Total and Volatile Solids

From Table 2, the result shows that the total solids of all substrates were high (above 40%), however, a high total solid value indicates inhibition to biogas production. TS values above 15 % may cause stirring problems in continuously stirred tank reactors (Agbede et al., 2019). This may be due to the fact that biogas produced from materials of high total solid values will be too hard to decompose. The optimum TS values lie between 8-10% which is favorable to biogas production (Mahmudul et al., 2021). For this reason, the TS values of this process were reduced by dilution with water to achieve optimum biogas production.

Additionally, the results from Table 2 showed high volatile solid contents in all the substrates (above 70%). This means that the substrate can be easily volatilized (i.e. converted to gas), and will likely support biogas production. This shows the suitability of each substrate for the production of biogas.

3.3 Carbon Content

The carbon content of each substrate is seen in Table 2. It was noticed from the result that the carbon contents of the substrates were within the range of 0.4, which showed lower carbon contents. The lower values (below 1) obtained in the result revealed that the substrates are feasible for the production of biogas. Carbon serves as a source of energy for microorganisms and nitrogen forms ammonia gas. The optimum C/N ratio for anaerobic digestion is 20 – 30. If the C/N ratio is too low, pH increases which produces a toxic effect on methanogens when it exceeds 8.5. Additionally, if the C/N ratio is too high, nitrogen is used up by the methanogens and is no longer available to react with leftover carbon in the material which consequently reduces biogas production (Matheri et al., 2017).

3.4 Moisture Content

The moisture content or water content of each substrate is seen in Table 2. It was observed from the result that the moisture content ranged from 5 to 50%. The optimum moisture content for effective performance is 90% (Abbasi et al., 2012). Since the moisture range was below the optimal moisture content, water was added to increase the moisture content for the process for effective production.

Table 2: Proximate and Ultimate Analysis of Substrates

	Pig dung	Cow dung	Banana waste	Corn waste
Total Solids (%)	46.5	29.4	25.1	41.8
Volatile Solids (%)	76.56	76.87	78.09	89.23
Carbon content (MgC)	0.425	0.427	0.434	0.494
Moisture content (%)	41.35	5.5	9.05	50.05

3.5 Effect of Co-substrates and Sugar Wastewater on Biogas Yield and Startup Time

The effects of co-substrates (banana and corn waste) on biogas yield and startup time were studied by varying the combination of co-substrates with substrates. The result of the digestion process evaluated at different combinations of co-substrates with substrates are presented in Figures 6 to 9.

3.5.1Effects of Cattle Dung, Pig Dung and Water on Biogas Yield and Startup Time

The effect of animal waste (pig and cattle dung) and water on biogas yield was shown in Figure 6, which shows that the cumulative biogas yield (3.35kg) was obtained during 240 hours of anaerobic digestion. It was observed from the result that the gas production started on the 48^{th} hour. Also, lag phase occurred but was not evident due to the adaptation of the bacteria to the nutrient medium and that the medium was rich in essential metabolites. This production was quite early compared to other research works, which takes at least four days to produce gas. For instance, According to Putria *et al*, 2012, the results of the study showed an increased biogas production from day 4 to day 24. This early onset of biogas production is this study is attributed to the low total solid content, high volatile content and low carbon content of the substrates. According to Di Maria *et al,* 2014, that low total solid value was favorable to biogas production.

It was observed that the exponential phase started on the 48^{th} hour and produced 1.2 kg of biogas until it reached its peak at 3.35kg on the 168^{th} hour. The production peak was early when compared to the results from Putria *et al*. (2012), where peak occurred at day 25. The exponential phase experienced maybe due to the nutrients in the medium and the working suitable pH for the microorganism. The stationary phase occurred at the peak and lasted for another 24 hours. This may be due to the nutrient depletion or product accumulation in the medium. The declining phase, which occurred at the 192^{nd} hour and declined steadily till the 216^{th} hour may be due to the depletion of nutrient and lack of formation of daughter cells. At the 240-hour, death phase occurred which may be due to nutrient exhaustion or lack of cell uniformity in their physiological characteristics.

In conclusion, the Figure 6 showed the result of the biogas production from the mixture of substrates increased with time before declining to 0.5kg. This shows the positive effect of the substrate combination on the yield of biogas produced.

3.5.2 Effects of pig dung, banana waste and sugar waste water on biogas yield and startup time

Biogas production yield for the digestion of pig dung, banana waste and sugar waste water are shown in Figure 7. It was observed from the graph that biogas production increases as time increases. The production yield in this mixture was however slightly lower when compared to the previous mixture of pig, cattle dung and water alone. This occurrence may be attributed to the higher lignin content present in the banana waste. It was observed from the graph that gas production began on the 48th hour and this early production may be attributed to the higher nutrients such as potassium, cellulose and hemicellulose in banana as co-substrate, and are favorable to biogas production. This is contrast to what was reported by Akindele *et al*, 2018 that no gas was produced from co-digestion till on the fourth day. It is also noteworthy that the occurrence of lag phase in the process may be due to the adaptation of the bacteria to the medium.

The maximum biogas production was attained on the 288th hour producing 3.1kg of biogas. The production then attained its stationary phase from the 288 to 384th hour, followed by the declining phase before death occurs, which may be due to nutrient exhaustion, producing 1kg of biogas. This result is synonymous to the co-digestion process of Tamrat *et al.*, (2013) which utilized rumen fluid, cow manure, and organic kitchen waste for co-digestion process and which showed a high cumulative biogas yield, even higher than the digestion of main substrate alone. However, for this experiment, the biogas yield was slightly lower in the co-digested sample than in the digested sample.

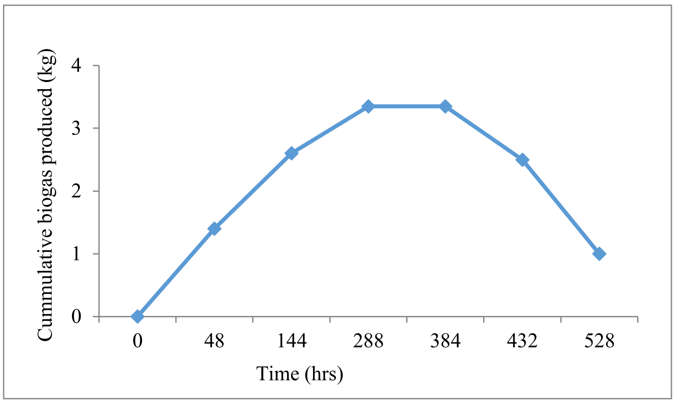

Figure 6: Cumulative biogas production of pig dung, cattle dung and water

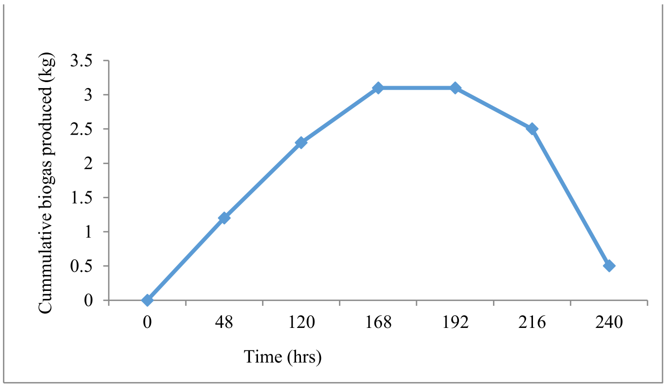

Figure 7: Cumulative biogas production of pig dung, banana waste and sugar waste water

3.5.3 Effects of pig dung, corn waste and sugar waste water on biogas yield and startup time

The cumulative biogas production obtained during 648 hours digestion process of the mixture of pig dung, corn silage and sugar waste water are shown in Figure 8. It was noticed that the production started on the 192nd hour and produced 1.2kg of biogas, the total production of gas was 2kg and the production reached its peak at the 312nd hour. However, cumulative biogas yield of the mixture of pig dung, corn silage and sugar waste water were lower than the mixture of pig, cattle dung and water alone and the mixture of pig dung, banana waste and sugar waste water. The low yield may be due to the high carbon content, the carbon-nitrogen imbalance or low potassium in corn waste. The lower pH value experienced in the process resulted into lower biogas yield and this resulted into accumulation of VFAs, which causes toxicity to methane-generating bacteria (Kemka et al.,2025;Franke *et al.,* 2014). This production is however similar to the findings of Akindele et al, 2018 in which mixture of animal and poultry waste alone had higher production than poultry waste with corncob. This may be attributed to the accumulation of volatile fatty acids (VFAs) caused by the corn waste in the digester.

Additionally, it is seen from Figure 3.3 that lag phase was not evident, and may be due to the adaptation of the bacteria to the nutrient medium. Also, the growth became exponential on the 192nd hour, before peaking on the 312th hour and producing 2kg of biogas. Stationary phase occurred at the 312 to 504th hour before reaching death phase, producing 0.8kg of biogas.

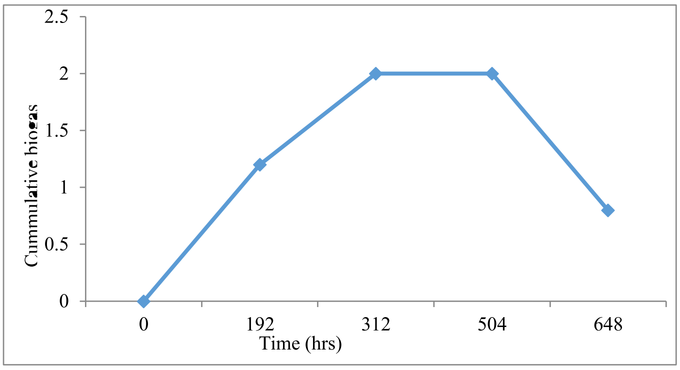

Figure 8: Cumulative biogas production of pig dung, corn waste and sugar wastewater

3.6 Effect of Co-substrates on Hydraulic Retention Time

The effect of co-substrates on retention time when digested with pig dung, fruit waste (banana, and corn) with sugar water and animal waste (pig and cattle dung) alone with water are shown in Figure 9. It was observed that both mixtures of pig dung, cattle dung and water and pig dung, banana waste and sugar waste water started production on the 48th hour and peaked at a time of 288 and 168 hours respectively before remaining constant at the peak. However, the mixture of pig dung, corn waste and sugar waste water started production on the 192nd hour before peaking at the 312nd hour. This delay in production of the mixture of pig dung, corn waste and sugar waste water may be attributed the high carbon content, low nitrogen, hemicellulose and cellulose in corn waste compared to the other substrates. This production also shows a longer time required to attain its peak, as it attained at 312 hours. In general, start-up time was lowest in the mixtures of pig dung, cattle dung and water alone and in the mixture of pig, banana and sugar waste water than in the mixture of pig dung, corn waste and sugar waste water. Therefore, the mixtures of animal waste and water alone and the mixture of pig, banana and sugar waste water seemed most efficient for the production of biogas. The efficiency of these two experiments may be due to the physiochemical properties of each substrate which improve the process stability. The result is similar to the observation from Matheri *et al.* (2017).

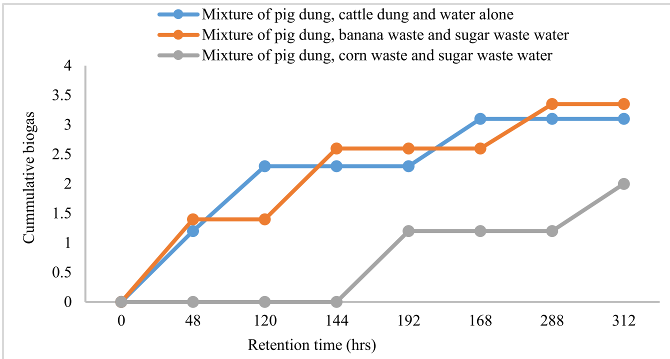

Figure 9: Effect of co-substrates on hydraulic retention time

3.7 Maximum biogas and Biomethane Production

The maximum cumulative biogas production for all experiments is shown in Figure 10. The result indicates that the mixture of animal waste alone (pig, cattle and water) had the highest yield which was higher compared with co-digestion by 7.5 and 40.3% and this may be due to the presence of high nitrogen in the cattle dung. Also, the second highest was observed in the mixture of pig, banana and sugar waste water, which had a yield of 3.1kg and it was also noticed that the biogas yield increased by 35.5% when compared with the mixture of pig dung, corn waste and sugar waste water and may be attributed to the high cellulose and hemicellulose content in the banana. The pH levels in the mixtures reflects the efficiency of the process as the pH values fall within the needed optimum pH range of 6.4 to 7.6 and this resulted into higher production level (Anderson and Yang, 1992). Since anaerobic microorganisms cannot grow under high acidic conditions, increase in pH is favorable to biogas production as it leads to higher methane yield (Matheri *et al*., 2017). The cumulative biogas yield obtained are 3.35, 3.1, and 2kg for pig dung, cattle dung and water; pig dung, banana and sugar waste water; pig dung, corn and sugar waste water respectively. The first experiment with a peak production of 3.35kg seemed most efficient of the three as it had the highest biogas production. This result is consistent with the findings of Otun *et al,* 2015 which showed that biogas production was highest in animal waste and can be attributed to the high organic content, lower carbon etc. in animal waste than other organic wastes.

The pH measurement also highlights the stability of the digestion process. Since the pH of the process ranges within the optimal range (5.5 - 8.2), it shows the digestion process is stable (Akindele *et al,* 2018). A stable pH range ensures optimal conditions for microbial growth and biogas production. Large fluctuations in pH (either low or high) levels can inhibit microbial activity and thereby reduce biogas yield. Therefore, a pH range of extremely acidic (greater or equal to 3) or extremely alkaline (greater or equal to 12) can be inhibitory to acidogenesis (Liu *et al*, 2012). Since the pH of the process ranged from 6-7, it showed there were no large fluctuations and the process was relatively stable. This explains the high yield of biogas obtained from the mixture of pig dung, cattle dung and water and mixture of pig dung, banana waste and sugar waste water and the mixture of pig dung, corn waste and sugar waste water.

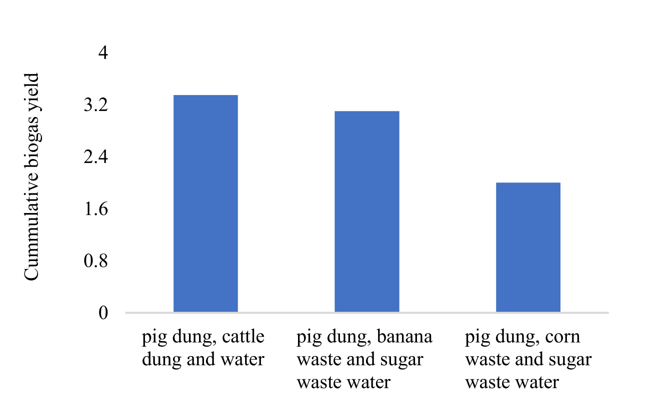

Figure 10: Cumulative biogas yield for all samples

3.8 Bacteria Counts

The substrate obtained after anaerobic digestion, also called digestate, was periodically sampled every five (5) days to determine the bacterial population kinetics. This analysis aimed to evaluate the effects of dilution ratio and retention time on bacterial growth. The result of the samples analyzed using a colony counter are presented in Table 3. The data revealed a notable trend: the bacterial count decreased with increasing dilution ratio. From the table, it was seen that at dilution 10^{-2}, the bacterial count was at 87. However, for dilution 10^{-4}, the bacterial count reduced to 69. This observation suggests that higher dilution ratios lead to reduced bacterial populations. Furthermore, the results also indicated that bacterial counts increased with prolonged retention times. This finding is consistent with the notion that longer digestion periods allow for more microbial growth and hence better yield of biogas (Kigozi *et al*., 2014).

Monod kinetics equation was used to calculate the specific growth rate (μ) and doubling time (td). The results of these parameters are depicted in Table 4.

Table 4: Result of Specific Growth Rate and Doubling Time

	Specific growth rate (μ)	Doubling Time (t_d)
1	0.01 hr^{-1}	69.3hrs
2	0.002 hr^{-1}	346.6hrs

4. Conclusion

This research work has demonstrated the feasibility and efficiency of biogas production from different mixtures of organic substrates, including cattle dung, pig dung, banana waste, corn waste and sugar wastewater as the values of their physiochemical characteristics were within the optimal range. It was also observed that an optimal pH range of 6.8 to 7.2 was found to be ideal for biogas production. Finally, the bacteria counting showed that longer retention time equals increased microorganisms and thereby increase in biogas production. This study contributes to the development of sustainable biogas production methods, promoting the utilization of organic waste sources. The findings of this research have important implications for the energy sector, highlighting the potential for biogas production to contribute to cleaner and more efficient energy sources. Integrating biogas production with existing energy infrastructure is critical for widespread adoption. Therefore, it is recommended that biogas production is integrated while focusing its applications in rural areas, agricultural industries, and waste management sectors. Also, collaboration with stakeholders and training programs for operators can ensure efficient operation and improvement.

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Table 3: Result of Bacterial Count

Time / *Bacterial count*	10^{-1} dilution (cfu\ml)	10^{-2} dilution (cfu\ml)	10^{-3} dilution (cfu\ml)	10^{-4} dilution (cfu\ml)	10^{-5} dilution (cfu\ml)
0 hrs	-	2.8	-	52.1	-
120 hrs	-	87	-	69	-

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