TITLE PAGE

PRODUCTION OF BIOMETHANE FROM FRESH CASSAVA WASTEWATER IN A LIQUID PHASE ANAEROBIC BIO- DIGESTER.

A PROJECT REPORT SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES IN PATIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF DEGREE OF MASTER OF SCIENCE (M.Sc) DEGREE IN ENVIRONMENTAL BIOCHEMISTRY, DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF NIGERIA, NSUKKA.

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CERTIFICATION

This is to certify that Egwim, Chukwuemeka Franklyn, a postgraduate student with registration number PG/M.Sc/16/82221 in the Department of Biochemistry, Faculty of Biological Sciences, University of Nigeria, Nsukka has satisfactorily completed the requirement for course work and research for the degree of Masters of Science (M.Sc) in Biochemistry (Environmental). The work embodied in this report is original and has not been submitted in part or full for any other diploma or degree of this or any other University.

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Supervisor	Supervisor
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Head of Department	

DEDICATION

This work is dedicated to God Almighty and my family

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I give all glory to God Almighty for his abundant grace and strength throughout the period of this work. My sincere appreciation goes to my supervisors, Prof. I.N.E. Onwurah and Dr C. S. Ubani for their support, encouragement and advice that led to the success of this work.

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ABSTRACT

One of the greatest challenges facing the societies today is the increased emission of greenhouse gas from fossil fuel combustion, with consequent change in climatic conditions. Hence the need for cleaner and renewable energy source. This study was carried out to produce biomethane from fresh cassava wastewater in a liquid phase biodigester. Fresh cassava wastewater (FCWW) (1000 ml) was charged into 5 liter capacity fixed bed bioreactor and were subjected to anaerobic digestion for a period of 28 days at ambient temperature. The physicochemical parameters and the persistent organic compounds in the wastewater were determined before and after 28 days of biodigestion using standard methods. Isolation, characterization and determination of microbial population were carried-out at the end of biodigestion using plate count method. The main problem of biogas production from cassava waste effluents is the acid forming-bacteria that produces acids resulting in the decline in pH below 7 thus reducing the growth of methane forming bacteria in the biodigester. One of the methods to overcome this challenge as adopted in this work is the use of $Ca(OH)_2$ to regulate pH by sequestrating CO_2 which can be used in the production of economically important substances such as biocarbonic acid. After 28 days of biodigestion, the composition of flammable biogas were determined using a Bacharach combustible gas analyzer. The following composition of gases were obtained: CO_2 = 12%, CO = 8%, NO = 3%, H_2 = 3%, CH_4 = 74% indicating that FCWW is a good substrate for biogas generation. The microbial isolation and characterization after 28 days of digestion indicated the presence of both Gram positive and Gram negative bacteria (*Proteus, Vibrio, Bacillus, Staphylococcus, Escherichia and Salmonella*) and a microbial population of 5.5×10^8cfu. The fresh cassava wastewater had a pH of 5.70 which decreased to 3.01 after biodigestion. GC-MS analysis were conducted to determine the persistent organic compounds in the FCWW and the Sludge. The chromatogram for the FCWW showed 5 peaks with various organic compounds. The chromatograph for the Sludge showed 3 peaks and the presence of two organic compounds in each peak, indicating that anaerobic digestion is an effective means of bioremediation. The anaerobic digestion of cassava wastewater and other organic waste substrates in the production of biogas will contributes to proving the domestic energy need. This will also improve the quality of the environment by ridding the processing sites of pollution and reducing the emission of greenhouse gases.

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ABREVIATIONS

GC: Gas Chromatography

GC-MS: Gas Chromatography-Mass Spectrophotometer

FCWW: Fresh Cassava Wastewater

S: Sludge

BOD: Biochemical Oxygen Demand

COD: Chemical Oxygen Demand

TVC: Total Viable Counts

CAS: Chemical Abstract Series

RT: Retention Time

PK: Peak

POC: Persistent Organic compound

CHAPTER ONE

INTRODUCTION

Biogas technology is also known as anaerobic digestion (AD) technology is the use of biological processes in the absence of oxygen for the breakdown of organic matter and the stabilization of these material, by conversion to biogas and nearly stable residue (digestate) (Ngumah *et al*., 2013). Alessando Volta first discovered biogas in 1776, while Humphrey Davy was the first to pronounce the presence of combustible gas known as methane in the farm yard manure as early as 1800.

The anaerobic fermentation of organic materials has long been used to generate useful resources which have been harnessed for the use of mankind (Uri, 1992; US EPA, 2001). As early as the 18th century, anaerobic process of decomposing organic matter was known, and in the middle of the 19th century, it became clear that anaerobic bacteria are involved in the decomposition process, also called fermentation.

Anaerobic digestion provides some exciting possibilities to global concerns such as alternative energy production, handling human, animal, municipal and industrial wastes safely, controlling environmental pollution, and expanding food supplies (Uri, 1992; Ofoefule and Uzodinma, 2006).

As the demand for energy is increasing, the fossil based fuels become scarce and more expensive, and carbon dioxide emission levels become a greater concern. Biogas is a by-product of anaerobic fermentation and as a renewable energy source it has been recognized globally as a means of solving the problem of rising energy prices, waste treatment /management and creating sustainable development (Rao and Seenayya, 1994; Ofoefule and Uzodima, 2006). Biogas is a colorless, flammable gas produced via anaerobic digestion

(fermentation) of animal, plant, human, industrial and municipal waste to produce methane (50- 80%), Carbon dioxide (20-40%) and traces of other gases such as nitrogen, hydrogen, ammonia, hydrogen sulphide, water vapour etc. (Ofoefule and Ibeto, 2010). However, the composition of these mixture depends on the source of biological waste and management of digestion process (Ofomatah, 2011). The natural generation of biogas is an important part of biochemical reaction which takes place under anaerobic condition in the presence of highly pH sensitive microbiological catalyst that are mainly bacterial (Uzodinma and Ofoefule, 2009). Biogas production comprises of three biochemical process, which includes; hydrolysis, acidogenesis/acetogenesis, and methanogenesis (Nagamani and Ramasamy, 1999). Complex molecules (carbohydrate, protein, fats) are broken down into a broad spectrum of end products (.i.e. acetic acid, H_2/CO_2, monocarbon compounds and organic fatty acids larger than acetic) by fermentative bacterial (Uri, 1992; EPA, 2001)

Fatty acids longer than acetate are metabolized to acetate by obligate hydrogen-producing acetogenic bacteria (Ntengwe *et al*, 2010). Hydrogen and carbon dioxide can be converted to acetate by hydrogen oxidizing acetogen or methane by aceticlastic methanogens (methanogenesis), (Nagamani and Ramasamy, 1999). At pH 6.0-8.0 and ambient temperature between 28^0C-40^{o}C in a bioreactor or digester under anaerobic condition. (Ntengwe *et al*, 2010). Ntengwe *et al* (2010), reported that the production of biogas from biomass is dependent on the amount of acid formed which depends on the types of substrate (feedstock) used. (The biogas production rate was found to be different for different substrate or raw material).

Various efforts to increase the biogas production has been proposed and developed. Some patent provides methods of increasing the production of biogas using bacterial inoculums such as U.S. Patent No. 20080124775, U.S. Patent No. 20070062866, and U.S. Patent No.7560026 (Budiyono and Kusworo, 2012). Furthermore, implementation of biogas technology from cassava starch effluent has been investigated by many researchers (Manilal *et al*., 1990;

Anunputtikul *et al*., 2004). However, biogas production rate from cassava starch effluent is still very low unlike biogas production using poultry dung, grasses, swine dung, bambara nut, cow dung, sawdust etc. Research on the production of biogas using cassava waste water has not been thoroughly investigated. The most important consideration in biogas production from cassava effluent are nitrogen source to support the growth of the methane bacteria, pH control during biogas production to keep methane bacteria alive and the management of the digesteion process (Kossmann *et al*, 2008). Therefore, biogas technology is currently dominated by the efforts to improve concentration and retention time in the bio-digester in order to increase the rate of biogas production (Viswanath *et al*., 1992). The research project is expected to contribute toward enhancement of knowledge in biogas technology by finding new technology of enhancing biogas production.

1.1. Biogas

Biogas is a biological gas, an alternative and renewable energy source which originates from bacteria in the process of biodegradation (fermentation) of organic material (from plants, animals and sometimes human origins) under anaerobic (oxygen free) conditions (Sárvári *et al*., 2016).

1.1.2. Composition of Biogas

Biogas is composed of methane (CH_4) and carbondioxide (CO_2) (Cvetković *et al*., 2014). Depending on the source of the organic matter and the management of the anaerobic digestion process, such as pH, temperature, ionic strength or salinity, nutrients and inhibitory substrates, small amounts of other gases such as ammonia (NH_3), hydrogen sulfide (H_2S) and water vapour (H_2O) may be present (Ogejo *et al*., 2009). In general, biogas consists of 55-80% methane and 20-45% carbon dioxide (CO_2), with other gases such as hydrogen sulfide (H_2S)

0-3%, 0-1% hydrogen, nitrogen and ammonia (Uzodinma *et al*., 2011). It is also characterized based on its chemical and physical properties (Uri, 1992).

1.1.3 Physical Properties of Biogas

Depending on the composition, (biogas) a gas considerably lighter than air, is colourless, it produces twice as less energy by combustion with equal volume of natural gas. Biogas burns with an almost odourless blue flame with heat of combustion equivalent of 21.5MJ/M^3 (Ossai, 2012). Relative density of biogas compared to air of about 0.8 kg/m^3. Auto-ignition temperature in the range of 6500^{o}C – 7500^{o}C compared to petrol 5000^{o}C – 6000^{o}C and, 8000^{o}C – 8500^{o}C. Like any pure gas, the characteristic properties of biogas are dependent on pressure and temperature. They are also affected by the moisture content (Uri, 1992; Ogejo *et al*., 2009).

1.1.4. Purification of Biogas/ Biogas Scrubbing

Biogas scrubbing involves the separation of unwanted components such as hydrogen sulphide (H_2S) and carbon (IV) oxide (CO_2) that can combine with water vapour to form acids. These acids can cause corrosion of metal parts. Again, these gases do not support combustion.

Hydrogen sulphide and carbon (IV) oxide can be separated from biogas by passing the biogas through concentrated solution of sodium hydroxide (Dioha *et al*., 2003). Gas chromatography can also be used as a better method (Dioha *et al*., 2003) and also the use of calcium hydroxide (quicklime), as shown in the chemical equation below.

By using NaOH

$$2NaOH + CO_2 \longrightarrow Na_2CO_3 + H_2O$$

$$Na_2CO_3 + excess\ CO_2 \longrightarrow 2NaHCO_3(s)\ ppt.$$

By using $Ca(OH)_2$

$$Ca(OH)_2 + CO_2 \longrightarrow CaCO_3 + H_2O\ ppt$$

Ammonia is also separated or retained by charcoal; while hydrogen sulfide (H_2S) can be removed by passing the biogas over iron fillings or iron (III) oxide mixed with wood shavings, as shown in the equation below.

$$Fe_2O_3 + 3H_2S \longrightarrow Fe_2S_3 + 3H_2O$$

$$2Fe_2S_3 + 3O_2 \longrightarrow 2Fe_2O_3 + 3S_2$$

These purification gives a pure biogas (biomethane) which burn with a high production of heat. (Young, 1982; Ogejo, *et al*., 2009).

1.2.0 Methane, a Component of Biogas

Methane is a simple chemical molecule, with the chemical formula CH_4. It is the principal component of biogas (natural gas), (Reay *et al*., 2011). Methane occurs naturally as a component of natural gas, it is odourless, lighter than air and highly flammable. Methane can form mixtures with air that are explosive at concentration 5-15%. Methane is not toxic, but can cause death due to asphyxiation by displacing oxygen in confined environments or spaces. The heating value of pure methane is 1,000 BTU per cubic foot (Ogejo *et a*l., 2009). Additionally, methane is considered a powerful greenhouse gas that can remain in the atmosphere for up to 15 years, with a global warming potential (GWP) of 30. (This means that every kilogramme of methane emitted to the atmosphere has the equivalent forcing effect on the earth's climate of 30 times that of carbon dioxide over a two-year period) (Ogejo *et al*., 2009). Other gases, such as CO_2, H_2S and NH_3, in biogas are not useful because CO_2 limits the combustion power of methane and lead to bad quality of flames. In addition, H_2S and NH_3 are toxic, corrosive and have an irritating smell (Uzodinma *et al*., 2011).

1.2.1 Digesters/ Culture plant for Biogas Production

Biogas plant is also called a bioreactor or a biodigester. It is an air tight container in which organic wastes and waste water are fermented by bacteria in the absence of oxygen to produce methane. It contains a system for gas collection and storage (Ukonu, 2011). Digesters are made of concrete, steel, brick or plastic. They look like silos, troughs, basins or ponds and may be placed under ground or on the surface. Metal digesters are made with iron (steel) to avoid poisoning of the bacteria during the digestion.

The modes of operation of the digestion include batch, semi-continuous and continuous operation. In batch culture operation system, the biodigestor is loaded with the substrate or organic material and allowing it to digest. Once the digestion is complete, the effluent is removed and the process repeated. One technical shortcoming of batch system is the risk of blockage of the leaching process caused by clogging of the perforated floor. This problem is alleviated by mixing the feedstock with bulking material (e.g. wood chips) and by limiting the thickness of the fermenting wastes in order to limit compaction (Vandevivere *et al*., 2003). Although batch systems have not succeeded in taking a substantial market share, especially in more developed countries, the system is attractive to developing countries. The reason is that the process offers several advantages as it does not require fine shredding of waste, sophisticated mixing or agitation equipments, or expensive, high-pressure vessels, which consequently lower the investment costs (Vandevivere *et al*., 2003; Koppar and Pullammanappallil, 2008).

For semi-continuous operation, the digester is fed on a more regular basis usually once or twice daily. The digested organic matter is also removed at the same interval.

In continuous operation, the organic material is fed constantly into the digester. The material moves mechanically or by the force of the new feed pushing out digested material. This kind

of operation is most suitable for large scale operations. There is steady availability of usable biogas (Ofomatah, 2011).

1.2.2. Types of Bio Digester

Digesters are made of concrete, steel, brick or plastic. Several different types of anaerobic bioreactors are used worldwide for municipal, industrial-food, and agricultural waste treatment (Ogejo *et al*., 2009).

The two commonly used biodigesters are: floating drum digester and fixed dome digester

Other types of bio digester include: Bag/balloon plant, Plug flow plants, Ferro cement plants and prototype bioreactor plant.

1.2.3. Floating Drum Digester

The floating drum digester consist of a cylindrical or dome shaped digester and a moving, floating gas holder or drum. The gas holder floats either in the fermenting slurry or in a separate water jacket. This type of digester is popularly known as the gobar gas plant. The gas is collected in the gas drum, which rises or moves down, according to the amount of gas stored. The gas drum is prevented from tilting by a guiding frame. The advantages are the simple and easily understood operation (Ukonu, 2011). The volume of stored gas is directly visible. The gas pressure is constant, determined by the weight of the gas holder. The construction is relatively easy, construction mistakes do not lead to major problems in operation and gas yield. The disadvantages are high material costs of the steel drum, the susceptibility of steel parts to corrosion. Because of this, floating drum plants have a shorter life span than fixed dome plant (Uri, 1992). The floating drum plants were mainly built in India

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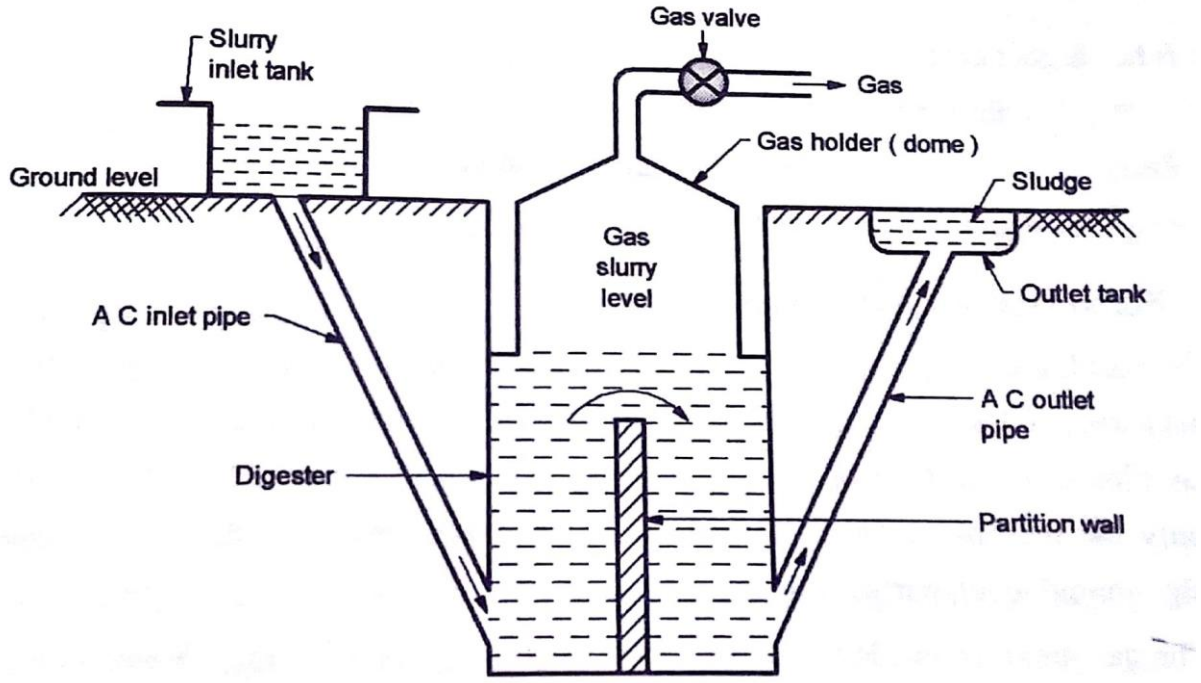

Fig 1: Floating drum biodigester

(Source: Ofoefule and Uzodima, 2009).

1.2.3 Fixed Dome Digester

This consists of an underground airtight pit constructed with bricks, stone or concrete with a dome shape cover. The fixed dome digester is a popular digester used in most places such as Nepal, India and China. In this type, the fermentation chamber and gas holder are combined as one unit (Iloeje, 1998). Fixed-dome plant is relatively low in its cost of construction because of the absence of moving parts and rusting steel parts (Ukonu, 2011). If well-constructed, fixed-dome plant have a long life span. The underground construction saves space and protects the reactor from temperature changes. The construction also provides opportunities for skilled local employment. The disadvantages of fixed-dome plant are mainly the frequent problems with gas-tightness of the brickwork gas holder (a small crack in the upper brickwork can cause heavy losses of biogas); fixed-dome plant are, therefore, recommended only where

construction can be supervised by experienced biogas technicians. The gas pressure fluctuates substantially depending on the volume of the stored gas (Omer and Fadalla, 2003).

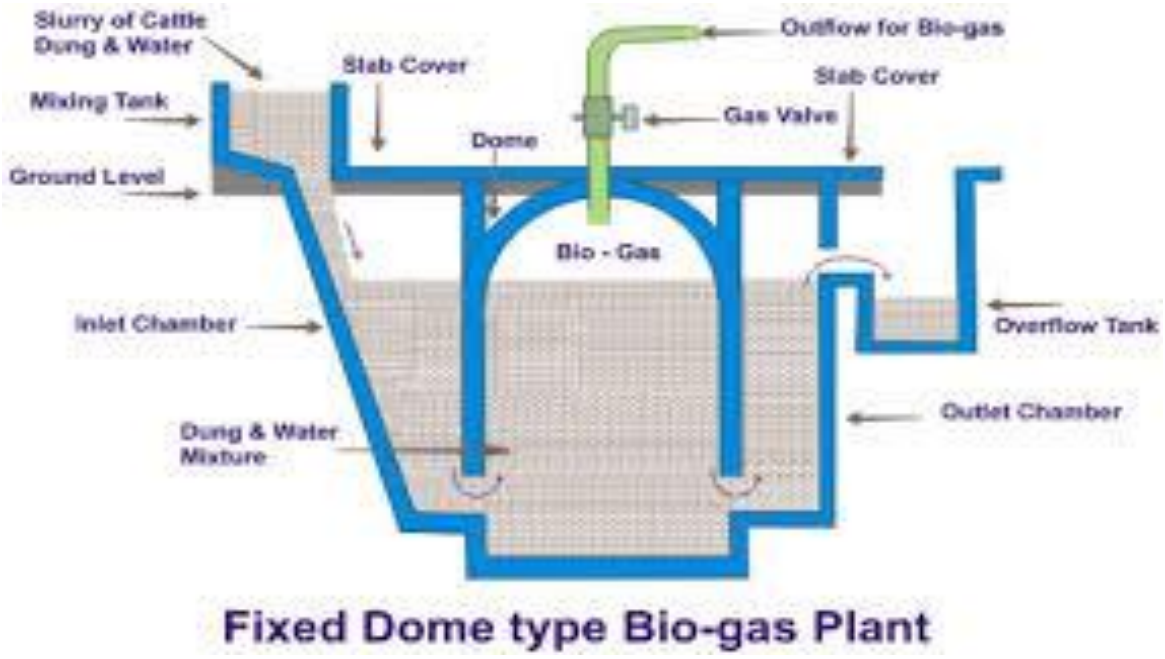

Fig 2: Fixed Dome Type Bio-gas Plant

(Source: GATE and GTZ, 2007)

1.3.0. Feed Stock Substrate (Cassava waste water)

Cassava (*Manihot esculenta*) also known as manioc is a woody, perennial shrub of the Euphorbiaceae (spurge family) which grows from 1-5 m (9ft) in height, has large tuberous roots with leaves deeply divided into 3–7 lobes. The shrub is often grown as an annual, and propagated from stem cuttings after tubers have been harvested. It is one of agriculture's oldest crops originating from South America but today spread all over the worlds tropical and subtropical regions (FAO, 2013). Because of its tolerance against drought and for marginal soils it is commonly grown by poor farmers in developing countries, and today millions of small-scale farmers in more than 100 countries grow cassava (FAO, 2013) and it is the third most important source of calories in the tropics after rice and maize (FAO, 2008). Cassava is a

truly versatile crop and in fact the whole plant can be used. The roots, which are the main product, is somewhat dark brown in colour and grows up to 2 feet long, can be processed in to a variety of food products and animal fodder, but they can also be used for industrial purposes. Such as for making noodles and cakes, for frying meat and fish, in textiles, pharmaceuticals, cardboard, monosodium glutamate (MSG), glucose, maltose and plywood; these are all just a few examples of how the roots can be used. The leaves, which contain up to 25 % protein, can be used as animal fodder and the stem as firewood (FAO, 2013). However, since the whole plant contains high levels of cyanide compounds including linamarin (cyanogenic glucoside) and hydrocyanic acid it is always processed in some way such as roasting, soaking, or fermentation before consumption to avoid intoxication (FAO, 2013).

The composition of cassava roots is 60-65 % moisture, 20-31 % carbohydrate (of which 60-65 %is starch), 0.2-0.6 % ether extracts, 1-2 % crude protein and comparatively low concentrations of vitamins and minerals (Tewe, 2004). The peel of the cassava generally has higher concentrations of protein, fat, fibre and minerals (Montagnac *et al*., 2009), but also cyanogenic glycosides (Tewe, 2004). The crop is highly efficient in producing starch, cassava starch is one of the best fermentable substrate for bioethanol production and can also be used for biogas production although not much research has been done on this part.

Cassava waste substrate (fresh) is obtained from a mill where cassava are milled and processed for human consumption. Cassava waste water is of two types; cassava *sievates* (waste product from *garri* processing) and cassava *offal* (waste from *fufu* production). Cassava *sievates* are the waste substrate that are removed from the mill as the cassava is being milled. They have a high concentration of starch and high organic load because they have not undergone fermentation (sun *et al*., 2012). They are very fresh and contains the normal microbial floral.

1.3.1. Microbial Floral in Cassava waste Water Substrate

The experiment on the micro floral and total cell population of microorganism in cassava waste water indicated the presence non lactose fermenters, positive with indole and urease *proteus mirabelis, g*lucose fermenting microorganisms: *Vibro spp, Bacillus subtillis*, non-mannitol fermenters and catalase positive microorganism: *Staphylococcus spp, salmonella spp* and *Bacillus licheniformis* (Etinosa *et al*., 2015) . Furthermore, it is the nature of the substrate that determines the types and extent of the fermentative bacteria in the reactor (Ogejo *et al*., 2009).

1.4.0. Biochemistry of Biogas Production

Anaerobic digestion is a complex biochemical reaction carried out in a number of steps by several types of microorganisms that require no oxygen to survive. In this process, the organic waste decays in the absence of oxygen to produce biogas whose main components are methane and carbon (IV) oxide gas. The quantity of biogas produced varies with the amount and type of organic waste fed to the digester (Ofomatah, 2011). Changes in environmental conditions can affect this steps and result in the build-up of intermediates which may inhibit the overall process.

The reaction take place in the following steps:

1. Hydrolysis

$$(C_6H_{10}O_5)_n + n(H_2O) \rightarrow n(C_6H_{12}O_6)$$

Complex carbohydrate Simple Sugar

2. Acidification

$$C_6H_{12}O_6 \xrightarrow{acetobacter} 3CH_3COOH$$

3 Methaniation

$$3CH_3COOH \xrightarrow{methanogen} CH_4\,(g) + CO_2\,(g)$$

The third step can be obtained in two ways

$$CO_2 + 4H_2 \xrightarrow{reduction} CH_4 + 2H_2O$$

$$3CH_3COOH \xrightarrow{Methanogen} CH_4 + CO_2 + Other\ gas + Residue$$

(Ofomatah, 2011).

1.4.1. Hydrolysis

In this stage, complex organic materials consisting mainly of carbohydrate, lipids and proteins are solubilized into simpler ones with the help of extra-cellular enzymes released by hydrolytic bacteria in the presence of water. This stage is also known as polymer breakdown stage. The monomers that are formed are easily available to any acid- producing bacteria. Proteins are converted into amino acids, carbohydrates into simple sugars and fats into long chain fatty acids. The breakdown of cellulose, lignin and other complex compounds to simple monomers is not so easy and can be the rate-limiting step in anaerobic digestion (NAS, 1997)). The rate of hydrolysis is dependent on substrate and bacterial concentrations as well as other parameters such as temperature and pH.

$$(C_6H_{10}O_6)_n + nH_2O \longrightarrow (C_6H_{12}O_6)\ Hydrolysis$$

1.4.2. Acidiogenesis

Acidification is a fermentative process where acid-forming bacteria, also known as acidogens, convert the products of hydrolysis into simple organic acids. The principal acids produced into this process are acetic acid, propionic acid, butyric acid and ethanol (Ukonu, 2011).

1.4.3. Acetogenesis

This is third stage of anaerobic digestion. In this stage simple molecules created through the acidogenesis phase are further digested by acetogens to produce largely acetic acids, as well as carbon dioxide and hydrogen. This pathway of single acid forming stage aims to reduce biochemical oxygen demand (BOD) and chemical oxygen demand (COD) values. Generally, high pH values inhibit the growth rate of acetogenic bacteria. Acetogenic bacteria are also known as obligatory hydrogen-producing acetogens (OHPA) as they exhibit a metabolism of proton reduction and are mandatory dependent on hydrogen removal (Arsova, 2010). Acetogenesis provides the two main substrate for the last step in the methanogenic conversion of organic materials, namely hydrogen and acetate. Both the acidogenesis and acetogenesis produce the methanogenic substrate, acetate, hydrogen and carbondioxide. The important distinction between these two stages is that the fermentative bacteria have the possibility of using various electron acceptors for the disposal of electrons. The acetogenesis is an obligate proton reducer and can utilize only protons as electron acceptors and only when the hydrogen concentration is low. At very low hydrogen concentration, however, methanogenesis from hydrogen and carbon dioxide becomes unfavourable (Ukonu, 2011).

$$nC_6H_{12}O_6 \xrightarrow{acetobacter} nCH_3COOH$$

1.4.4. Methane Formation/ Methanogenesis

This is the terminal stage of anaerobic digestion. In this stage, the principle acids produced in stages 1, 2 and 3 are processed by methanogenic bacteria to produce methane (CH_4) and other products such as NH_3, H_2S, CO etc. Methane, the main component in biogas, is produced through a syntrophic relationship between acetate-oxidizing bacteria and hydrogenutilizing methanogens (Arsova, 2010). The growth rate of the methanogens is generally slower than that of acetobacter in stage 2 or hydrolyses in stage 1.

$$3CH_3COOH \xrightarrow{methanogens} CH_4(g) + CO_2(g)$$

The third stage can be achieved in two ways

$$CO_2 + 4H_2 \xrightarrow{reduction} CH_4 + 2H_2O$$

$$3CH_3COOH \xrightarrow{methanogens} CH_4 + CO_2 + Other\ gases + Residue$$

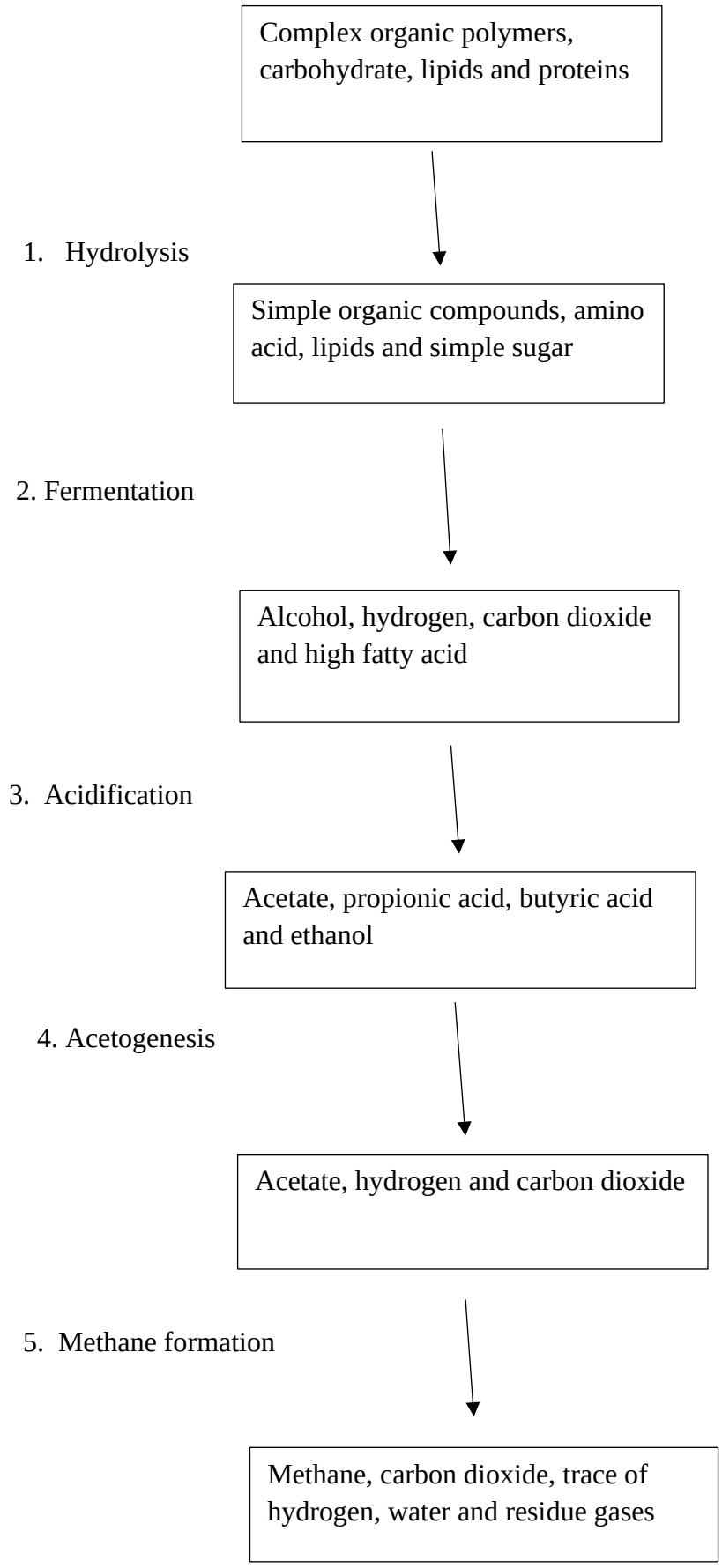

Fig 3: Stages of biomethanation production (Ofomatah, 2011).

The methanogens utilize acetic acid, methanol or carbon (iv) oxide and hydrogen to produce methane (CH_4). Acetic acid or the acetates are the most important compounds used in the production of methane as the very major product in the anaerobic process. The remaining methane may come from CO_2 and H_2. The methanogenic bacteria also regulate and neutralize the pH of the digester slurry by converting volatile fatty acids into methane and other gases. The conversion of hydrogen into methane helps to reduce the partial pressure of the hydrogen in the digester slurry that is beneficial to the activity of the acetogenic bacteria (Garba, 1999). If the methanogens fail to function effectively, there will be little or no methane produced from the digester and so waste stabilization will not be achieved. The organic compounds in the waste will only be converted to volatile free fatty acids that can cause further pollution if discharged into a river, stream or ocean or on land.

There are four main groups of bacteria involved in the digestion of wastes namely: Hydrolytic and fermentative bacteria, acetate and hydrogen producing, Methane forming bacteria and Hydrogen utilizing bacteria (Niemi *et al*., 2009).

1.4.5. Kinetics of Anaerobic Fermentation

Several kinetic models have been developed to describe the anaerobic fermentation process (Nagamani and Ramasamy, 1999). The mechanism of the reaction is first; the fermentative action of acid forming microbes on the substrate (S) to produce alcohol, hydrogen (H_2), acids and carbon dioxide (CO_2) and Second the action of methane-forming bacteria (methanogensis) to produce methane (CH_4) and CO_2 as indicated below (where SE is the intermediate product, E is the enzyme, K_1 and K_2 are rate constant (s^{-1}).

$$S + E \overset{K1}{\rightarrow} SE \overset{K2}{\rightarrow} E + CH_4 + H_2 + CO_2 \quad ----------(1)$$

The rate of reaction is assumed to depend on the concentration of SE, temperature, pH, and the geometric of the bioreactor. The breakdown of S has been reported to follow the Michaelis Menten mechanism (Ukonu, 2011). The rate of reaction of S can be given as below, which yield first order kinetics

$$R_1 = K_1[S][E] = R_{-1} + R_2 = K_{-1}[SE] + K_2[SE] \quad -------- (2)$$

$$\frac{ds}{dt} = K_2SE/(K_{-1} + K_2)/K_1 + S = K_2SE/KM + S \quad -------- (3)$$

$$\frac{S_1}{S_2} = K_2E/KM + S\,Xdt = Kdt \quad ---------- (4)$$

Where T is the time, K_{-1} is the rate constant (S_{-1}), R_1, R_2 R_{-1} are the rates of reaction ($Kmols^{-1}$) R_1, R_2 are the initial and final substrate concentration ($Kmols^{-1}$), Km is a constant. Equation (4) can be used to evaluate the first order kinetic of biogas production at a given conditions of temperature and pH (Ntengwe *et al*., 2010). Hashimoto *etal* (1980) developed an equation, which attempts to describe kinetics of methane fermentation in terms of several parameters. According to this equation, given below for a given loading rate so/q daily volume of methane per volume of digester depended on the biodegradability of the material (BO) and kinetic parameters μm and k.

$$Yv = (BO.So/q)\{1 - (k/q\,\mu m_{-1} + k)\} \quad --------(5)$$

Where

Y_v = is volumetric methane production rate, $(CH_4)^{-1}$ digester d^{-1}

S_o is influent total volatile solid (VS) concentration (gl^{-1}).

B_o is ultimate methane yield, CH_4 (g^{-1} VS) added.

q Is hydraulic retention time d^{-1}

μm is maximum specific growth of microorganism's d^{-1}

K. is kinetic parameter, dimensionless.

1.5.0. Factors That Influence Biogas Production

Biogas production is a microbial process and as such, it requires the maintenance of suitable growth conditions for biogas producing bacteria. To maintain a viable micro-organism and hence maximum yield of methane, the following factors must be considered (Ofomatah, 2011):

1.5.1. Temperature

For maximum efficiency, a suitable temperature is necessary. The two kinds of bacteria that will bring about this production operate at two different temperatures: Mesophilic and Thermophilic ranges. Any chosen environment for the digestion must maintain one of these temperature ranges. The methanogens are inactive in extreme high and low temperatures. The optimum temperature is usually 40^{o}C for the Mesophilic range while that for the thermophilic fermentation is 55^{o}C. When the temperature of the ambient goes down to 10^{o}C, gas production virtually stops. Useful gas production takes place at the mesophilic range between 25^{o}C and 40^{o}C (Kardos *et al*., 2011; Gou *et al*., 2014) and 45^{o}C to 55^{o}C for the thermophilic range. Different bacteria dominate at different temperatures. Imbalance between different bacteria groups may develop, causing methane production to be reduced and other gases to be given off. So it is important to maintain the temperature within these range. Higher temperatures shorten the retention time but can lead to increased rate of biogas production.

1.5.2. pH

pH is a measure of the acidity or alkalinity of a solution. The acetogens and methanogens are easily affected by pH. Optimum biogas production is achieved when the PH value in the digester is between 6.5 and 7.5 (Garba *et al*., 1996).

The pH is a function of the bicarbonate alkalinity, the CO_2 partial pressure and the volatile acids concentration as well as the retention time. Ofomatah, (2011) reported that biogas production would always continue as long as the digester slurry pH is maintained within a range of 6.6 to 7.6 with optimum range between 7.0 and 7.2. Below 6.2, the bacteria become inactive. The methanogens are very sensitive to pH and do not survive below a value of 6.5. Later, as the digestion continues, the concentration of ammonia rises due to nitrogen which can increase pH value above 8. When the methane production level is stabilized, the pH range remains buffered from 7.2 to 8.2 (Sustainable Development, 1997). A pH value higher than 8.5 will show toxic effect.

1.5.3. Nature of Feedstock (substrate)

The characteristics of solid wastes determine the successful anaerobic digestion process (e.g. high biogas production potential and degradability). All organic waste materials except mineral oil and lignin are suitable substrates for the production of biogas. Some organic materials such as animal manure, vegetable matter and the effluents of some industries are more easily digested. It has been found that dry vegetable matter produces more gas than fresh green vegetable matter (Ossai, 2012).

The quality of the substrate is also affected by animal diet, manure handling, and storage method (Uzodimnma and Ofoefule, 2009). Substrate from animal fed with higher energy feed

(e.g. Grain-based diets) has the potential to yield more methane gas compared to substrate from animals fed with roughage diet (Ramasamy *et al*., 1990).

1.5.4. Carbon/ Nitrogen Ratio

The composition of waste also determines the relative amounts of organic carbon and nitrogen present in the waste substrate (C/N ratio). For optimum biogas production, it is important to mix various materials in accordance with the carbon- nitrogen ratio requirement for fermentation. A carbon – nitrogen ratio of 20:1 to 30: 1 is considered adequate for anaerobic digestion, though a C/N ratio of 30:1 is optimum. It should not exceed 35:1 (Garba *et al*., 1996). A solid waste substrate with high C/N ratio is not suitable for bacterial growth due to deficiency of nitrogen which limits the growth and activity of bacteria. As a result the gas production rate and solid degradability will be low. On the other hand, if the C/N ratio is very low, the degradation process leads to ammonia accumulation which is toxic to the bacteria. High nitrogen wastes such as human and animal urine can be added to high carbon content cellulosic waste such as grass, straw and bagasse to bring the ratio closer to optimum (Ofomatah, 2011).

1.5.5. Agitation

The production of biogas requires stirring from time to time to enhance contact between the micro-organisms and the organic waste and this increases reaction rate. Biological activities are increased when digester fluid are mixed to provide homogenous temperature and nutrient condition throughout the digester. If the sludge is left without stirring, scum will form at the top and this can lead to blockage of the digester. Manual stirring device is very suitable for this purpose (Ofomatah, 2011).

1.5.6. Absolute Anaerobic Environment

All microbes that play important role in biogas production are strictly anaerobic. They include acid producing bacteria and methane producing bacteria. The latter are so sensitive to oxygen that digestion could be inhibited by even the slightest trace of oxygen (Ofomatah, 2011).

1.5.7. Loading Rate

The ability of a digester to convert organic material into methane is related to its loading rate. Loading rate is commonly defined as the amount of volatile solids fed to the digester per day per unit volume of the digester. Volatile solid is the measure of the amount of digestible organic material in a feedback. In general, materials with high volatile-matter content produce more biogas if digested properly (Sorathia *et al*., 2012).

1.5.8. Design of Digester

Floating gas holder type and fixed dome type are the two first designs that are been widely employed, though economics use of the model and their suitability are well documented

1.5.9. Redox potential

In the anaerobic digester, low redox potential is necessary. Methanogenic archaea need redox potential between -300 and -330 mV for the optimum performance. Redox potential can increase up to 0 mV in the digester; however it should be kept in the optimum range. To achieve that, no oxidizing agents should be added to the digester, such as oxygen, nitrate, nitrite or sulphate (Ukonu, 2011).

1.6.0. Inhibitory Factors Affecting Biogas Production

Inhibition in anaerobic digestion process by the presence of toxic substances can occur to varying degrees, causing upset of biogas production and organic removal or even digester failure (Noraini *et al*., 2017). These kinds of substances can be found as components of the

feeding substrate (organic solid waste) or as by-products of the metabolic activities of bacteria consortium in the reactor. Publications on anaerobic digestion show a wide variation in the inhibition/toxicity levels for most substances. The main reason for these variations is the significant influence by microbiological mechanisms such as acclimation, antagonism, and synergism (Chen *et al*., 2008). Acclimation is the ability of microorganism to rearrange their metabolic resources to overcome the metabolic block produced by the inhibitory or toxic substances when the concentrations of these substances are slowly increased within the environment. Antagonism is defined as a reduction of the toxic effect of one substance by the presence of another, whereas synergism is an increase in the toxic effect of one substance by the presence of another. Several substances with inhibitory/toxic potential to anaerobic digestion, such as ammonia, sulfide, light metal ions, heavy metals and organic substances and biotics (bacitracin, flavourycin, lasalocid, monensin, spriamcin, etc.) and detergents used in livestock husbanding have been shown to exhibit inhibiting effect on the process of bio methane production.

Also carbon nitrogen ratio is a major inhibitory factor in biomethane production, Microorganisms need both nitrogen and carbon for assimilation into their cell structures. Various experiments have shown that the metabolic activity of methanogenic bacteria can be optimized at C/N ratio of approximately 2-5, where by the optimum point varies from case to case, depending on the nature of the substrate. Low nitrogen concentration may inhibit the process of fermentation. Noticeably, inhibition occurs at a nitrogen concentration of approximately 1,700mg ammonium-nitrogen (NH_4-N) per liter substrate, nevertheless, with time, the methanogens are capable of adapting to NH_4-N concentrations in the range of 5000-7000mg/l substrate. The main prerequisite being that the ammonia level (NH_3) does not exceed 200-300mg NH_3-N per liter substrate. The rate of ammonia dissolution in water depends on the process temperature and pH value of the slurry (Maduekeh *et al*., 2014).

1.7.0. Residues from Biogas Production

The residue from biogas production is what remains after anaerobic digestion of the waste has been completed. The residue is a high quality organic fertilizer containing expired bacteria bodies, undigested or partially digested organic matter (STP, 2003). Analysis of the residue shows that it contains double the concentration per weight of nitrogen, phosphorous, potassium and minerals that were in the manure fed originally to the digester (Dioha *et al*., 2003). This is possible because only carbon, hydrogen and oxygen were elements removed in the process of the digestion.

1.7.1. Advantages of Biogas Technology

Anaerobic fermentation/ biogas technology provides some exciting possibilities and solutions to such global concerns as alternative energy production, handling human, animal, municipal and industrial wastes safely, controlling environmental pollution, and expanding food supplies (Schröder *et al*., 2008; Rao *et al*., 2010). The usage of biogas as a renewable energy source has great potential to minimize the emission of methane gas into environment (Cvetković *et al*., 2014). Biogas has great potential to reduce global climate change since, the greenhouse effect for methane is 23 times higher than that of carbon dioxide (Gerlach *et al*., 2013). The recovery of this significant energy by anaerobic treatment helps to reduce fossil fuel and greenhouse gases (GHG) (Gupta *et al*., 2012). Biogas production has been paid close attention because of its potential as renewable and versatile energy source for heat and electricity generation, and transportation fuel (Lagerkvist *et al*., 2012).

1.8.0 Rationale of Study

A large mass of waste is produced in Nigeria. These wastes ranging from agro, domestic and industrial wastes are littered all over the towns and cities of the country. A lot of these waste

are dropped or washed into streams and rivers. As a result, they cause both land (soil) and water pollution.

As an agricultural country, Nigeria has abundance of biomass wastes such agricultural wastes include the cassava starch waste water. The production of starch commonly named tapioca and flour from cassava roots generates big amounts of high organic content wastewater which if not treated has a big negative impact on the environment causing air pollution and the worsening of soil fertility through the depletion of organic matter.

Cancado *et al* (2006) reported that air pollution from waste substrate causes damage to the respiratory system, leading to an increase in respiratory hospital admissions. Pathogenic micro-organisms that feed on these wastes are also agents of various diseases. However, anaerobic digestion of waste substrate will help in general environmental sanitation of the country and reduce land, water and air pollution from waste effluent. It will also reduce respiratory sicknesses associated with the offensive smell of cassava waste water substrate.

Production of biogas from cassava waste water could reduce the great pressure on the use of wood since biogas can be used for cooking at home. Also reduces the effect and emission of greenhouse gases thereby mitigating the effect of global warming, ozone layer depletion and greenhouse gas effect.

1.9.0. Aim and Objectives of the Study

The aim of this project was to produce biomethane from fresh cassava wastewater.

Specific Objectives of the Study

i. To determine the physicochemical properties of the fresh and fermented cassava wastewater.

ii. To produce biomethane from fresh cassava wastewater from the processing mill using a fixed bed biodigester.

iii. To determine the microbial population after biodigestion.

iv. To isolate and characterize the microorganism that convert the cassava wastewater to biomethane

v. To identify the persistent organic pollutant in the cassava wastewater substrate before and after biodigestion using GC-MS method.

CHAPTER 2

MATERIALS AND METHOD

2.1 Materials

2.1.1. Collection of Samples

Fresh cassava wastewater substrate were obtained from a cassava mill located at Ogurugu road in Nsukka, Enugu State of Nigeria.

2.1.2. Reagents and Chemicals

All the chemicals used in the research were of analytical grade and were obtained from from Merck (Germany) or BDH chemicals Ltd (poole England). These includes : Catalyst mixture (Mixture of 20g potassium sulphate, 1g copper sulphate and 0.1g selenium powder), concentrated tetraoxosulphate (VI) acid, (conc. H_2SO_4), distilled water, 40% NaOH, Boric acid indicator solution, NaF and 1ml of diphenylamine indicator, Hydrochloric acid, A.C.S grade, SP.gr.1.19., Zinc oxide, C.P grade, Potassium hydroxide, pallets A.C.S. grade, Sulphuric acid, A.C.S, grade, SP.gr.1.84, Sodium molybdate, reagent gradek, Potassium dhydrogen phosphate, A.C.S grade, Drt at 101®C, for 2hours before use, Standard potassium heptaoxochromate (VI) $K_2Cr_2O_7$, solution, 0.125M, Ag_2SO_4- H_2SO_4 solution, Mercury (II) sulphate, Sulphamic acid, Standard Iron (II) ammonium sulphate (A.R) solution, The sterile glucose enriched molten agar.

2.1.3. Equipment/Apparatus

Bioreactor (prototype) of 2000ml capacity constructed at the University of Calabar and mounted at National Centre for Energy Research and Development University of of Nigeria, Nsukka were used.

Combustible BACHARACH gas analyzer of model number PCA2, made in USA; Mercury in glass thermometer (0-100OC); pH meter (SEARCHTECH) of model number PHS-3C, made in USA; Gas burner; incubator, Micro-Kjedahl digestion flask (500ml capacity) (Make: Barloworld U.K, model Fk 500/3l) Ohaus weighing balance (0.001g accuracy, model AR3130, Made in England), micro Kjedahl distillation unit (make: Barloworld, UK model 734205) 100 ml conical flask. (Receiver flask), Soxhlet extraction apparatus, porcelain crucibles, weighing balance. 50 ml Burette (Pyrex, England), Pipette (Pyrex, England), (Pyrex, England), Petri dishes, Spatula, Light Microscope, muffle furnace (make: Vecstar, model LF3, made in U.K), Desicator (make: Vecstar, model LF3, made in U.K), Vijcor crucible- 50ml capacity, Watch glass- 75mm diameter, Electric hot plate, rheostat control or 3- step dial control, Glass funnel, short stem-50mm diameter, Filter paper whatman No.42.90 mm or equivalent, Wash bottle, 1litter with cork or asbestos covered neck, Volumetric flasks, glass stopper, 50ml, 100ml, 250ml and 500ml, Transfer pipettes 10 2.5 .10, and 25ml., Pipettes, mohr types, 10ml graduated in 0.1ml. subdivision, Spectrophotometer, meeting the requirements of A.O.C.S method Ce 13c-50, Cuvettes, meeting requirement of A.O.C.S. method Ce 13e-50, (Reflux apparatus consisting of 250,300 or 400 ml Erlenmeyer flasks with quick fit ground-glass necks to which are fitted sizeable Liebig condensers), Hot plate or heating mantle of the Gallenkamp type to insure adequate boiling of the contents of the reflux flasks, Winchester bottle (2.5 litres), GC/MS-QP2010 Agilent Plus, Instrument name: Atomic Absorption Spectroscopy (AA),ASC,GFA, Model Name: AA-7000, ROM Version: 1.01, S/N A30664700709

Other equipment used includes test-tubes, beakers, conical flasks, syringes; measuring cylinder (Pyrex); crucible; Buchner funnel; muffle furnace; hose pipe; water trough; graduated (transparent) bucket.

2.2. Methods

2.2.1. Preparation of Wastewater

Varied quantities of the fresh cassava waste water were collected and sieved using a cloth sieve of 0.02 mm to remove unwanted particles. Fresh waste water substrate was collected for analysis before charging into a 1000ml bioreactor. The fresh water was stored in a refrigerator at $4^{o}C$.

. **Fig**

4: Experimental set up for the production of biomethane.

2.2.2. Experimental Design

A fixed bed bioreactor of 5-liter capacity constructed at the National Centre for Energy Research and Development, University of Nigeria, Nsukka was used. Fresh cassava wastewater of 1000 ml was collected and loaded into the bioreactor and allowed to ferment for a period of 28days at ambient temperature. The digester contents were stirred periodically to ensure homogenous dispersion of the chemicals in the mixture. The bioreactor tap was opened at 4 days interval. CO_2 produced will be collected in a gas jar containing aqueous $Ca(OH)_2$ which is expected to turn milky. This will reduce the concentration of CO_2 in the anaerobic tank, regulating pH and favouring methanogenesis. Biomethane produced will be collected in a gas cylinder together with other lighter gases. The residue (Sludge) left after 28 days of fermentation was analysed for the physicochemical content, persistent organic compound and microbial content**.**

2.2.3. Nitrogen/Crude Protein Determination of Cassava Waste Water Substrate

The micro-Kjedahl method as described in Pearson (1976) was used. This method involves the estimation of the total nitrogen in the waste and the conversion of the nitrogen to protein with the assumption that all the nitrogen in the waste is present as protein. Using a conversion factor of 6.25, the percentage protein in the waste was calculated

% crude protein = % Nitrogen x 6.25.

Digestion of Fresh Cassava Wastewater

1g of the ground waste sample was weighed into the Kjedahl digestion flask. 1g of the catalyst mixture was weighed and added into the flask. 15 ml of conc. H_2SO_4 was also added. Heating was carried out cautiously on a digestion rack in a fume cupboard until a greenish clear solution

appeared. The digest was allowed to clear for about 30 minutes. It was further heated for more 30 minutes and allowed to cool. 10 ml of distilled water was added to avoid caking. Then the digest was transferred with several washings into a 100 ml volumetric flask and made up to the mark with distilled water.

Distillation

Apparatus used: micro Kjedahl distillation unit (make: Barloworld, UK model 734205) 100 ml conical flask. (Receiver flask)

Reagents used: 40% NaOH, Boric acid indicator solution

Procedure

A 10 ml aliquout was collected from the digest and put in the flask. A 100ml receiver flask containing 5ml boric acid indicator solution was placed under the condenser of the distillation apparatus so that the tip was 2cm inside the indicator. 10ml of 40% NaOH solution was added to the digested sample through a funnel stop cork. The distillation commenced by closing the steam jet arm of the distillation apparatus. The distillate was collected in the receiver flask (35 ml).

Titration

Titration was carried out with 0.01M standard HCl to first pink colour.

% Nitrogen =

Where M= molarity of std HCl

Percentage (%) crude protein = % N x 6.25

Equation of the Reaction

$$N\ \ in\ waste + conc.H_2SO_4 \xrightarrow{catalyst} (NH_4)_2SO_4$$

$$(NH_4)_2SO_4 + 2NaOH \longrightarrow Na_2SO_4 + 2H_2O + 2NH_3$$

The ammonia generated was collected in excess boric acid.

$$NH_3 + H_3BO_3 \longrightarrow NH_4BO_2 + H_2O$$

After complete ammonia distillation, the ammonium borate solution is titrated with a standard HCl solution. Strong acid (HCl) displaces weak boric acid from its salt.

$$NH_4BO_2 + HCL \longrightarrow NH_4CL + HBO_2$$

1 mole of ammonia is equivalent to 1 mole of ammonium borate which is equivalent to 1 mole of HCL. Knowing the amount of 0.01 M HCL used for the titration, the amount of ammonia bound to borate can be calculated. From this amount, the quantity of nitrogen in the sample can be calculated.

2.2.4. Determination of Moisture Content of Cassava Waste Water

The AOAC (1990) method was used. Porcelain crucibles were washed and dried in an oven at 100^{o}C for 30 minutes and allowed to cool in a desiccator. One gramme of the sample was placed into weighed crucibles and then put inside the oven set at 105^{o}C for 4 hours. The samples were removed from the oven after this period and then cooled and weighed. The drying was continued and all the samples with the crucibles weighed until a constant weight was obtained.

$$Percentage\ (\%)\ Moisture = \frac{A - B}{A} \times \frac{100}{1}$$

A = Original weight of sample

B = Weight of dried sample.

2.2.5. Ash Content Determination of Cassava Waste water

The residue remaining after all the moisture have been removed and the fats, proteins, carbohydrates, vitamins and organic acids burnt away by ignition at about 600^{o}C is called ash. It is usually taken as a measure of the mineral content of the raw waste.

Using AOAC (1990) method, 1g of the finely ground samples were weighed into porcelain crucibles which have been washed, dried in an oven at 100^{O}C, cooled in a desiccator and weighed. They were then placed inside a muffle furnace and heated at 600^{o}C for 4 hours. After this, they were removed and cooled in a desiccator and then weighed.

$$Percentage\ (\%)\ \ Ash = \frac{A - B}{C} \times \frac{100}{1}$$

A = Weight of crucible + ash

B = Weight of crucible

C = Weight of original sample

2.2.6. Fat Content Determination of Cassava Waste Water

Pearson (1976) method was used. This involves the use of Soxhlet extraction apparatus. This method involves continuous extraction of waste with organic solvent such as petroleum ether for 4 hours or so depending on the volume of sample. To carry out the extraction, the flask was washed and dried in an oven. It was then cooled in a desiccator and weighed.

1g of the ground sample was accurately weighed and transferred into a rolled filter paper and then placed inside the extraction thimble. The thimble was placed inside the extractor. Some quantity of petroleum ether was poured inside the extraction flask (usually three-quarter of the volume of flask). The condenser and the flask were connected to the extractor. The whole unit was place on a heating mantle for 4 hours after which the petroleum ether was recovered. The

oil collected in the flask was dried in an oven at 105^{o}C. It was then weighed and the percentage fat calculated as shown below.

$$Percentage\ (\%)\ Fat = \frac{C - A}{B} \times \frac{100}{1}$$

C = weight of flask +oil

A = weight of empty flask

B = weight of original sample.

2.2.7. Crude Fibre Content Determination of Cassava Waste Water

This determination is done to have an idea of the materials that are indigestible in the waste. It is largely made up of cellulose and small lignin.

Crude fibre is obtained as an organic residue left behind after the raw waste has been subjected to standard condition with organic solvents, dilute mineral acids and sodium hydroxide.

The AOAC (1990) method was used. 1g of the sample was weighed (w1) into a 600ml beaker and 150ml of preheated 0.128M H_2SO_4 was added to it. This was heated for 30 minutes and filtered under suction and washed with hot distilled water until the washings were no longer acidic. The residue was then transferred to a beaker and boiled for 30 minutes with 150ml of preheated KOH (0.223M). It was filtered and washed with hot water until the washings are no longer alkaline. The residue was washed three times with acetone and dried in an oven at 105^{o}C for 2 hours. It was then cooled in a desiccator, weighed (W2) and ashed in a muffle furnace (make: Vecstar, model LF3, made in U.K) at 500^{o}C for 4 hours. The ash obtained was cooled in a desiccator and weighed (W3).

$$Percentage\ (\%)\ Crude\ fibre = \frac{W2 - W3}{W1} \times \frac{100}{1}$$

Where:

W_1 = weight of sample

W_2 = Weight of dry residue

W_3= Weight of ash.

2.2.8. Carbon Content Determination of Cassava Wastewater

Walkey and Black (1934) method was used. 0.05g finely ground waste sample was weighed into a 500ml conical flask. 10ml of 1M potassium dichromate was poured inside the flask and the mixture was swirled. 20ml of conc. H_2SO_4 was added and the flask was swirled again for 1 minute in a fume cupboard. The mixture was allowed to cool for 30 minutes after which 200ml of distilled water; 1g NaF and 1ml of diphenylamine indicator were added. The mixture was swirled and titrated with ferrous ammonium sulphate.

The blank was also treated in the same way.

$$Percentage\ (\%)\ Carbon = \frac{B-T\times M\times 1.33\times 0.003\times 100}{g}$$

Where B = Titration volume (Blank)

T = Titration volume (Sample)

M =Molarity of Fe solution

2.2.9. Determination of Hydrocyanic Acid Content of Cassava Waste Water

This was determined according to the method described by Oboh and Akindahunsi (2003). 20g of the waste sample was placed in an extraction flask followed by the addition of 100ml of

distilled water. It was allowed to stand for 2 hours to free all bound hydrocyanic acid. After 2 hours, 100ml of distilled water was added to the slurry and steam distilled. The distillate was collected in 20ml 0.01N $AgNO_3$ that has been acidified with 1ml HNO_3. The distillation proceeded for 40 minutes. After getting 150ml of distillate, the distillate was filtered with little water and the excess $AgNO_3$ was titrated with 0.02N KSCN using ferric alum indicator. The end point was observed by the appearance of faint reddish colour upon the addition of 0.02N KSCN.

Calculation

Volume of $AgNO_3$ consumed to complex CN = 20-2V

V=Volume of titre

1ml 0.01N $AgNO_3$ = 0.27mg HCN.

2.2.10. Conductivity Determination of Cassava WasteWater

The conductivity meter was standardized with 0.01M KCL solution. The electrode was rinsed with deionized water, wiped and dipped into the water sample and left for some time for the reading to stabilize. The reading displayed on the screen was then recorded in micro Siemens per centimetre (uS/cm).

2.2.11. Chemical Oxygen Demand of Cassava Waste Water

Preparation of Solution

a. Standard potassium heptaoxochromate (VI) $K_2Cr_2O_7$, solution, 0.125M: 12.259g $K_2Cr_2O_7$, A.R, previously dried at 103°C for 2 hours was dissolved in distilled water and made it up to 1 litre mark. Ml sample

b. Ag_2SO_4-H_2SO_4 solution: 11g Ag_2SO_4 crystals, A.R was dissolved in a Winchester bottle (2.5 litres) of conc. H_2SO_4, (s.g 1.84); 1 to 2 days are required for dissolution.

c Mercury (II) sulphate, A.R., 0.4g powder was needed for each determination.

d. Sulphamic acid: This was needed if NO_2-N were known to be present in the sample. About 2mg sulphamic acid crystals was needed for each determination.

e. Standard Iron (II) ammonium sulphate (A.R) solution, 0.05M: 39g $FeSO_4$, $(NH_4)_2SO_4.6H_2O$ was dissolved in distilled water, 20ml conc. H_2SO_4 was added, cooled and diluted to 1 litre mark, was well shaken and standardize daily against standard $K_2Cr_2O_7$ solution.

(f) Ferroin indicator solution: 1.485g 1.10-phenanthroline monohydrate and 695mg Iron (II) sulphate heptahydrate, $FeSO_4.7H_2O$ was dissolved in distilled water and diluted to 100ml mark and shaken well.

Standardization of Solution

10ml standard $K_2Cr_2O_7$ solution was diluted to about 100ml, 30ml of concentrated H_2SO_4 was added and cooled, then the solution was titrated against Iron (II) ammonium sulphate solution using 2 or 3 drops of ferroin solution as indication, to red- brown end point, the molarity of the ferrous ammonium sulfate was calculated as usual.

Procedure of Standardization

0.4g $HgSO_4$ was placed in refluxing flask, 20ml sample or aliquot diluted was added to 20ml, then about 2mg sulphamic acid was also added. By the aid of pipette, 10ml standard $K_2Cr_2O_7$ solution was added and then several glass beads previously dried at 600^oC for 1 hour also added, slowly and with gentle swirling, 30ml Ag_2SO_4 solution was as well added. The flask was connected to the condenser. A blank mixture was prepared, then the mixture refluxed for

2 hours. Cooled, and the condenser was washed with distilled water into Erlenmeyer flask and dilute to about 150ml., then cooled to room temperature and then titrated the excess dichromate with standard ferrous ammonium sulfate using 2 to 3 drops of ferroin as the indicator ml sample.

Calculation of Chemical Oxygen Demand (COD)

$$COD(mg/l) = \frac{(V_b - V_s) \times M \times 16{,}000}{ml\ sample}$$

Where Vb = ml FAS used for blank.

Vs = ml FAS used for sample

M = molarity of FAS.

2.2.12. Biochemical Oxygen Demand Determination of Cassava Waste Waster

BOD was determined by AOCAC (1990) method. Solution A contains $MnSO_4.H_2O$ (m.wt 169.01). $MnSO_4.H_2O$ 0.1M was prepared by weighting out 4.23gm/250cm^3 of distilled water, Solution B (Alkaline) KI solution was prepared by weighing out 125g of NaOH and 40gm KI and then dissolved in 250cm^3 of distilled water.

Starch Solution Indicator preparation

Starch (1g) was dissolved in a little quantity of cold water and boiling water added to make up to 100cm^3 to clear consistency. A little quantity of chloroform was added for its preservation.

Sodium Thiosulphate (mwt =248.18, $Na_2S_2O_3.5H_2O$)

$Na_2S_2O_3.5H_2O$ (0.1M) was made by dissolving 6.21gm of the salt in 250cm^3 of distilled water

Sulphuric acid – Sp. Gravity 1.84

Procedure

Solution A (1.0cm^3) was added to the sample (250cm^3) in the sample bottle filled nearly to the brim and 1.0 cm^3 of solution B, using a pipette. The sample was stoppered and shook thoroughly, inverting several times. Allowed to settle, to observe white precipitation of Mn $(OH)_3$. Then 1.5cm^3 of conc. sulphuric acid was added, and the bottle was restoppered and mixed thoroughly to dissolve the precipitate. Then with a pipette 25cm^3 of the sample was withdrawn into a titrating flask and titrated with standard 0.1M with $Na_2S_2O_3$ to nearly faint yellow solution. Then the starch indicator was added and continued adding the titrant until the blue solution turned white.

This was the end-point for the titration

The process was repeated for the same volume of the water kept for 5days at the same time of collection.

Equation for the calculation

$$MnSO_4 + 2NaOH \longrightarrow Mn(OH)_2 + Na_2SO_4$$

$$2Mn(OH)_2 + O_2 + H_2 \longrightarrow 2Mn(OH)_3$$

$$2Mn(OH)_3 + 2KI + H_2SO_4 \longrightarrow I_2 + 2MnSO_4 + K_2SO_4 + 6H_2O$$

$$I_2 + 2Na_2S_2O_3 \longrightarrow 2NaI + Na_2S_4O_6$$

By combining these equation

1cm^3 of 0.1M $Na_2S_2O_3$ = 0.4mg (O_2)

For calculation, the titre volumes of $Na_2S_2O_3$ for (ii) was subtracted from (i) and the oxygen demand was calculated bearing in mind that 1cm^3 of 0.1M $Na_2S_2O_3$ = 0.4 mg (O_2)

2.2.13. Microbial Analysis of Cassava Waste Water

The microorganisms in the waste were cultivated and identified using plate count method (Miles and Misra 1938).

Preparation of media

Glucose enriched agar (0.5%w/v): A 28g of nutrient agar powder was dissolved in 1000ml of distilled water, and was allowed soaking for 10minutes. The agar suspension was brought to melt by boiling in a water bath. A 4 g of glucose was added into the molten agar and mixed well. A 20 ml aliquot of the molten agar was dispensed into a bijou bottles, cocked, and sterilised in an autoclave at 121^{o}C for 15minutes. The sterile molten nutrient agar was stored at 42^{o}C until use.

2.2.14. Determination of original cell population (Total Viable Counts) of the samples.

The original cell population of the sample was determined via surface viable count method. A 1g of the sample was suspended in 10ml of sterile water under aseptic condition. The suspension was allowed for 10 minutes with constant shake. A 10^{-6} dilution was obtained from the suspension using 10 fold serial dilution techniques. A 0.02ml of 10^{-6} dilution was dropped at the centre of the segments. The culture plates were allowed to stand for 15mins for proper absorption before incubation. The culture plate were incubated in inverted position at 37^{o}C for 48hours. Thereafter, the plates were observed for growth, and the colonies were counted to determine the mean colony count per drop.

Thus, the total Viable Count (TVC) was calculated as followed (Prescott *et.al*., 2000).

The original cell population of the sample was calculated using the formula:

$$\text{Original cell population (OCP) (cfu/ml)} = \frac{\text{mean colony count/drop} \times \frac{1}{dilution}}{\text{volume/drop}}$$

Where: Mean colony count/drop (mcc/d) is obtained

Dilution factor = 10^{-2}

Volume per drop = 0.02ml

2.2.15. Gas Chromatography-Mass Spectrophotometer Analysis of Cassava Waste Water with Purge and Trap Method (P & T)

GC-MS analysis was done by purge and trap method as described by (Victor *et al*., 2017). This GC-MS method is also known as the dynamic headspace. This functions by separating volatile compounds from the sample matrix (Cassava wastewater) by passing an inert gas such as helium or nitrogen through the matrix (purging). The target, volatile compounds will desorbed from the aqueous phase to the gas phase (purged) and are then separated from the stream of gas (trapped) by adsorbent filters (Lee *et al*., 2005). The adsorbent material was then heated in a stream of GC carrier gas (pure helium). This released the trapped substances into the carrier gas, the target analytes were introduced to GC, and analyzed. Typical trapping (adsorbent) materials are porous polymer beads, activated charcoal, silica gel, other GC column packing materials, or combinations of such materials. A quantity 1μL of the extracted sample was analyzed with Agilent US EPA 8270 GCMS. See Section 2.2.16 for operational conditions: The chromatograms were calibrated with internal standards. The calibration standard used for the cassava wastewater consists of the following components. (1) Naphthalene, (2) Acenaphthylene, (3) Fluorene, (4) Acenaphthene, (5) Phenanthrene, (6) Anthracene, (7) Pyrene, (8) Benzo(a) antracene, (9) Chrysene, (10) Benzo(b)floranthene, (11) Benzo(k)floranthene, (12) Benzo(a)pyrene, (13) Indeno(1,2,3-cd)pyrene, (14) Fluoranthene, (15) Dibenzo(a,h)anthracene and (16) Benzo(ghi)perylene.

2.2.16. Operational Conditions of the GC-MS

Gas chromatographic tandem mass spectroscopy technique was used with the following conditions. GC/MS-QP2010 Agilent Plus, ion source temperature: 200.00°C, interface temperature: 250.00°C, solvent cut time: 2.50 min, detector gain mode: MS, detector gain: 0.00 kV, threshold: 2000, column oven initial temperature: 70.0°C, injection final temperature: 250.00°C, injection Mode: Split, flow control mode: linear velocity, pressure: 116.9 kPa, total Flow: 40.8 mL min−1, column flow: 1.80 mL min−1, linear velocity: 49.2 cm sec−1, trap and purge flow: 3.0 mL min−1, Split Ratio: 20.0, high pressure injection: OFF, Carrier Gas: Helium and Splitter hold: OFF. While oven rating was as follows: Oven Temp. Program Rate Temperature (°C) Hold Time (min) Initial: 0.00 70.0 0.00 Final: 10.0 280 5.00 (Victor *et al*., 2017).

2.2.17. Product Estimation for Industrial Scale Production of Biomethane Using Fresh Cassava WasteWater

Period of fermentation was 28 days (4 weeks)

Temperature condition at Ambient/ Room temperature 25^{o}C

pH of cassava waste water substrate before digestion 5.70

pH of cassava waste water after 28days of digestion 3.01

Volume of cassava waste water charged into the bioreactor was 1,000ml

The microbial dilution for 10^{-6} serial dilution per ml was 5.5×10^{8}cfu bio-load

Mathematically, if 1ml of substrate at a serial dilution of 10^{-6} gave 5.5×10^{8}cfu bio-load, 1000ml of substrate at same serial dilution rate would give α cfu bio-load.

$$\alpha = \frac{1ooo ml \times 5.5 \times 10^{8} cfu}{1 ml} = 5.5 \times 10^{11} cfu$$

2.2.18. Microbial Population Estimation

10,000 ml of substrate at 10^{-6} serial dilution will require

$$\frac{10{,}000 ml \times 5.5 \times 10^{8} cfu}{1{,}000 ml} = 5.5 \times 10^{12} \text{cfu}$$

This implies that 5.5×10^{12}cfu microbial load is required to digest 10,000 ml of cassava waste water substrate to produce bio-methane.

For 100,000 ml of substrate at 10^{-6} serial dilution 5.5×10^{13}cfu microbial load is required to digest 100,000 ml substrate to produce bio-methane.

2.2.19. Methane Estimation

The biogas produced in the digester was analysed using a combustible gas analyzer (BACHARACH, Model PCA2: made in China).The gas analyzer is equipped with sensors for the determination of the percentage concentration of CO_2, NO, NO_2, CO, and O_2. The percentage concentration of methane in the biogas was determined by subtracting the percentages of other gases from 100.

$$100 - (\%CO_2 + Other\ gases)$$

$$CH_4 = 100 - (12 + 3 + 3 + 8)$$

$$CH_4 = 100 - (26)$$

$$CH_4 = 74\%$$

If 1000 ml of cassava waste water substrate with 5.5×10^{11}cfu at ambient temperature was required to produce 74% bio-methane after 28 days of digestion.

10,000 ml of cassava waste water substrate with 5.5×10^{12}cfu bio-load would be required to produce α% of biomethane at same condition and days.

$$1000\ ml \longrightarrow 74\%\ biomethane$$

$$10000\ ml \longrightarrow \alpha\%\ biomethane$$

$$\alpha\%\ CH_4 = \frac{10{,}000\ ml \times 79}{1{,}000ml} = 740\%\ biomethane$$

This implies that 10,000 ml of cassava waste water substrate at ambient temperature and a bioload of 5.5×10^{12}cfu in 28 days produces 740% biomethane.

Also 100,000 ml of cassava waste water substrate at ambient temperature and a microbial population of 5.5×10^{13}cfu in 28 days will produce 7400% biomethane.

CHAPTER 3

RESULTS

3.1. Physiochemical properties of Cassava WasteWater

The physiochemical analysis on the fresh and the sludge after 28 days of bio digestion. The fresh substrate sludge showed the following parameters: moisture 89.40%, Ash content 0.31%, crude fat 0.55%, crude fibre 0.80%, carbohydrate 7.98%, crude protein 0.96%, carbon 4.31%, nitrogen 0.15%, HCN 2.7, BOD 46.40mg/l, COD 154.40mg/l, conductivity 121.80uS/cm and the pH 5.70. The fermented sludge showed: moisture 88.60%, Ash content 0.88%, crude fat 0.40%, crude fibre 0.30%, carbohydrate 5.89%, crude protein 3.93%, carbon 3.22%, nitrogen 0.63%, HCN 0.54, BOD 27.20mg/l, COD 90.40mg/l, conductivity 79.40uS/cm and pH 3.01.

Comparing the fresh substrate and the sludge it can be inferred that there were high concentration of the following parameters in the fresh waste substrate than in the fermented substrate by the following differences: moisture content 0.8%, crude fat 0.15%, carbohydrate 2.09%, carbon 1.09%, Crude fibre 0.5%, BOD 19.2mg/l, COD 64mg/l and HCN 2.16. There was an increase in the concentration of the following parameters in the sludge than in the fresh waste substrate by the following differences: Ash content 0.57%, Crude protein 2.97% and Nitrogen 0.48%.

Table1: Physiochemical Analysis of Cassava Wastewater Substrate (Fresh and Fermented)

Parameters	Fresh Cassava Wastewater	Fermented Cassava Wastewater (sludge)	Difference
% Moisture	89.40	88.60	0.80(decrease)
% Ash Content	0.31	0.88	0.57(increase)
% Crude Fat	0.55	0.40	0.15(decrease)
% Carbohydrate	7.98	5.89	2.09(decrease)
% crude Protein	0.96	3.93	2.97(increase)
% Crude fibre	0.80	0.3	0.5 (decrease)
Total	100	100	
% Carbon	4.31	3.22	1.09(decrease)
% Phosphorus	0.12	0.24	0.12 (increase)
% Nitrogen	0.15	0.63	0.48 (increase)
HCN	2.7	0.54	2.16 (decrease)
BOD (mg/l)	46.40	27.20	19.20(decrease)
COD (mg/l)	154.40	90.40	64.00(decrease)
Conductivity(uS/cm	121.80	79.40	42.80(decrease)
C/N Ratio	28.73	5.11	
pH	5.70	3.01	

3.2.0. Microbial Analysis of Fermented Cassava Waste Water Substrate

Microbial population of the fermented cassava waste water substrate in the bioreactors after 28 days; measured in colony forming unit per ml (cfu/ml). The result showed a mean colony of 11 and serial dilution factor of 10^{-6} gave a microbial load of $5.5\times10^{8}{}_{CFU.}$ This indicated that for1000ml volume of substrate loaded in the bio digester $5.5\times10^{11}{}_{CFU}$ microbial population was

present and responsible for the fermentation of the cassava waste water substrate to produce biomethane.

Table 2: Microbial Analysis of Fermented Cassava Waste Water Substrate

Bioload Result

	Mean colony	Dilution	Total viable count (cfu/ml)
	11	10^{-6}	$550000000 \cong 5.5x10^{8}$

3.3.0. Cultural characteristics of the isolates

Results for the isolation and identification of isolates of bacteria in cassava waste water substrate. The result showed seven isolates namely: isolates A, B, C, D, E, F, and G. These isolates are characterised on the bases of their shape, surface texture, chromogenesis, elevation and opacity as shown in the table below.

Table 3: Cultural characteristics of the isolates

Sample	Shape	Surface texture	Chromogenesis	Edge	Elevation	Opacity
A	Circular	Glistering	No pigment	Entire	Flat	transparent
B	Circular	Smooth	No pigment	Entire	convex	Transparent
C	Irregular	Rough and Doul	No pigment	Undulated	Flat	Opaque
D	Circular	Smooth and shining	No pigment	Entire	Raised	Opaque
E	Circular	Smooth	No pigment	Entire	Convex	Transparent
F	Circular	Smooth and shining	Bromide yellow	Entire	convex	Transparent
G	Circular	Smooth	No pigment	Undulated	Raised	Opaque

3.4.0. Gram's Characterisation of Isolates

The results of cell wall/ membrane characteristics of bacteria in Cassava waste water substrate after 28 days of fermentation at ambient temperature. Isolate A, E, F were rod shaped and negative to Gram test, isolate D had a cocci shape with a purple colouration so a Gram positive organism, isolate B is a Gram negative organism unlike other Gram negative isolate it had a curved shape. Isolate C and G were rod sharped with a purple coloration indicating a Gram positive organism.

Table 4: Gram's Characterisation of Isolates

Sample code	Shape	arrangement	Colour	Gram's Character	Spore character
A	Short rod	Single	pink	negative	Negative
B	Curve	single	pink	negative	negative
C	Rod	Long chains	purple	positive	positive
D	Cocci	Clusters	purple	positive	negative
E	Rod	Single	pink	negative	negative
F	Rod	Single	pink	negative	negative
G	Rod	Single with swollen head	purple	positve	positive

3.5.0. Biochemical Identification of Microorganism Present in Cassava Waste Water

The result of the biochemical identification of the microorganism in cassava waste water after 28 days of fermentation.

Sample A did not ferment lactose, positive with indole and urease reagent thus identified as *Proteus mirabilis*.

Sample B fermented glucose and produced acid, oxidase positive, is identified as *Vibrio spp*

Sample C hydrolyzed starch, reacted positive with Voges-Proskauer test. It utilized citrate, and did not grow in 6.5% NaCl enriched agar at 55^{o}C. Thus sample C was confirmed to be *Bacillus subtillis.*

Sample D was identified as *Staphylococcus spp* since it did not ferment Manitol, and it is catalase positive.

Sample E fermented lactose, reacted positive with indole reagent and negative with citrate thus, *Escherichia coli* confirmed.

Sample F did not ferment lactose, indole, and urea reagent. It reacted positive with H_2S thus, *Salmonella spp* identified.

Sample G hydrolyzed starch, positive with Voges proskauer, and citrate, and growth in 6.5% NaCl enriched agar at 55^{o}C. Thus, *Bacillus licheniformis.*

Table 5: Biochemical Identification of Microorganism Present in Cassava Waste Water

Sample	Microbial Observation	Probable isolate
A	Did not ferment lactose, positive with indole and urease reagent	*Proteus mirabilis*
B	Fermented glucose and produced acid, oxidase positive.	*Vibro spp*
C	Hydrolyzed starch, reacted positive with voges Proskauer test. It utilized citrate, and did not grow in 6.5%NaCl enriched agar at 55^{o}C.	*Bacillus subtillis*
D	Did not ferment Manitol, and it is catalase positive.	*Staphylococcus spp*

E	Fermented lactose, reacted positive with indole reagent and negative with citrate.	*Escherichia coli*
F	Did not ferment lactose, indole, and urea reagent. It reacted positive with H_2S.	*Salmonella spp*
G	Hydrolyzed starch, positive with voges proskauer, and citrate, and growth in 6.5%NaCl enriched agar at 55^{o}C.	*Bacillus licheniformis*

3.6.0. GC-MS Analysis for Persistent Organic Compounds in Fresh Cassava Wastewater.

GC-MS analysis for fresh cassava waste water showed five peaks of fifteen compounds with their retention time (RT), peak area percentage, and Molecular weight, molecular formula and chemical abstract series (CAS). Each peak was comprised of three organic compounds. It was shown that peak five (5) with 85.86% area concentration were the major compounds present. This peak contained organic compounds like 9,12-octadecadienoicacid(z,z)-2,3-dihydroxypropylester, E,z-1,3,12 Nonadecatriene and 2-butyl-5-hexyloctahydro1-indene, followed by peak two (2) with 6.20%, peak four (4) having 2.77% , peak three(3) with 2.59%

and the lowest peak area of 2.58% was observed in peak one(1) with organic compounds such as Hexamethylene diacrylate, 1,2-cyclohexanediol,cyclic sulphite. These compounds in the waste water have been seen to be toxic to but human and environment.

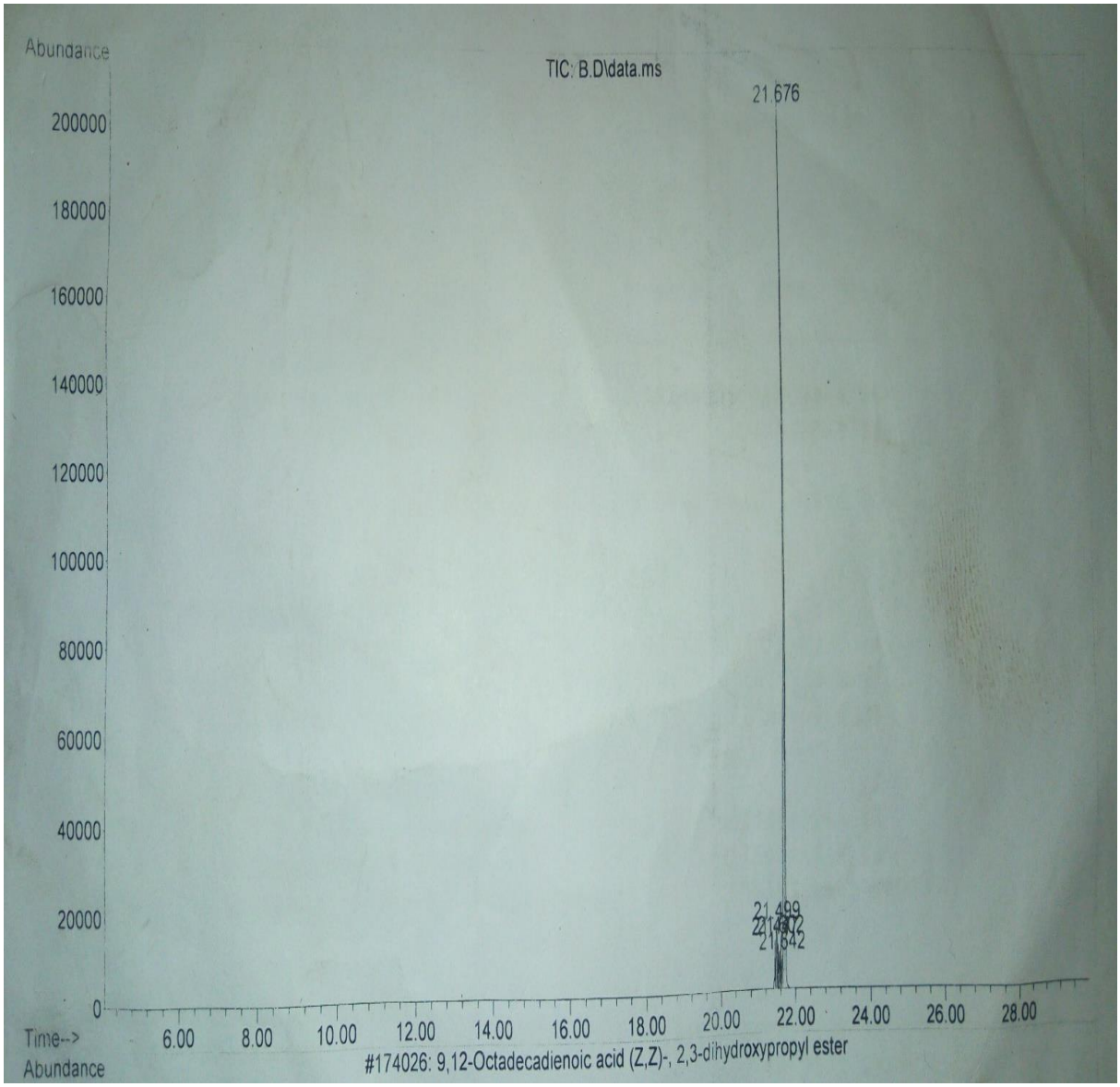

Fig 5: GC-MS Chromatogram of Fresh Cassava Waste Water.

Table 6: Persistent Organic Compounds (POC) in Fresh Cassava Waste Water Substrate

PK	RT	%Conc	Compounds	Molecular Formula	CAS	Molecular Weight (g/mol)
1	21.447	2.58	Hexamethylenediacrylate	$C_{12}H_{18}O_4$	013048-33-4	226.272
			1,2-cyclohexanediol,cyclic sulfite	$C_6H_{10}O_3S$	019456-18-9	162.203
			2-cyclopentene-1-undecanoic acid, methyl ester	$C_{17}H_{30}O_2$	024828-56-6	266.425
			1,14-Tetradecanediol	$C_{14}H_{30}O_2$	019812-64-7	230.392
2	21.499	6.20	1,2-diethyl cyclohexene	$C_{10}H_{18}$	001674-10-8	138.2499
			1,6-dimethyl cyclohexene	C_8H_{14}	001759-64-4	110.197
			1,2:4,5:9,10-Triepoxydecane	$C_{10}H_{16}O_3$	052338-90-6	184.235
3	21.602	2.59	8-methoxy-1,6-octadiene	$C_9H_{16}O$	014543-49-8	140.226
			1-pentanol,5-(methylenecyclopropyl)	$C_9H_{16}O$	1000157-89-1	140.226

			10-undecyne-1-ol			
4	21.642	2.77		$C_{11}H_{20}O$	002774-84-7	168.28
			1- Decyne			
				$C_{10}H_{18}$	000764-93-2	138.258
			1,2:4,5:9,10-Triepoxydecane			
				$C_{10}H_{16}O_3$	052338-90-6	184.235
5	21.676	85.86	9,12-octadecadienoic acid ZZ, 2,3-dihydroxypropyl esther	$C_{21}H_{40}O_4$	002277-28-3	356.539
			E,E,Z-1,3,12-Nanodecatriene			
				$C_{19}H_{34}$	1000131-11-3	262.48
			2-butyl-5-hexyloctahydro-1-1ndiene			
				$C_{19}H_{36}$	055044-33-2	264.497

Where PK: Peak

RT: Retention Time

CAS: Chemical Abstract Series

% Conc: Area % Concentration

3.7.0. GC-MS Analysis for Persistent Organic Compounds in Fermented Cassava Waste Water Substrate

The GC-MS analysis for fermented cassava wastewater substrate was conducted after a fermentation period of 28days at ambient temperature, the result showed three peaks and two organic compounds with their retention time (RT), peak area percentage and chemical abstract series. It was shown that peak three (3) with 34.81% area concentration was the major compounds present, followed by peak two (2) 32.91% and peak one (1) 32.28%. These peak contained compounds such as propanenitrile and 2-propyn-1-amine which have been proven to be less toxic to the ecosystem when compared to the compounds present in the fresh waste water substrate.

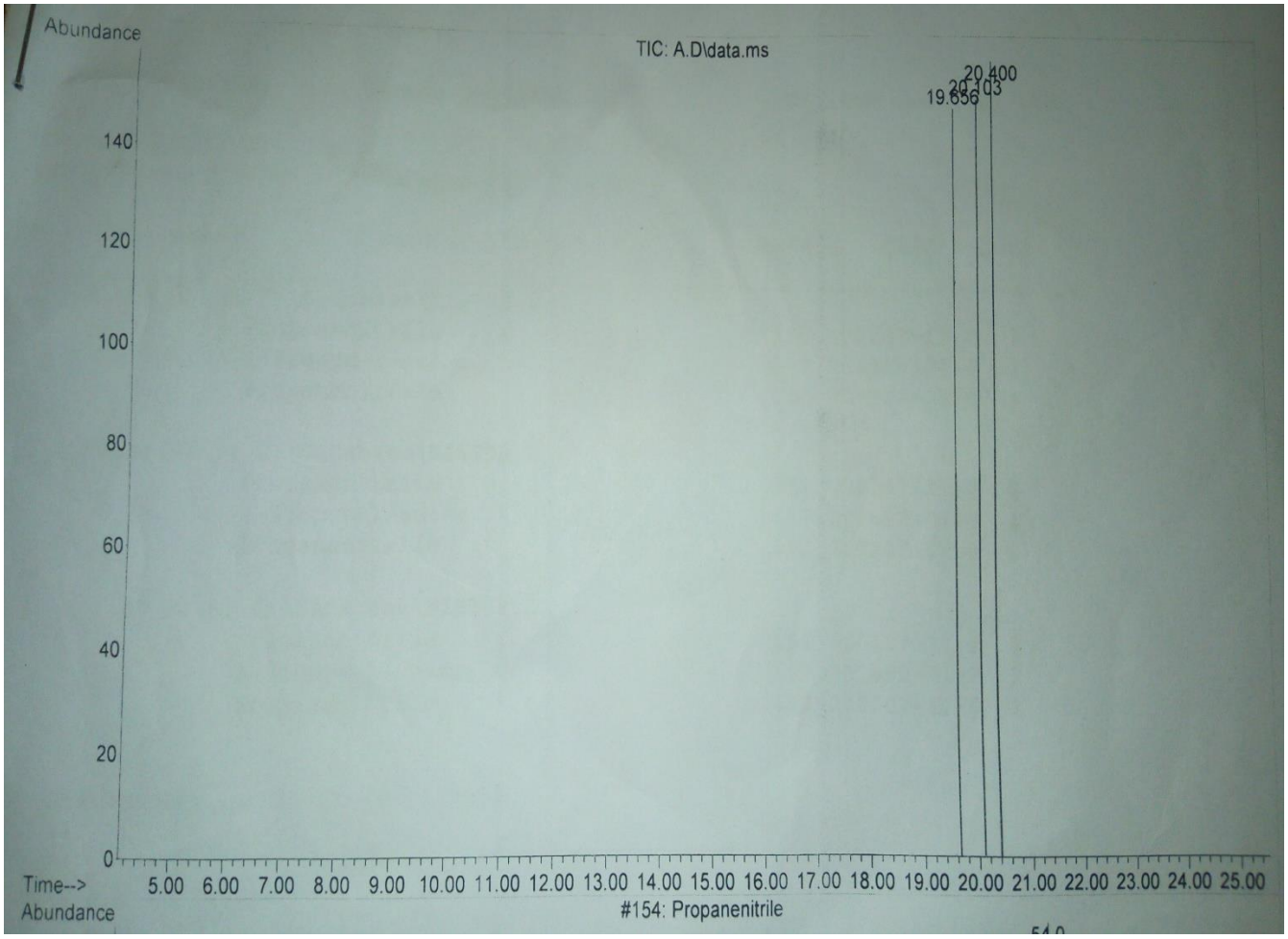

Figure 6: GC-MS Chromatogram of Fermented Cassava Waste Water

Table 7: Persistent Organic Compounds (POC) in Fermented Cassava Waste Water.

Pk	Retention Time	% Conc	Compounds	Molecular formula	Molecular Weight(g/mol)	CAS
1	19.656	32.28	Propanenitrile	$C_9H_{15}N_3$	165.24	000107-12-0
			Prop-2-yn-1-amine	C_3H_5N	55.08	002450-71-7
			Propanenitrile	$C_9H_{15}N_3$	165.24	000107-12-0
2	20.103	32.91	Propane nitrile	$C_9H_{15}N_3$	165.24	000107-12-0
			Prop-2-yn-1-amine	C_3H_5N	55.08	002450-71-7
			Propane nitrile	$C_9H_{15}N_3$	165.24	000107-12-0
3	20.400	34.81	Propanenitrile	$C_9H_{15}N_3$	165.24	000107-12-0
			Prop-2yn-1-amine	C_3H_5N	55.08	002450-71-7

	Propanenitrile	$C_9H_{15}N_3$	165.24	000107-12-0

Where Pk: peak

CAS: Chemical Abstract Series

% Conc: Area % Concentration

3.8.0. Composition of Gases in Cassava Waste Water

The result showed that after 28 days of biodigestion, cassava waste water was composed of the following gases in their percentage concentration. NO 3%, H_2 3%, CO 8%, CO_2 12% and CH_4 74%. This showed that fresh cassava wastewater is a good substrate for biogas generation.

Table 8: Composition of Gases in Cassava Waste Water

S/N	PARAMETER	% CONCENTRATION
1	CO_2	12
2	CO	8
3	NO	3
4	H_2	3
5	CH_4	74
		100

3.9.0. Product Estimation for Pre-factory production of Bio-methane from Cassava Waste Water Substrate

A pre-factory analysis for the large scale production of bio-methane from fresh cassava waste water using a serial dilution of 10^{-6} at an ambient temperature and a digestion period of 28 days. From the mathematical result obtained 1,000 ml of substrate at the stated conditions produced 74% methane using a bio-load of 5.5×10^{11}. 10,000 ml of substrate produced 740% bio-methane requiring a microbial population of 5.5×10^{12}. 100,000 ml of substrate produced 7400% bio-methane using a microbial load of 5.5×10^{13}. Higher volume of substrate can be digested using a bigger bio reactor, the microbial load and methane concentration can be extrapolated for commercial purposes changing waste to wealth.

CHAPTER FOUR

4.0. Discussion

This research was aimed at converting fresh cassava waste water into bio-methane. The pH of the fresh cassava wastewater was 5.70. This implies that the fresh cassava wastewater substrate is acidic in nature and this can be attributed to the high cyanide content (Uzochukwu *et al*., 2001). Effluent from cassava processing plants are therefore regarded as harmful and should not be allowed to spread over farmlands (Eze and Onyilide, 2015).

After 28days of fermentation, there was a drop in pH from 5.70 to 3.01. The drop in pH was caused by acid forming bacteria which produce acetate, hydrogen gas, carbon dioxide, and few other volatile fatty acid such as propionic and butyric acid. A low pH value inactivated microorganisms involved in the biogas production especially methanogenic bacteria (Caceres *et al*., 2009). This can be attributed to the formation of secondary metabolites by the microorganism during the period of fermentation.

Result of the physiochemica analysis on the fresh substrate showed the following parameters: moisture 89.40%, Ash content 0.31%, crude fat 0.55%, crude fibre 0.80%, carbohydrate 7.98%, crude protein 0.96%, carbon 4.31%, nitrogen 0.15%, HCN 2.7, BOD 46.40mg/l, COD 154.40mg/l and conductivity 121.80uS/cm . The fermented sludge showed: moisture 88.60%, Ash content 0.88%, crude fat 0.40%, crude fibre 0.30%, carbohydrate 5.89%, crude protein 3.93%, carbon 3.22%, nitrogen 0.63%, HCN 0.54, BOD 27.20mg/l, COD 90.40mg/l and conductivity 79.40uS/cm.

Comparing the fresh substrate and the fermented sludge it can be inferred that there were high concentration of the following parameters in the fresh waste substrate than in the fermented

substrate by the following differences: moisture content 0.8%, crude fat 0.15%, carbohydrate 2.09%, carbon 1.09%, Crude fibre 0.5%, BOD 19.2mg/l, COD 64mg/l and HCN 2.16. There was an increase in the concentration of the following parameters in the sludge than in the fresh waste substrate by the following differences: Ash content 0.57%, Crude protein 2.97% and Nitrogen 0.48%. The decrease in these parameters: moisture content by 0.8% could be attributed to the fact that the micro-organisms utilized the moisture present in the substrate for growth thereby reducing the samples' moisture content (Adeleke *et al*., 2017). Crude fat decreased by 0.15%, carbohydrate 2.09% the decrease in carbohydrate could be attributed to the possible transformation of some of the carbohydrate, which could be used as carbon sources for synthesis of protein or fat (Lehninger, 1987). Crude fiber decreased by 0.5%, this decrease could be attributed to the ability of the fermenting microorganisms to degrade the crude fibre of fermenting cassava waste water, secrete hydrolyzing and oxidizing enzymes involving in conversion of recalcitrant compounds in the waste into utilizable compounds (Obueh and Ikenebomeh, 2014) and abundant production of organic acids resulting from fermentative dissimulation of carbohydrate (Akinfala and Tewe, 2004). HCN decreased by 2.16% this suggest that microorganisms involved in the fermentation process is capable of utilizing cyanogenic glycosides and the breakdown products to less toxic compounds (Tweyongyere and Katongole, 2002). Carbon decreased by (1.09%) this can be attributed to the emission of gases. Biological oxygen demand (BOD) and chemical oxygen demand (COD) of the wastewater exceeded the WHO permissible levels of 4 mg/l and 10 mg/l respectively (Shittu *et al*., 2008) the high BOD and COD levels from this study might be attributed to the presence of high organic matter in the effluent (Shittu *et al*., 2008). After 28 days of fermentation there was a decrease in BOD and COD by (19.2mg/l) and (64mg/l) respectively this can be attributed to high microbial count and the low oxygen concentration in the bioreactor.

The result also showed an increase in the following parameter after 28 days of digestion: ash content 0.57% this increase in the ash content could have been as a result of microbial fermentation and the hydrolysis of such chelating agents like phytate which is highly concentrated in cassava waste products (Aro *et al.*, 2010). There was a 2.97% increase in crude protein, this observation could be due to the possible secretion of some extra-cellular enzymes (proteins) such as amylase, linamarase and cellulase (Oboh and Akindahunsi, 2003) into the bioreactor by the micro-organisms in an attempt to make use of these wastes as a source of carbon (Jokotagha and Amoo, 2012). Nitrogen and phosphorus concentration increased by 0.48% and 0.12% respectively indicating that the fermented cassava waste water substrate can be effective in the production of bio-fertilizer.

The microbial analysis conducted to determine the microorganism present and the microbial bio-load responsible for the conversion of cassava waste water substrate to methane showed the presence of both Gram-positive and Gram-negative bacteria, six genera bacteria (*Proteus, Vibrio, Bacillus, Staphylococcus, Escherichia and Salmonella*) were isolated and characterized using Biochemical tests assay techniques, see Tables 3 and 4. And a microbial population of 5.5×10^{8}cfu.

The identified pathogenic microbes from the wastes can cause numerous diseases in human beings and animals (Ofomatah, 2011).They include skin infections, urinary tract infectious, wound infections and food poisoning. The identification of these microbes makes it imperative for the users of biogas to be extremely careful in handling the wastes. It has also exposed the dangers of harbouring dirt and wastes in our environment. From the result it can be inferred that bacteria are the predominant microorganisms that carry out biogas production.

The substrate contained compounds which are toxic and possibly cancerous to human beings and ecosystem if not properly disposed. These organic compounds can be referred to as

persistent organic compounds or pollutant (POCs). They are chemicals of global concern due to their potential for long-range transport, persistence in the environment, ability to bio-magnify and bio-accumulate in ecosystems, as well as their significant negative effects on human health and the environment causing a serious imbalance in the living and non-living entities of the ecosystem (Elango *et al*., 2007). The chromatogram showed five peaks with their various organic compounds, area concentration, retention time and their chemical abstract series (CAS). Peak five with organic compounds such as 9,12-Octadecadieonic acid (ZZ)-2,3-dihydroxypropylester, E,Z-1,3,12-Nanodecatriene had the highest concentration of 85.86%, retention time of 21.676 is the major persistent organic compound in the fresh substrate.

The chromatogram of fermented cassava waste water showed three peak with their organic compounds, retention time, area concentration, molecular weight, molecular formula and their chemical abstract series. The three peaks had the same organic compounds with varying area concentration. The organic compounds present were propane nitrile and 2-propyn-1-amine. This implies that the secretion of certain endogenous enzymes by the microorganisms might enhance the degradation of recalcitrant substances in nature, this implies that anaerobic digestion can be used as a form of bioremediation. Therefore, fermentation of cassava could facilitate the decontamination of waste disposed into the environment for a desirable products formation, reducing environmental pollution and creating a balance in the ecosystem (Adeleke *et al*., 2017).

The relative percentages of these gases in biogas depend on the type of waste substrate and the management of the digestion process (Ofomatah, 2011). Contrary to the previous rearch report on biogas production by Eze (2000) and Yongfu (1989) on the composition of biogas, the composition of CO_2 in cassava waste water substrate was below the standard concentration of (20-50%) having a percentage concentration of (12%) this can be attributed to the management of the digestion process and the design of bio-digester. The use of aqueous $Ca(OH)_2$ regulate

pH by sequestrating CO_2 which can be used in the production of economically important substances such as biocarbonic acid reducing the carbon foot print. Also using fresh cassava wastewater gives a pH within the range which allows methanogens to thrive. The 74% values obtained for methane was high (50-80%) concentration as reported in previous research. This indicates that fresh cassava wastewater have excellent potential for anaerobic digestion because of the high organic load and carbohydrate content. In addition, they are generated in large quantities, reaching 60 m3 of effluent per ton of processed cassava (Sun *et al*., 2012).

4.1. Conclusion

The increasing demand for renewable energy compels the exploration of new substrates and the development of new technologies for biogas production. This study was aimed at using fresh cassava wastewater to generate biomethane. Anaerobic digestion of cassava wastewater usually face problems with low pH due to a fast acidogenesis which creates an acid environment that is not suitable for methanogenesis. Since reduction in pH of the system impedes methanogenesis, removal of CO_2 using $Ca(OH)_2$ as adopted in this work could regulate pH by sequestrating CO_2 which can be used in the production of economically important substances such as biocarbonic acid. This increases the activities of methanogens increasing methane yield. Using fresh cassava wastewater gives a pH within the range which allows methanogens to thrive.

The anaerobic digestion of cassava wastewater and other organic waste substrate in the production of biogas will go a long way in contributing to the domestic energy need. This will also improve the quality of environment by ridding the processing sites of pollution, reducing the emission of greenhouse gases, thus converting waste to wealth.

4.2. Recommendations

The following recommendations are made as a result of the findings in this study

- Effort should be made to optimize the pH at which the activities of methanogen are increased.
- Sequestration of carbon dioxide and production of biocarbonic acid could be commercialized after research.
- More study should be carried out on cyanide toxicity and detoxification.
- The sludge an important residue after biodigestion could be researched on for bio fertilizer production.

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