STUDIES ON BIOGAS AND BIOLIQUID PRODUCTION BY FUNGAL DEGRADATION OF BANANA (*MUSA SAPIENTUM*) LEAVES

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A thesis in the Department of CHEMISTRY Faculty of Natural Sciences
Submitted to the School of Postgraduate Studies, University of Jos, in
Partial fulfillment of the Requirements for the award of degree of **DOCTOR OF PHILOSOPHY IN APPLIED PHYSICAL CHEMISTRY** of the

UNIVERSITY OF JOS

JANUARY, 2011

DECLARATION

I hereby declare that this work is the product of my own research efforts; undertaken under the supervision of **Prof. M.M. Ekwenchi** and has not been presented elsewhere for the award of a degree or certificate. All sources have been duly distinguished and appropriately acknowledged.

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MUHAMMAD NASIR YARO(*PGNS/UJ/0020/04*)

CERTIFICATION

This is to certify that the research work for this thesis and the subsequent preparation of this thesis by **Muhammad Nasir Yaro** (PGNS/UJ/0020/04) were carried out under my supervision.

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_____

(Supervisor) ***Signature*** ***Date***

Dr. S.J. Salami _____

_____

(Head of Chemistry Dept.) ***Signature*** ***Date***

ACKNOWLEDGEMENT

All praises be to Allah (S.W.T.), Who spared my life to this time and blessed me with health and strength with which I undertook this research work.

I wish to greatly acknowledge with full respect my supervisor, Prof. M.M. Ekwenchi for the guidance, remarkable assistance, intellectual interpretation and valuable suggestions rendered during the course of this research work.

My sincere appreciation goes to Prof. T. Shambe, Prof. K.I. Ekpenyong, Prof. B.T. Nwufo, Prof. Y.N. Lohdip and Prof. (Mrs.) E.A. Adelakun for their positive contribution towards the success of this research work.

My sincere appreciation also goes to Dr. S.J. Salami, the Head of Chemistry Department, Faculty of Natural Sciences, University of Jos for his assistance and interest shown in this research.

I must extend my profound thanks and gratitude to Associate Prof. D.A Dashak for the advice, guidance and assistance offered me throughout the period of this research work.

I am very grateful to all other academic staff of the Department of Chemistry, Faculty of Natural Sciences, University of Jos for their contributions in one way or the other.

I am also very grateful to the following technical staff of the Department of Chemistry, Faculty of Natural Sciences, University of Jos: Rev. Ayewa, Mr. Dasam, Mallam Yakubu, Mr. Chaba, Mr. Yusuf, Miss. Makplang, Mrs. Bako, Mr. Sunny, Mr. Richard and Mr. Tony for the technical assistance given to me during the course of this research.

I appreciate the efforts of all the non academic staff of the Department of Chemistry, Faculty of Natural Sciences, University of Jos for their contribution in one way or the other.

I am highly indebted to Prof. A. A. Zuru and Prof. S.M. Dangoggo all of the Department of Pure and Applied Chemistry, Usmanu Danfodiyo University , Sokoto for laying my research foundation in biogas technology.

I am also highly indebted to my secondary school chemistry teacher, Mr. Irfan Ahmad Siddique, for laying my foundation in Chemistry. His contribution shall never be in vain.

I owe special respect and honour to my mother, Mallama Binta Abubakar Didare for the moral and financial supports offer me all the times.

I owe special love and regards to my beloved wife, Bilkisu Bala Muhammad; my sister, Bilkisu ('Yar-inna); and my Children: Halima, Khadijat, Fatima , Zulaihat and Muhammad for their tireless patience and endurance for leaving them alone throughout the period of this research.

I appreciate the remarkable efforts of Mr. Emeka Nweke, the director Janico Business Centre for typesetting the write up.

I finally thank all my friends and relatives especially the members of Tururuwa group for their contributions in one way or the other.

May Allah (S.W.T) help us in all our undertakings.

DEDICATION

This research work is dedicated to Allah (S.W.T.) and my parent.

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Abstract

Anaerobic biodegradation of banana leaves by cellulolytic fungus (yeast) was conducted at optimum operational conditions of concentration, temperature and pH for biogas and bioliquid production. Proximate analysis of both the fermented and unfermented substrates was carried out. Effects of nutritive additives on biogas and bioliquid production were investigated. The bioliquid generated was extracted by soxhlet extraction and, its components were separated into maltenes (soluble component) and asphaltenes (insoluble component) by precipitation. The maltenes contents of both the fermented and unfermented substrates were separated into fractions by column chromatography. Gas-chromatographic identification of organic component of the biogas generated was carried out using flame ionization detector (FID) while the heavier hydrocarbons in the bioliquid were analysed using gc-mass spectrometer. The analyses carried out revealed that, all the physio-chemical prametres employed, enhanced the production of biogas and bioliquid; the quantity of bioliquid generated from the fermented substrate was smaller than the quantity of the bioliquid generated from the unfermented substrate while the maltenes contents of the fermented substrate were higher than those in the unfermented substrate; the fractions of maltenes of the fermented substrate were in the order: saturates> polyaromatics > monoaromatics> diaromatics> resins and polars while the fractions of maltenes of the unfermented substrate were in the order: polyaromatics > monoaromatics >diaromatics >resins and polars> saturates; and the organic gas identified in the biogas was methane (CH_4) while the heavier hydrocarbons detected in the bioliquid were n-alkanes, iso-alkanes and cyclo-alkanes.

CHAPTER ONE
INTRODUCTION

1.1 BACKGROUND TO THE STUDY

The cost and scarcity of improved petroleum products used for agricultural, industrial and domestic fuels are drastically increasing. This makes it very difficult for most people to rise beyond subsistence level especially in developing countries like Nigeria. There is the problem of environmental pollution due to the release of by-products such as SO_2, PbO, CO_2, etc. when petroleum products are used in internal combustion engines. The use of firewood as fuel for domestic energy supply also causes environmental pollution as well as desertification, erosion and reduced biodiversity due to the frequent indiscriminate felling of trees. There is also the problem of tremendous amount of biodegradable wastes produced everyday due to increase in population, which of course constitutes nuisance to the environment and reduces the aesthetic (beautiful) value of the environment.

If the subsistence and the developmental needs of such developing countries are to be met as well as reduce environmental hazards, there is therefore the need to think about alternative energy sources, which are cheap, abundant and environmentally friendly.

Biogas technology appears to have promise as an alternative way of getting energy and at the same time, could alleviate environmental problems and enhance agricultural production.

1.1.1 **Alternative Sources of Energy**

The major alternative sources of energy are broadly divided into two (2) categories. They are: **renewable** and **non-renewable**.

Renewable energy is the energy obtained from the continuing or repetitive currents of energy occurring in the natural environment (Twindell & Anthony, 1990). The following are the major alternative sources of renewable energy: hydroelectric power; wind power; geothermal power, solar power and the power obtained from waste (Dangoggo, 1984).

Hydroelectric power is obtained from oceans and stream currents and it is limited by the number of rivers and water waves that can be utilized to drive generators. The total exploitation of hydropower potential of Nigeria was estimated at over 10,000MV and it is capable of generating 36,000 GWh of electricity annually (Sambo, 1992). However, only one-fifth of this power is being exploited as reported by Ahmad (2000).

Wind energy is obtained as a result of seasonal changes, it is used for sailing ships, windmills are used for pumping of water and milling as reported by Dangoggo (1984).

Geothermal energy is released from the interior of the earth core by conduction and heat convection from hot springs and volcanoes. However, the use of this form of energy is restricted because volcanoes and hot springs are not found everywhere (Dangoggo, 1984).

Solar energy is the most reliable because it is available everywhere, it cannot be depleted and it can be used in its original form or after conversion. Nigeria lies within a high sunshine belt and within the country solar radiation is fairly well distributed. The annual average total solar radiation varies from about 3.5 KWh/m^2/day in the coastal latitudes to about 7.0KWh/m^2/day in the far north. Solar radiation intensities are diluted when compared to the volumetric concentration of energy in fossil fuels (Sambo, 1992).

Power from waste is principally derived from biomass resources such as wood, forage-grasses and shrubs, animal wastes arising from forestry, agricultural, municipal and industrial activities as well as aquatic biomass. It has been reported by Garba (1998) that the biomass resources of the nation have been estimated at about 8 x 10^2 MJ.

Non-renewable energy is the energy obtained from static store of energy that remains bound unless released by human interaction (Twindell & Anthony, 1990). Petroleum (Crude oil), coal, natural gas and plants are the examples of non-renewable energy resources available in Nigeria.

(a) Liquid petroleum (Crude oil) is a complex mixture of organic compounds, principally hydrocarbons. Of these hydrocarbons, straight chain alkanes predominate, cycloalkanes (ring or cyclic alkanes), such as cyclopentane, cyclohexane, and their derivatives, are present in lesser

proportions, and there are even smaller amount of aromatic hydrocarbons. Traces of sulphur, nitrogen and oxygen compounds are also present in petroleum as contaminants. The predominance of alkanes (i.e. saturated hydrocarbons) in petroleum, and the absence of alkenes (i.e. unsaturated hydrocarbons), indicate that fats and possibly proteins were the likely starting points for petroleum formation (Hill & Holman, 1982).

Petroleum which means rock oil in Latin occurs as a dark viscous liquid in huge subterranean deposits, in many part of the world. It is a mixture of gaseous, liquid and solid alkanes (containing about one to forty carbon atoms per molecule), cycloalkanes, aromatic hydrocarbons, and others. It is generally believed to have been formed from the remains of microscopic plants and animals which lived in the warm inland seas millions of years ago. The chemical effects of pressure, heat and bacteria have converted these remains into petroleum (Ababio, 1985).

The actual proportions of the petroleum constituents depends on the source of the oil, but alkanes and cycloalkanes form the larger majority, with aromatics making only about 10% of the total hydrocarbons (Hill & Holman, 1982).

Petroleum has no significant use in its raw form (i.e. in crude form). To provide useful products, it components must be partly separated and, if necessary, modified. Petroleum has been a source of energy for heating, lighting and locomotion and, in particular, it provides

the most convenient of all fuels for the internal combustion engines. In fact, practically, all the petroleum processed up to about 1920 was used as fuel. It was only after 1920 that petroleum emerged as an important source of raw materials for industrial uses in

the chemical industry; and its importance in this field has had a phenomenal growth ever since as reported by Bamkole and Ogunkoya (1997).

Other sources of petroleum includes **bituminous (tar) sands** and **shale oil (kerogen).**

Bituminous sands – petroleum is some times found impregnated into sand or clay relatively near the earth surface. The exact mode of formation of such "sand" is not clear, but they are believed to be similar to conventional crude oil formation. The proportions of oil and water vary from 12 – 16% oil and 3 – 15% water. The separation of the oil from finely divided solids is a difficult matter, but considerable amount of experiments have been carried out which indicate that a practical method can be found (Mailabari, 1983).

Oil samples separated during exploration work showed considerable variation of properties, but all the oils are of the asphaltic type, with specific gravity ranging from 1.002 – 1.027 and 4 – 5% of Sulphur as reported by Mailabari (1983).

Shale oil (kerogen) – The reserves of shale in the world are considerable, amounting in oil equivalent to 20,000 million tons, but in view of the greater availability of petroleum, its utilization at present is comparatively negligible. Workable deposits are widely distributed in many parts of the world. In Britain, the production of shale oil was formerly an important source of fuel oil as reported by Mailabari (1983).

Just like in the formation of petroleum, similar conditions led to the formation of natural gas that is often found associated with petroleum as well as in deposits on its own as reported by Hill and Holman (1982). Natural gas is a convenient raw material for producing hydrocarbons with a low relative molecular mass. The major part of natural gas is methane (82-98%), the remainder being ethane, propane and buthane (Oganesian, 1989). Besides the use of natural gas as domestic and industrial fuel, Oganesian also reported that large amount of carbon black is obtained from natural gas, which is used in making Vehicle tires. He also reported that numerous organic substances such as some acids and alcohols are produced from the hydrocarbons contained in the natural gas.

(b) Coal: It is believed that about 350 million years ago there existed flat swamps and forest of huge trees in many parts of the earth. This period was known as carboniferous period (Bajah, Tebo, Onwu & Obikwere, 1988).

Coal originates from the vegetation of carboniferous era which was protected from complete decay by overlaying water-washed earth deposits. Decomposition occurred gradually under pressure and in the absence of air. Carbon IV oxide, methane and water were liberated, leaving behind a material that contained a very high percentage of carbon. During this process of carbonization, the vegetative materials were converted in stages into peat, lignite (or brown coal), bituminous (or

soft coal) and finally the anthracite (known as hard coal), which is about 95% pure carbon with some nitrogen content in it,

sulphur and phosphorus as impurities (Ababio, 1985). Carbon with naturally occurring hydrocarbons and other compounds are also found in coal (Bajah & Godman, 1975). Coal is widely used for domestic heating and power stations for generating electricity. Enormous quantities of coal are made into gas and coke for use in various furnace as pointed out by Bajah et al (1988). Besides the fact that coal is sued as fuel, it also provides valuable raw materials for the chemical industries.

The usefulness of coal is achieved after its destructive distillation; i.e. the industrial distillation in which it is heated to a very high temperature (1,200^{o}C) in the absence of air to give four main products, namely coal gas, coal tar, ammonical liquor and coke. The volatile products are collected at different temperatures. The composition and the uses of the industrial distillation products of coal as pointed out by Bajah et al (1988) are as follows:

Coal gas - consists of a mixture of gases such as hydrogen, methane, carbon II oxide, ethane and minute impurities such as sulphur IV oxide and hydrogen sulphide. Coal gas is used as fuel in industries. Coke, the residue from the destructive distillation of coal, is light and porous, but chemically similar to hard coal. It contains about 90% amorphous carbon. Coke is extensively used as both industrial and domestic fuel. It is also

used as reducing agent in the extraction of metals from their ores and in the manufacture of carbide. Gasification of coke gives rise to producer gas and water gas, which are also used as fuel.

Coal tar -This is a thick, brownish black liquid. It is a mixture of many organic chemicals, among which are benzene, toluene, phenol, nepthalene and anthracene. Coal tar is used to produce a host of useful chemicals, such as disinfectants, explosives for blasting rocks, pain killing drugs, etc., it is also used in making roads' surface.

Ammonical liquour - contains mainly ammonia. It is used in the preparation of ammonium tetraoxosulphate VI which is used as fertilizer.

(C) Plants (Vegetative biomass)-Plants (the total dry mass accumulated excluding the moisture) obtained from aquatic plants, natural vegetation and agricultural crops, which are used for domestic fuel for most rural populace and some urban dwellers. The use of plants for fuel is achieved by direct burning of the plants for immediate heat. Dry homogenous input is preferred for efficient energy production and effective heating. In addition to fuel benefit, some plants are used for making of shelter and other construction purposes.

1.2 CONCEPT

Scientific researches are conducted in order to come up with the new idea (s) that could greatly help in solving problems and /or uplifting the standard of living as well as lowering the cost of living. The current

fuel scarcity in Nigeria, and the environmental hazards associated with the use of petroleum products and fire – wood as fuel as well as the use of plants as ources of organic compounds could possibly be alleviated through the use of biogas and bioliquid as alternative sources of fuel and organic compounds respectively. This could only be possible when the necessary information needed about biogas and bioliquid technology are carefully studied and obtained.

1.3 GENERAL OBJECTIVE OF THE RESEARCH

The general objective of this research is to produce biogas and bioliquid from banana leaves by fungal degradation at optimum operational conditions.

1.4 SPECIFIC OBJECTIVES OF THE RESEARCH

The specific objectives of this research work are to:

(i) assess the potentiality of banana leaves as a substrate for biogas and bioliquid generation;

(ii) determine the effect of physio-chemical parameters - concentration, temperature and pH on biogas and bioliquid generation;

(iii) determine the influence of nutritive additives such as: urea, blood meal as source of protein), sugar, lipid (oil) and ethanoic acid on biogas and bioliquid generation;

(iv) assess the combined effect of buffering and addition of urea on biogas production and its composition;

(v) determine the moisture, ash (inorganic), volatile solids (organic), carbon and nitrogen contents as well as carbon to nitrogen ratio of the fermented and unfermented substrates (banana leaves);

(vi) determine the lignin, crude fibre, crude fat, crude protein, total carbohydrates and reducing sugar contents of the fermented ad unfermented banana leaves;

(vii) assess the maltenes (deasphaltened oils) and asphaltenes contents of the biolioquid extracted from fermented and unfermented banana leaves;

(viii) analyse the maltenes contents of both the fermented and unfermented banana leaves in terms of saturates, mono-aromatics, di-aromatics, poly-aromatics, and resins and polars contents by column chromatographic method;

(ix) identify by gas-chromatographic method the organic component of the gaseous degradation products (biogas) using flame ionization detector (FID), and the inorganic component (CO_2) using thermal conductivity detector (TCD); and

(x) identify the heavier hydrocarbons in the liquid biodegradation products (bioliquid) by G-C mass spectrometric analysis.

1.5 SCOPE AND LIMITATIONS OF THE RESEARCH

This research will consist of the laboratory analyses and assessment of the feasibility of generating biogas and bioliquid from banana leaves, and their potentials as sources of energy and other useful

organic products like saturates, aromatics and resins. The study will also assess the effect(s) of some physio-chemical parameters and the effect(s) nutritive additives on the biogas generating rate and yield as well as the influence of these

physio-chemical parameters on the composition of biogas produced. Percentage fungal degradation of the substrates, percentage gaseous degradation product (biogas) and percentage liquid degradation product (bioliquid) will be treated. Compositional analysis of the substrates (fermented and unfermented), column chromatographic separation of maltenes, gas-chromatographic analysis of biogas and G-C mass spectrometric analysis of bioliquid will also be treated.

The major limitation of this research was electricity power fluctuation, which if not supplemented with boiled water, could have rendered the microbes less active at temperatures below 33^0C and impair degradation. The problem of electricity power fluctuation was overcome by using boiled water and maintained the temperature of the water bath within which the digesters were immersed at 33^0C.

1.6 RESEARCH PROBLEM

There is the problem of over-reliance on petroleum products, which are scarce and expensive for domestic and industrial fuels as well as the ecological problems associated with the use of petroleum products, indiscriminate felling of trees for fire-wood and improper ways of disposing agricultural wastes (banana leaves inclusive) by our local

farmers. In order toreduce/minimize environmental pollution and nuisance in our environment the practice of biogas and bioliquid production from wastes would greatly be of paramount importance.

1.7 SIGNIFICANCE OF THE RESEARCH

Banana leaves are among the abundant crops residues (as agricultural wastes) in most gardens and in some farms, and most farmers dump it on the swampy farmlands to decay aerobically to be used as green manure. Some farmers also burn them to ash, to supplement fertilizer. The practice of dumping banana leaves leads to the generation of large quantity of CO_2 by aerobic fermentation; through hydrolysis followed by oxidation, which causes environmental pollution like "green-house effect" and ozone layer depletion. The burning of banana leaves is a wasteful process, which also generates gaseous pollutants in the atmosphere, and also causes the destruction of some useful micro-organisms in the soil. In addition, dumping of organic substrates (banana leaves inclusive) under favourable anaerobic fermentation condition leads to the generation of methane gas in the atmosphere without control. This also causes a serious environmental hazard because methane gas is a greenhouse gas that remains in the atmosphere for considerable length of time as reported by EPA(1986); EPA (2005). It was also reported by Ayodele and Emmanuel (2007) that methane gas is more effective in trapping heat than carbon (iv) oxide.

The scientific perception of the problems associated with the dumping and burning of agricultural wastes of any kind (banana leaves inclusive) coupled with the high energy demands for crops processing in modern farming system nowadays, made it necessary to think about an appropriate and effective way of dealing with such wastes on farmlands and gardens, as well as to think about an environmentally friendly and cheaper sources of energy for crops processing and other energy demanding processes. The production of biogas and bioliquid is of course one of the ways through which biodegradable wastes of any kind can be converted into useful products (i.e. fuel and biofertilizer); the rate of felling trees for firewood could drastically be reduced, which would in turn save the environment from erosion and desert encroachment; and would also minimize the utilization of petroleum products and natural gas, whose combustion also cause total environmental pollution (i.e. air, land and water pollution) via the formation of some solid and gaseous by-products.

CHAPTER TWO

LITERATURE REVIEW

2.1 APPROACHES TOWARDS THE DEVELOPMENT OF BIOGAS TECHNOLOGY

It has been reported by Ahmad (2000) that the techniques of biogas production have been in existence since 1850s. Methane was first recognized as having practical and commercial value in the 1890s in England, where a specially designed septic tank was used to generate the gas for the purpose of lighting streets (Garba, 1998). In India, for example, methane generating units and plants using cow dung had been in operation for years; in Taiwan, more than 7,500 biogas generating devices utilizing pig manures have been constracted; in Europe, for example during second world war, methane was produced in order to provide fuel for automobiles; and in the USA, there has been considerable interest in the process of anaerobic digestion as a means to generate safe and clean fuels as well as biofertilizer (Bryant,1979). Presently, in Bangladesh, there are about 22 million cattle that excrete about 0.11 million tones of dung per night that can produce an average of 1.48 x $10^9 m^3$ of biogas per year which is equivalent to 0.75 x 10^6 tons of kerosene or 1.52 x 10^6 tons of coal (Cheremisionff, 1980).

In order to develop the technique as well as to improve the quality of biogas and maximize its utilization, several but different researches were conducted.

For instance, Ekwenchi, Akunwanne, Okeke and Ekpenyong (1989) investigated the possibility of obtaining gaseous fuel from fungal degradation of lignocelluloses from elephant grass at $33^{O}C$ using four cellulolytic fungi and a bacterium, which were harnessed from air, isolated in pure form and identified. The analysis of the gaseous products obtained showed that the biogas contained CH_4, C_3H_8 and CO_2. It was also found that the saturates content of the bioliquid of the fermented slurry was very high with no polars at all while the bioliquid of the unfermented slurring was rich in polyaromatics with very little saturates. The research also revealed that two species of fungi, Curvularia Spp. and Penicillium, were responsible for the degradation. The other two, Fusarium species and Aspergillus niger, including the bacterium gave no gaseous products.

In their own contribution, Singh, S.K, Singh, A. and Pandey (1992) studied the effect of additives on bioconversion of biomass into methane; they used Na, Mg, Fe, Al, Sn and Cd salts as additives on Enchorial Crassipes at different concentrations. The work revealed that addition of Na and Al salts enhanced biogas production; Fe, Mg and Cd salts showed inhibitory effects; and Sn salt completely stopped the production of the biogas.

Anaerobic biodegradation of lignocellulose from water hyacinth using specific fungus (Curvularia Spp.) was investigated by Airehrour (1994). The work revealed that at low temperature ($28^{o}C$), the activity of the Curvularia

was low compared with the activity at relatively higher temperatures (33^0C and 38^0C). But above 38^0C, the curvularia's activity drastically decreased.

In 1994, anaerobic biodegradation of the crude and extracted lignocelluloses from water hyacinth was investigated at room temperature by Audu. The work revealed that unextracted (crude) lignocelluloses gave better biogas yield than the extracted one.

In 1995, Ekpenyong, Arawo, Melaiye, Ekwenchi and Abdullahi studied the biogas production potential of unextracted nutrient – rich elephant grass. The work revealed that crushed unextracted elephant grass straw is biodegradable by mixed microorganisms as effectively as by pure cultures. The work also showed that support with potato dextrose agar as well as nutrients such as urea is essential for attaining substantial gas yield.

Machido, Zuru and Akpan (1996) investigated the effects of inorganic nutrients on the performance of Cow dung as substrate for biogas production. The work revealed that high or extremely low nitrogen content present undesirable factor in biogas production.

Effect of seeding on biogas production using pigeon droppings was investigated by Aliyu, Dangoggo and Atiku (1996). The study revealed that the production of biogas was enhanced by seedling with active sludge

at mesophilic temperature. It was also found that seedling of slurry helps in prolonged and uninterrupted biogas production.

In 1996, Garba, Zuru and Sambo studied the effect of slurry concentration on biogas production. The study revealed that the total volume of biogas produced in 6 weeks of digestion of the cattle dung was directly proportional to the slurry concentration in the range of 187.72 – 326.01kg/m^3.

Physio-Chemical studies on biogas production were conducted by Tambuwal, Dangoggo and Zuru (1997). The study revealed that biogas production was higher at higher temperature, and the effect of temperature out weighed that of seeding. It was also found that Chemical additives such as urea, NH_4NO_3, $(NH_4)_2SO_4$ and yeast did not favour the generation of biogas at temperature range of 22^{o}c.

Ezeonu (1997) worked on an experimental evaluation of energy economics of biogas production at mesophilic and thermophilic temperatures. The work showed that within the tropics where energy production is the priority, mesophilic digestion proved to be the more economically viable option while thermophilic digestion is suitable only where consideration for environmental hygiene (since infective pathogens are destroyed at this temperature) and rapid digestion of wastes is intended irrespective of the operation cost.

Effect of urea on the fungal degradation of lignocellulose from elephant grass was studied by Akor (1999). The study showed that low

concentration of urea enhanced the yield of methane while high concentration was toxic to the microbes, and thus killed them.

Yerima, Rahman and Ekwenchi (2001) studied the effect of buffering on biogas fermentation of chickens droppings. The work revealed that 0.3M buffer solution of pH 7.6 gave high biogas production.

Maduagwu (2002) studied the effect of pH on anaerobic biodegradation of the lignocellulose from maize cob and maize stalk using curvularia spp. The study revealed that pH 7.3 was the best and pH below 6.2 rendered the microbes inactive.

Chemical composition of total hydrocarbons and quantitative determination of n-alkanes in Nigerian bitumen was carried out by Mailabari in 1983. The investigation showed that the saturates content of the maltenes was relatively higher than the aromatics and polar compounds contents.

These efforts and many others are the motivating factors responsible for designing this research, in order to justify the effects of physio-chemical parameters and additives on biogas and bioliquid generation and their respective compositions from banana leaves.

2.2 SOURCES OF BIOGAS AND BIOLIQUID

The proportion and the relative composition of biogas and bioliquid obtained depend on the biodegradable portion of the biomass substrate, and is mainly determined by the nature of the biomass and prevailing physio-chemical conditions.

In nature, there are many raw materials from which biogas and biliquid can be extracted: human and animal manure, leaves, twigs,

grasses, stalks from crops, and so also agricultural and industrial wastes whose organic content is greater than 2% (Ariane, 1985). Practically any kind of watery organic substance can be used as slurry for anaerobic digestion for the production of biogas and bioliquid. Wastes from ruminant animals are very useful for starting the fermentation process, because they already contained the necessary methanogenic bacteria (Dangoggo & Fernando, 1986).

It should be noted that, whatever the source of biogas and bioliquid is, the important part from which they are produced is the biomass.

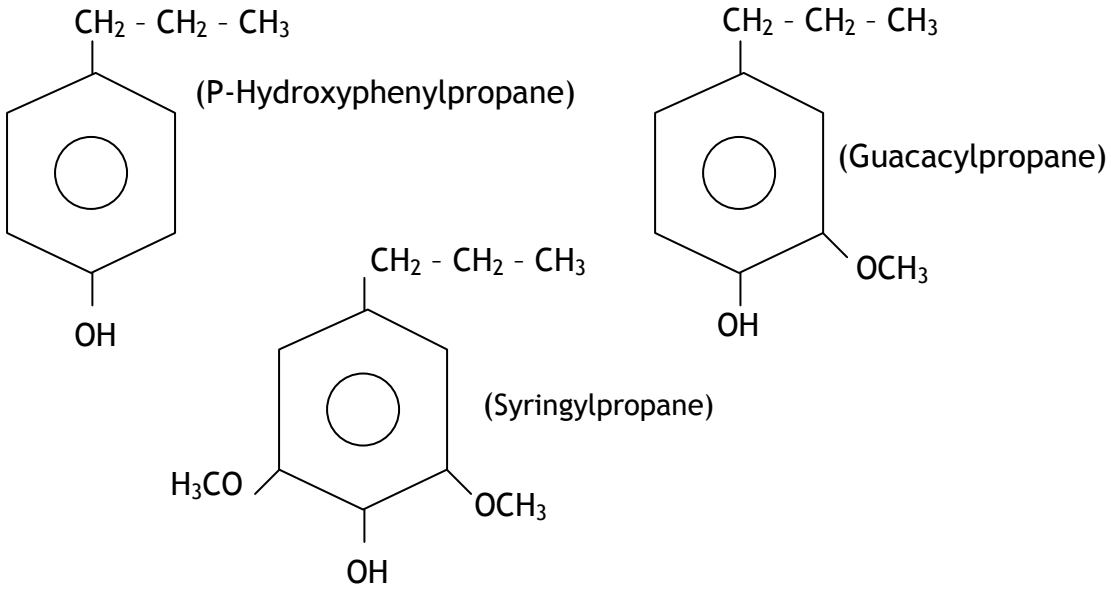
2.3 **BIOMASS**

Biomass is any material that is directly or indirectly derived from plant live excluding the moisture, and that is renewable in time period of less than 100 years. Typical biomass resources are energy crops farm and agricultural wastes and municipal waste. Animal wastes are also biomass materials in that they are derived either directly or via the food chains, from plants (Probstein & Hicks, 1982).

2.3.1 **Composition and Structure of Biomass**

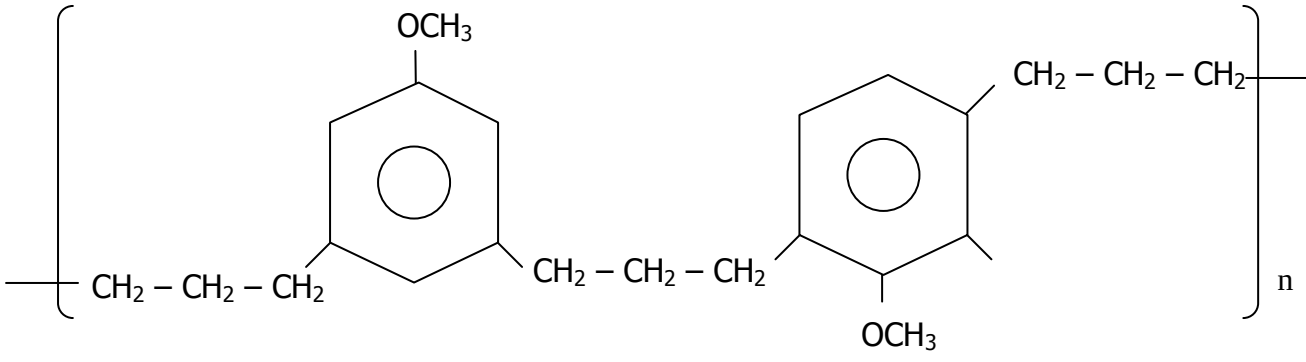
The lignocellulosic materials constituents of biomass consist of cellulose, hemicellulose and lignin in the ratio 4:3:3 as reported by Maduagwu (2002).

(a) **Lignin** - This is the non-cellulose resinous component of wood but a polymer of single benzene ring linked with aliphatic chains. The phenolic compound polyhydroxylphenyl propane is an important monomer group in lignin (Seymour & Carraher, 1992). Below are examples of lignin monomers:

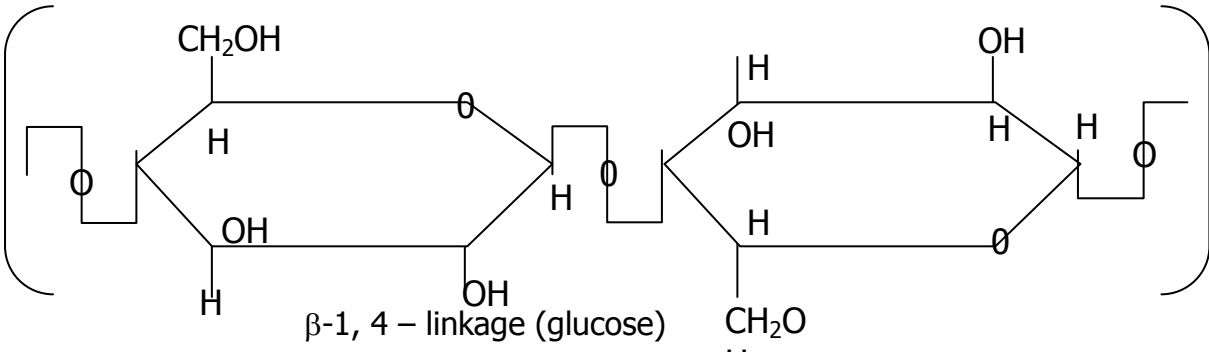


Lignin is a characteristic constituent of the walls of the cells, and act as natural glue providing the plant with added mechanical strength. In other words, it is the cementing agent that binds the matrix of cellulose fiber into a rigid woody structure known as lignocelluloses.

It is a phenyl propane polymer of amorphous structure comprising (about 15 – 30%) of wood. Its structure varies from plant to plant. Below is the skeleton structure of lignin as reported by Maduagwu (2002):



(b) **Cellulose** -This is the naturally occurring carbohydrates polymer consisting of repeating glucose units. It is insoluble and consists of β-D-glycospyranose units linked by β (1-4) bond to form long, straight chains strengthened by cross link hydrogen bonds. It cannot be digested by many mammals including humans because of the absence of a hydrolase (microbes) that attacks the β-linkage. Thus, it is an important source of 'bulk" in the diet. In the gut of ruminant and other herbivores, there are microorganisms that can attack the β-linkage, make cellulose available as a major – calorigenic source. This process can also take place to a limited extent in the human body as reported by Maduagwu (2002). The structure of cellulose is given below:

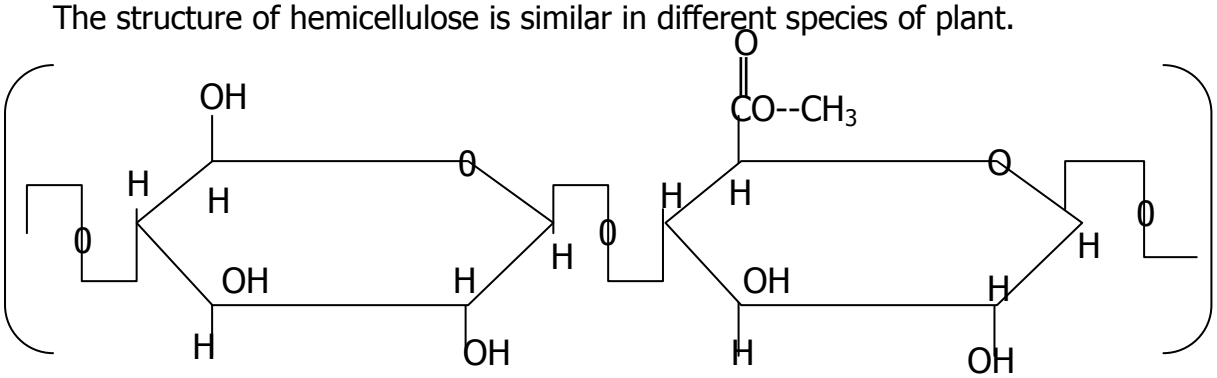


β-1, 4 – linkage (glucose)

Cellulose derives its strength from its fibrous composition, which results from the hydrogen bonding within the chain, maintaining a linear conformation (Emsley & stevens, 1994).

(c) **Hemicellulose -** This is a short chain polysaccharide formed from non glucose. Hemicellulose is water soluble polysaccharide similar to cellulose,

strictly described as pentosans. Sugar, for instance mannose, arabenose, xylose, etc. It assists x-cellulose with a lower degree of polymerization. The structure of hemicellulose is similar in different species of plant.



Partial structure of Hemicellulose.

(d) **Lignocelluloses** - This is the most important fraction of the total biomass existing for biogas and bioliquid generation. Lignin combines chemically with cellulose to yield lignocelluloses. It does not exist alone in nature, rather it co-exists with the cellulose fraction of the cell component, and the bonded cellulose constitutes what is known as lignocelluloses (Emsley & Stevens, 1994).

2.3.2 **Biomass Conversion Processes**

Biomass (lignocelluloses) has not only played role as a significant source of fuel in the world energy economy, its importance as a source of chemicals has also been realized (Twindell & Anthony, 1990).

Different methods of biomass conversion are now available. However, not all the conversions are brought about by biological means, but in all cases the substrate is a biomass of one form or the other. The methods available for biomass conversion are physical, biological and thermo chemical processes.

a. **Physical Process** -This includes particles size reduction of the biomass, the separation of the biomass into components and drying to remove all part of water in the biomass (Audu, 1994).

b. **Thermo-chemical Process** - This includes the following processes:

i. **Gasefication**: This is a process of heating biomass in the presence of limited supply of Oxygen to produce maximum amount of CO and H_2. The crude gas produced consists of varying amount of CO, CO_2 and H_2. Depending on the type of process and substrate employed, quantities of N_2, small amount of CH_4, and heavier hydrocarbons are also formed (Audu, 1994).

ii. **Pyrolysis** – This means the decomposition of a compound by the action of heat alone when applied to petroleum, the decomposition is referred to as thermal (cracking, and it is usually carried out at high temperature in the absence of air and often in the presence of catalysts (Bamkole & Ogunkoya, 1977). In this process, the organic matter is heated to break into fragments. The output depends on the temperature, type of input materials and treatment. In some processes, the presence of water is necessary and need not be dried. This process has been the source of many organic chemicals such as acetone, methanol oil, solid char and gases, usually the heating is done slowly over a long period of time

to give maximum yield of charcoal. It could be exothermic or endothermic, depending on the temperature of the system.

iii. **Burning**: This is a direct combustion of immediate heat. Dry homogenous input is preferred for efficient energy production.

(c) **Biological Process** - This includes fermentation of the substrate into ethanol and CO_2 by the action of enzymes (yeast).

Burning, pyrolysis and gasification are the thermo-chemical processes of producing fuel and energy from biogas while fermentation is a biological process from which biogas and bioliquid are produced.

2.4 BIOGAS AND BIOLIQUID

2.4.1 Biogas, Composition and Properties

Biogas is a flammable gas produced by microbes when organic materials are fermented in a certain range of temperature, moisture content and acidities, under airtight conditions. Biogas is a mixture of CH_4 (60 – 70%), CO_2 (30 – 40%), and small quantities of H_2S, N_2, H_2, CO, and several other hydrocarbon compounds (Ariane, 1985).

The fact that methane (CH_4) is the major component of biogas, the properties of biogas could therefore be deduced from those of methane.

(a) **Physical Properties -** Biogas (CH_4) is colorless, tasteless and odourless flammable gas, but the presence of hydrogen sulphide (H_2S) gives a slight smell of garlic or rotten egg to biogas. CH_4 is a

very stable hydrocarbon due to its low molecular weight. According to Ariane (1985), the weight of biogas (CH_4) is roughly half that of air:

$$\frac{1m^3 \text{ of } CH_4}{1m^3 \text{ of air}} = \frac{0.716Kg}{1.293kg} = 0.554$$

Ariane (1985) also pointed out that the solubility of CH_4 in water at 20^oC and atmospheric pressure is very low, only 3 units of CH_4 (volume) can be dissolved in 100 units of water.

(b) **Chemical Properties of Biogas -** Because the major component of biogas (i.e. CH_4) belongs to alkanes homologous series, and alkanes are non polar, they are generally unreactive toward common reagents under normal conditions. Thus, CH_4 undergoes no reactions in the cold with dilute acids, alkalis, oxidizing agent and most other reagents, and this gives rise to an early notion that CH_4 and other alkanes are inert and earned for them the name paraffins from latin 'parvum' means little and 'affins' means affinity or attraction (Bomkole & Ogunkoya, 1977). In fact, under appropriate and more drastic conditions, alkanes (CH_4 inclusive) undergo reactions. The following are important reactions of biogas (CH_4):

(i) **Halogenation** – Halogen, especially Chlorine and bromine reacts with biogas (CH_4) to form halogenated biogas (halogenated methane) as illustrated below:

$CH_{4(g)} + Cl_{2(g)} \rightarrow CH_3Cl_{(g)} + HCl_{(g)}$ …….. (1). the reaction can be brought about in two ways Viz: by exposing the mixture of biogas (CH_4) and halogen to light (U.V.); and by heating the mixture of biogas (CH_4) and halogen (Bamkole & Ogunkoya, 1977).When the reaction is brought about by light, it is described as **photochemical reaction**. When the reaction proceeded in the presence of heat, it is described as **thermo-chemical reaction**. The thermo chemical reaction can be achieved even in the absence of light but not at room temperature.

(ii) Combustion or Oxidation – Biogas (CH_4), in the presence of oxygen or strong oxidizing gents at high temperatures, burns to give CO_2 and H_2O. The oxidation or combustion of biogas (CH_4) to CO_2 and H_2O is exothermic, and this is the basis of its use as fuel;

$CH_{4(g)} + 2O_{2(g)} \rightarrow CO_{2(g)} + 2H_2O_{(l)}$ ……..(2) $\Delta H = -887.008KJ$.

On complete combustion, $1m^3$ of CH_4 can reach a temperature of 1400^oC and releases 35827.4KJ while complete combustion of $1m^3$ of biogas (CH_4) can release 23012 – 27196 KJ (Ariane, 1985).

2.4.2 **Bioliquid, Composition and Properties**

Bioliquid could be described as the extract of the liquid obtained through anaerobic fermentation of organic waste (fermented slurry) by soxhlet extraction. Bioliquid is relatively heavier than biogas, and usually

composed of Maltenes (saturates, monoaromatics, diaromatics, polyaromatic and small quantities of resins and polars); and asphaltenes (resins and polars).

2.4.3 **Maltenes (Deasphaltened Oils)**

Maltenes are a component of bioliquid that is soluble in hexane – methanol solution mixture of volume ratio 40:1 V/V per gram of maltenes at low temperature (usually below 21^{o}C). Maltenes consist mainly of C, H and very small quantities of N, S and O, and they are generally paraffinic in nature as reported by Oladapo (1988).

2.4.4 **Asphaltenes**

These are a component of bioliquid which is insoluble in hexane – methanol solution mixture but soluble in carbondisulphide. They consist mainly of C, H, and some quantities of N, S and O, an they are generally aromatics in nature (Oladapo, 1988).

2.4.5 **Properties of Maltenes and Asphaltenes**

Maltenes and asphaltenes belong to a suggested class of fermentation products called residua. Treatment of the residua with hexane-methanol solution at temperatures not exceeding 21^{o}C dissolves the maltenes (as soluble components) and precipitates out the asphaltenes (as insoluble component).

The maltenes always exceed the asphaltenes, though there is considerable variation in the ratio. The maltenes and the asphaltenes contain about half of the total nitrogen and sulphur in substrates.

Most of these are in the form of heteromolecules, which are condensed into both aromatic and naphthenic rings. The asphaltenes are more aromatic and contain more N and S as well as oxygenated compounds than maltenes as reported by Oladapo (1988).

A simple way to compare the paraffinicity and aromaticity of any fossil fuels or its fractions such as maltenes and asphaltenes is by showing the ratio of C to H. The C/H ratio drops sharply from maltenes to asphaltenes. This is because the asphaltenes are more compacted with more condensed aromatic rings having less hydrogen as reported by Oladapo (1988).

The lighter the bioliquid component, the more paraffinic and thus the maltenes are more paraffinic than ashaltenes. Therefore, maltenes are more combustible than asphaltenes.

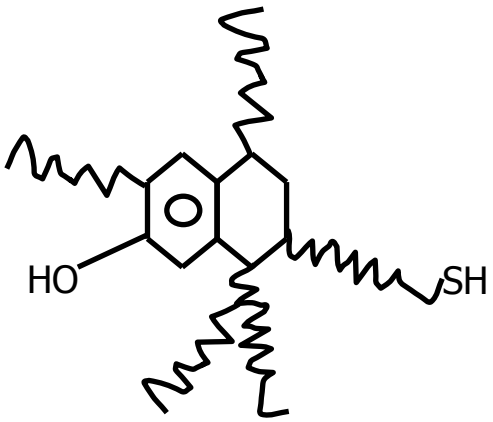
2.4.6 **Structure of Maltenes and Asphaltenes**

The structure and properties of these two compounds vary with bioliquid sources and the solvent used for the precipitation. This reveals the inadequacy of the present knowledge of maltenes and asphaltenes structure. Another reason that has contributed to the difficulty in structural investigation of asphaltenes is that, it is an extremely hetrogeneous mixture (Spears & Whitehead, 1969).

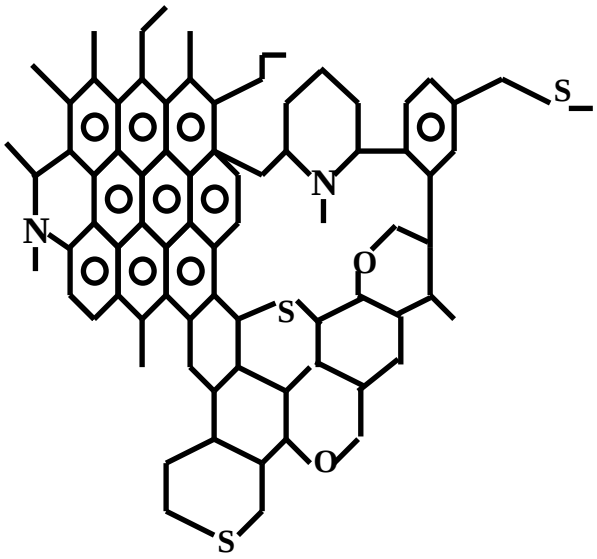
Some of the techniques that have been used in the past for the structural determination of matlenes and asphaltenes have been reviewed. These include: mass spectrometry (Clerk & O'neal, 1960).

Infrared (IR), Nuclear Magnetic Resonance (NMR), Electron Paramagnetic Spin Resonance (EPSR), X-Ray diffraction and electron microscopy (Dickie & Yen, 1966) have all been used to define the distinct outline of the asphaltenes molecules.

A true understanding of the structure of maltenes and asphaltenes has not yet been established but the structures proposed from several studies are given below as reported by Oladapo (1988):



(**Schematic Maltenes Molecule)**



(**Schematic Asphaltenes Molecule)**

2.4.7 **Classification of Biogas and Bioliquid**

Based on the fact that biogas consists mainly of CH_4 and CO_2, it could therefore be classified according to the CH_4 and CO_2 contents. Below are the three suggested classes of biogas:

(a) High Paraffinic Biogas – when the CH_4 content exceeds 60% and the CO_2 content is not up to 40%

(b) Moderate Praffinic Biogas – when the CH_4 content is 50 – 60% and the CO_2 content is 40% - 50%

(c) Low Paraffinic Biogas – when the CH_4 content is below 50% and the CO_2 content exceeds 50%.

Bioliquid, on the other hand, could be classified according to the number of side chains, number of rings and asphaltenes contents. Based on this, Brame and King (1967), classified bioliquid as follows:

(a) Praffinic bioliquid, if the side chains are over 75%

(b) Naphthenic bioliquid, if the naphthenic rings are over 70%

(c) Asphaltic bioliquid, if the asphaltenes content exceed 60%

(d) Paraffinic – naphthenic, if the side chains are 60 – 70% and naphthenic rings are over 20%

(e) Naphthenic – aromatic, if the naphthenic rings and aromatic exceed 30%.

2.5 CHEMISTRY OF BIOGAS AND BIOLIQUID PRODUCTION

Biogas production from organic substrate is a microbial process that involves the combined action of four groups of bacteria in four stages (Khandelwal and Mahdi, 1986). The stages are:

The **First Stage** is the degradation of higher molecular weight substances like cellulose, starch, fats, proteins etc. present in organic materials into low molecular weight compounds like fructose, glucose etc. that are able to pass through bacterial membrane by a group of hydrolytic bacteria. Thus polymers are transformed into monomers by enzymatic hydrolysis (Aireh-rour,1994).

$n(C_6H_{10}O_5)_{(s)} + nH_2O_{(l)} \rightarrow nC_6H_{12}O_{6(aq)}$ ---------- (3)

In the **Second Stage**, the product of the first stage is converted into organic acid and by-products like CO_2, H_2O, H_2, NH_3 etc by a group of bacteria known as acetogens, which are collectively called "acid former"

$n(C_6H_{12}O_6)_{(aq)} \rightarrow 3nCH_3COOH_{(aq)}$ ---------- (4)

The **Third Stage** is the conversion of hydrogen and simple carbon compounds produced in the second stage into ethanoic acid by a group of bacteria called homoacetogens.

$4H_{2(g)} + 2CO_2(g) \rightarrow CH_3COOH_{(aq)} + 2H_2O_{(l)}$ ---------- (5)

The **Fourth Stage** is the conversion of ethanoic acid and some other compounds like CO_2 and H_2 into CH_4 by a group of bacteria known as methanogens.

$CH_3COOH_{(aq)} \rightarrow CH_{4(g)} + CO_{2(g)}$ ---------- (6)

$CO_{2(g)} + 4H_{2(g)} \rightarrow CH_{4(g)} + 2H_2O_{(l)}$ ---------- (7)

Bioliquid (maltenes and asphaltenes), which is usually heavier than biogas is produced simultaneously with the biogas in the fermentation pit during fermentation. The bioliquid is obtained from the fermented liquid through soxhlet extraction. The asphaltenes, which usually contain resins and polars are separated from maltenes which usually contain saturates, monoaromatics, diaromatics, polyaromticas and small quantities of resins and polars by precipitations.

Usually in the biogas digestion unit, all the four stages are occurring simultaneously, and if any one stage gets out of hand, the

production of CH_4 will adversely be affected. This is particularly true of the acid forming stage, as for example acidity prevents methanogens from functioning (Airehrour, 1994).

Maduagwu (2002) has reported that the following stoichiometric equation is the general equation for methane formation by fermentation of any organic substrate:

$$C_aH_bO_{c(s)} + (a - {}^b/_4 - {}^c/_2)\, H_2O_{(l)} \longrightarrow ({}^a/_2 - {}^b/_8 + {}^c/_4)\, CO_{2(g)} + \text{-----(8)}$$
$$({}^a/_2 + {}^b/_8 - {}^c/_4)\, CH_{4(g)}$$

where a, b and c = 6, 10 and 5 respectively.

The production of CH_4 can also be achieved through the processes illustrated by the equations below:

$4H_2CO_{2(g)} + H_2O_{(l)} \rightarrow H_2CO_{3(aq)} \rightarrow CH_{4(g)} + 3H_2O_{(l)}$ ----- (9)

$2CH_3CH_2OH_{(aq)} + CO_{2(g)} \rightarrow CH_{4(g)} + 2CH_3COOH_{(aq)}$ —---- (10)

$2CH_3CH_2OH_{(aq)} \rightarrow 3CH_{4(g)} + CO_{2(g)}$ ..... (11)

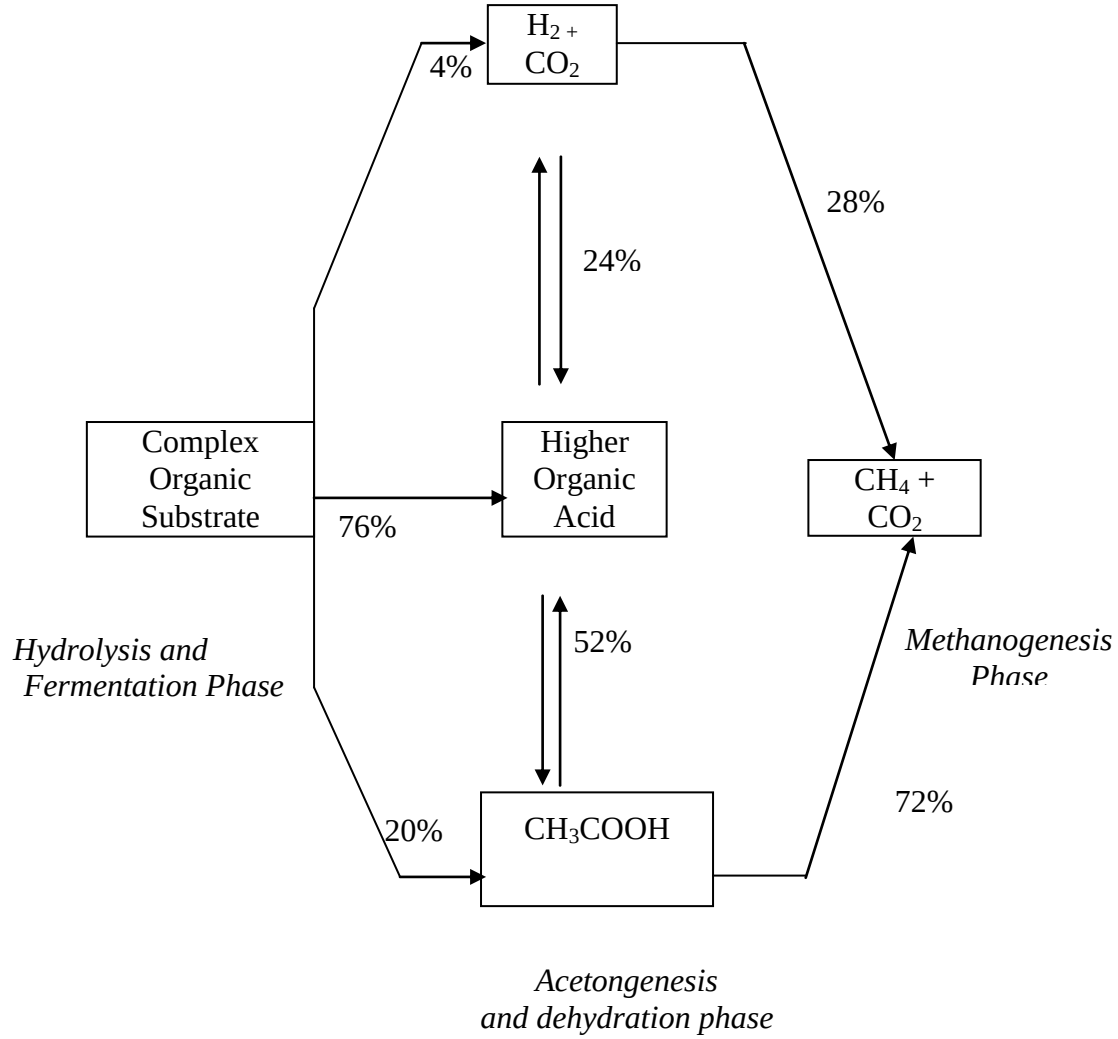
$2CH_3COOH_{(aq)} + H_{2(g)} \rightarrow 2CH_{4(g)} + 2CO_{2(g)} + H_2O_{(l)}$ --- (12)

$4CH_3CH_2COOH_{(aq)} + 2H_2O_{(l)} \rightarrow 7CH_{4(g)} + 5CO_{2(g)}$ –-(13)

$2CH_3CH_2CH_2COOH_{(aq)} + 2H_2O_{(l)} \rightarrow 5CH_{4(g)} + 3CO_{2(g)}$ –- (14)

$2CH_3CH_2CH_2CH_2COOH_{(aq)} + 2H_2O_{(l)} \rightarrow 7CH_{4(g)} + 3CO_{2(g)}$ --(15)

It has been reported by McCarthy (1964) that the activity of the microbes in the conversion of organic substrate into Methane can be represented diagrammatically as follows:



2.6 THEORY OF METHANOGENESIS

Methanogenesis simply means generation of methane from organic wastes by micro-organisms. It is also known as biomethanisation or simply methanisation (Jimenez, Cantagena & Arce, 1990).

The theory - The process in which methane is formed from organic substrates is an energy yielding oxidation–reduction process in which organic substrates (usually ethanoic acid formed by acetogens) serve as electron donors and acceptors (Seeley & Van Demark, 1972). It has been reported by

Airehrour (1994) that the basis of this theory is that the organic compounds

fermented by Methanogens are completely oxidized to Carbon IV Oxide and this oxidation process is followed by a reduction process in which some or all of the Carbon IV oxide formed is reduced to methane. This can best be explained by the Van Niel equation for fermentation of ethanoic acid.

The Van Niel process for the fermentation of ethanoic acid is as follows:-

Oxidation:

$CH_3COOH_{(aq)} + 2H_2O_{(l)} \rightarrow 2CO_{2(g)} + 8H^+_{(g)}$ ------ (16)

Reduction:

$8H^+_{(aq)} + CO_{2(g)} \rightarrow CH_{4(g)} + 2H_2O_{(l)}$ ------ (17)

Overall:

$CH_3COOH_{(aq)} \rightarrow CO_{2(g)} + CH_{4(g)}$ ------ (18)

2.7 METHANE – PRODUCING MICROBES

These are a group of extremely specific bacteria which are very sensitive to oxygen and oxides, and thus their anaerobisis is specific. They grow very slowly because they can only utilize simpler organic and inorganic materials as their substrates. It was reported by Okafor and Uzuegbu (1993) that when oxygen content in the fermenting liquid of $5cm^3$ reaches $0.8g/dm^3$, the growth of methanogenes will be damaged. All the methane-producing bacteria can use hydrogen and carbon IV oxide as their substrates to yield methane and most of them can utilize methanoic acid to generate methane as pointed out by Smith, Hein and Greiner

(1979). One of the species called Sarcina has been reported to utilize methanol, methylamine and ethanoic acid but not methanoic acid in many instances, ethanoic acid has been pointed out as the critical substrate for the production of methane, amounting to approximately 74% of the substrate (Kumar & Garde, 1979).

2.8 NON-METHANE PRODUCING MICROBES

It should be noted that some microbes contribute in the fermentation pits towards activities such as hydrolysis and other conversion processes but not methane formation. These kinds of microbes are known as non-methane producing microbes. These microbes are of three categories; namely: bacteria, fungi and protozoa. Of all, bacteria are the most essential (Garba, 1998).

(a) **Bacteria -** There are many kinds of non-methane producing bacteria that participate in methanogenesis. Those that have hydrolytic activity account for a small group of the overall colonies. Among these, the obligate anaerobes (those that cannot grow in the presence of even minute traces of oxygen) are 100 – 200 times more than the facultative anaerobes (those that can grow in the presence or absence of oxygen) and the anaerobes are those involved in non-methane producing step. Such microbes include Clostridium butyruim, bacillus lactum, many positive cocci e.t.c (Garba 1998).

(b) **Fungi -** They take part in the methanogenesis from which they got nutrients for growing e.g. mould and yeast.

(c) **Protozoa** - These are thought to play a minor role in the process (Kumar and Garde, 1979). They were detected during the process of methanogenesis and protozoa detected were mainly plasmodium (Smith et al, 1979).

2.9 PHYSIOLOGY OF MICROBES (FUNGI)

The majority of the known fungi live on dead organic matter, performing a useful service in returning to the soil nutrients originally extracted by plants, but there is a large group of species which are enemical to man's activities through their habit of parasitizing plants which are grown for food and clothing, and a smaller group which are parasitic on animal, including man himself. A number of species are capable of synthesizing, under suitable conditions, substances useful to man, e.g. biogas, bioliquid and biofertilizers. Because of these benefits, it is important to study the physiology of fungi, so as to know the favourable conditions that can stimulate their growth and activities.

The growth of fungi, like that of all other living things is profoundly affected by the environment. Water and oxygen are both absolutely necessary for growth of fungi and in addition, the following elements are known to be required: carbon, nitrogen, potassium, phosphorus, sulphur and magnesium. Iron is needed in small amounts by many species and

possibly by all as reported by Golueke (1958). The actual quantity of food material required to support growth is very small.

All fungi require oxygen for growth. Some fungi produce a typical structure when growing, submerged in liquid, but growth is slow and depends on the oxygen dissolving in the fluid. The spores of a great many filamentous fungi will germinate when immersed in a liquid medium, but grow exceeding slowly. Normal metabolism results in the breakdown of some of the organic food material to carbon IV oxide and unless this is continually removed and replaced by a fresh supply of air, growth ceases, whether on account of the diminishing of oxygen tension alone or by actual poisoning is not known (Airehrour, 1994).

A supply of water is absolutely essential for the growth of all fungi, although a few species can thrive in the presence of very small amounts of available moisture. The total amount of water contained in any particular substance does not necessarily determine its liability to fungal attack. Materials consisting mainly of cellulose are liable to attack if the moisture content exceeds about 8% as pointed out by Airehrour (1994).

Fungi, like other classes of living things are affected adversely by the presence of certain substances in their food as reported by Airehrour (1994).

Fungi show great differences in their response to temperature changes and in their resistance to heat and cold. Thermal dead points however cover a fairly wide range; some strains being killed by prolonged

exposure to a temperature of 33^{o}C, while fungi responsible for much trouble in the canning industry can survive the normal sterilization process for canned fruits in which, the temperature exceeds 90^{o}C for a short time. In the same way, the spores of many are killed by freezing in the presence of water. Some fungi will flourish at temperatures sufficient to inhibit the growth of the majority of species and to kill some of them as reported by Airehrour (1994).

2.10 FACTORS REQUIRED FOR MAXIMUM BIOGAS AND BIOLIQUID GENERATION

Since fermentation occurs as a result of the action of many sorts of anaerobic micro-organisms, the better the living environment of those micro-organisms, the faster the production of biogas, and if proper conditions cannot be maintained, biogas and bioliquid generation will drastically be reduced or ceased altogether (Chen & Inbar, 1991). Therefore, the optimal fermentation conditions for those microbes are:-

(a) **Air tightness** – None of the biological activities of anaerobic micro-organisms, including their development, breeding and metabolism, requires Oxygen: in fact they are very sensitive to the presence of oxygen. It has been reported by Ariane (1985) that the breakdown of organic materials in the presence of oxygen produces CO_2 while in the airless (absence of oxygen) produces CH_4.

(b) **Suitable Temperature** – Under suitable temperature conditions, the micro-organisms become more active and biogas is produce at a higher rate. CH_4 can be produced in a fairly wide range of

temperatures, depending on the prevailing conditions. It has been reported by Maishanu and Hussain (1990) that three types of fermentation are possible at high temperature (50–55^{o}C), medium temperature (30–35^{o}C) and ordinary temperature (10–30^{o}C). Digestion at higher temperature proceeds more rapidly than at lower

temperature with gas yield rates doubling at about every 5^{o}C rise in temperature (Zubr, 1988).

(c) **Necessary Nutrient** – There should be plentiful materials for the normal growth of the micro-organisms and the microorganism must be able to extract plentiful nutrients from the source of fermentation (Ariane,1985). The main nutrients are carbon, nitrogen and inorganic salts.

(d) **Carbon to Nitrogen ratio (C/N)-** For proper biogas production, a specific ratio of carbon to nitrogen must be maintained, between 20:1 and 25:1, and the carbon to nitrogen ratio varies for different substrates, and sometimes even for the same substrates (Ariane, 1985). For proper biogas and bioliquid production, the carbon to nitrogen ratio should not exceed 30:1 (Garba, 1998), and extremely low nitrogen content as compared to carbon in a substrate, always present undesirable factor in biogas production (Machido et al, 1996).It has been reported by Obinwanne (1999) that carbon is needed by the microbes for energy supply. Carbon is

also important for high yield of CH_4 and nitrogen is required for bacterial growth, and deficiency of nitrogen limits the growth ofbacteria while over supply of nitrogen is detrimental, because of the liberation of ammonia gas, which in excess is toxic to the microbes as pointed out by Garba (1998). The main sources of nitrogen are human and animal excrements while the main sources of carbon are the polymers in crops stalks as pointed out by Ariane (1985).

(e) **Water Content (Concentration of the Slurry) –** There must be a suitable water content as micro-organisms excretive and other metabolic processes require water. The water content should normally be around 90% of the weight of the total content (Ariane, 1985). Ariane also reported that both too much and too little water are harmful to micro-organisms during fermentation, because if the water content is too much, the rate of production per unit volume in the pit will fall, and if the water content is too low, ethanoic acid will accumulate, inhibiting the fermentation process and hence production, also a rather thick scum will form on the surface. It is worth noting that the water content should differ according to the difference in raw materials for fermentation.

(f) **Suitable pH** – The micro-organisms require a neutral or mildly alkaline environment – a too acidic or too alkaline environment will be detrimental, therefore a pH between 7 and 8.5 is best for

fermentation and normal gas production (Ariane, 1985), and pH below 6.2 renders microbes inactive (Yaro, 2003).

(g) **Mixing/Stirring or Shaking** – Biogas production is a biochemical process that is dependent on intimate contact between the microbes and the feed stock material. By stirring the slurry frequently, one can ensure contact between the methane forming bacteria and the fermentation materials, which will produce maximum biogas. Stirring the slurry with suitable device provided both effective contact and prevents scum formation, which will otherwise create obstruction. It was noted by Ogbeide (1988) that biogas production was low when the slurry was stirred continuously, and no biogas was produced when stirring was completely avoided due to scum formation. Therefore, stirring/shaking has to be done intermittently. When biogas pits (digesters) are not stirred, the fermentation materials settled into three layers: **the top layer** is scum with high content of fresh materials, very few microbes in both number and variety and much acid was produced; **the middle layer** is clearly fermented materials containing very little solids and also few microbes; and **the bottom layer** is sediment and residues rich in many kinds of microbes, but low in fresh materials because it was under a high hydrostatic pressure, the biogas produced was dissolved in the

fermenting liquid and was not easily released (Khandelwal & Mahdi, 1986).

(h) **Nature of the Substrate** – The chemical composition of a substrate determines the carbon content, nitrogen content and pH of the slurry (Dangoggo & Fernando, 1986). Dangoggo and Fernando also reported that for plant materials, the content of biodegradable and soluble substrate varies from species to species and generally decrease with the increase in the age of the plant, while cellulose, lignin, hemicellulose and polythronides (pectins, gums and mucilage) contents increase. The presence of lignin protects the cellulose from bacterial action (Amamatu, 1995). On the other hand, materials with high cellulose and hemi-cellulose produce more biogas as reported by Garba (1998). It was found that young plants produce more biogas than the old ones and dry vegetables produce more biogas than green ones (Dangoggo & Fernando, 1986).

Animal wastes vary in chemical composition, physical forms and qualities mainly due to the variability in the digestive physiology of the various species, the composition and the form of the diet, the stage of growth and productivity of the animals; and the management system of waste collection and storage. Also the quality and the composition of manure primarily depend on three factors as reported by Yaro (1993). The factors are:

(i) the amount of feed eaten and its digestibility. About 40-80% of the organic content of the feed reappears as manure (for instance cattle excrete about 33% of their fibrous fodder);

(ii) quality of fodder utilization; and

(iii) the life weight of the animals.

The quantity and composition of digestible and soluble materials in animal wastes vary with livestock. Poultry produces more volatile solids, nitrogen and phosphorus per unit weight of animals than any other animals, (Dangogo & Fernando, 1986). However, Dangoggo and Fernando also reported that the biogas yield from ruminant's wastes could be lower than that obtained from non-ruminants, because ruminant animals extract a higher percentage of nutrients out of the fodder, and the left-over lignin complexes from high fibre fodder are very resistant to anaerobic digestion.

(i) **Addition of Nutrients.** – Addition of various additives like cattle urine, urea and poultry excreta may stimulate and accelerate bacterial activity that would subsequently increase biogas yield as pointed out by Qasim, Warren and Udonsinrot (1984). Other additives may be toxic or inhibitory to the biogas production process. Thus, toxicity results in decrease of biogas production, sometimes to zero-gas production. The nutrients may generally be organic or inorganic substances. Small concentration of ammonium

nitrate increase the activities of anaerobic bacteria and remain effective in accelerating biogas production till the end of 40 days fermentation period (Garba & Sambo, 1995). The micro organisms that aid the formation of biogas and bioliquid are easily affected by many harmful materials, which interfere with their livelihood. Maximum allowable concentrations of some harmful materials are given in Table 1 below:

Table 1: **Maximum Allowable Concentrations of Some Harmful Materials**

S/N	Harmful Materials	Concentration (mg/dm^3)
1	Sulphate ion	5,000
2	Sodium Chloride	40,000
3	Copper	100
4	Chromium	200
5	Nickel	200 – 500
6	Cyanide ion	Below 25
7	Alkyl Benzene Sulphonate	20 – 40
8	Ammonia gas	1,500 – 3,000
9	Sodium	3,500 – 5,500
10	Potassium	2,500 – 4,500
11	Calcium	2,500 – 4,500
12	Magnesium	1,000 – 1,500

Source: Ariane (1985)

Alkali and alkaline earth metal salts may be stimulatory or inhibitory to biogas production depending upon the concentration. The toxicity is associated with cations rather than the anions portion of the salt. Some cations are antagonists while others are synergists, such that their combination become quite complex (Amamatu, 1995).

Some organic materials that may inhibit digestion process include alcohols, which are toxic at high concentrations. But when introduced gradually at low concentration can be degraded as rapidly as they are introduced. Sodium oleate has been found to be toxic at concentrations over 500mg/dm^3. However, concentrations as high as 200 – 300mg/dm^3 can be tolerated (Amamamatu, 1995).

(j) **Seedling with Bacteria –** Normally after feeding the digester, it takes some time before the gas production starts, but when seeded with bacteria, the production commences immediately as reported by Contois (1959).

Enzymes can also be added to the slurry in order to alter (promote) the speed of biogas and bioliquid generation from the substrate. Enzyme reactions are reported to proceed in two stages, (Sharma, 1981). Viz:

i) Formation of complex between the enzyme and the substrate as shown below:

$$E + S \rightleftharpoons ES \quad \ldots\ldots\ldots\ldots \quad (19)$$

where, E is the enzyme used, S is the substrate and ES is the complex formed between the enzyme and the substrate.

ii) Decomposition of the complex into reaction products with regeneration of enzyme, ES $\xrightarrow{K}$ products + E ------ (20).

2.1.11 BIOGAS AND BIOLIQUID TECHNOLOGY

The development of biogas in rural China emphasized on economy, self reliance and mobilization (Ariane, 1985).

In order to achieve maximum gas production and lasting biogas plant, it is worth encouraging to work hard and build solid and practical biogas pits of simple construction, with low demand of materials, low in cost (less expensive but qualitative) and easy to build. Each and every pit or digester constructed should meet the technical specifications so as to avoid unnecessary waste of resources. Therefore, before a biogas digester or pit is built, there should be exhaustive study and discussion of its size, the model to be used, the location and the materials.

Different digesters are available for use as household plants which are small scale type of about 8 – 10cm^3 in capacity, community scale plant having capacity greater than 40cm^3, digester plant associated with disposal of industrial wastes and plant associated with intensive animal dung. In general, biogas digesters can be divided into two main types: continues process type; and batch type digester.

2.11.1 Large Scale Continuous Processes

This category consists of three tested and field worthy designs of biogas units, they are (a) floating gas holder type (b) fixed – dome type and (c) Ganesh Model. About 250,000 floating gas holder type units and 200,000 fixed dome type biogas have already been set up in India, and another model known as Ganesh has been approved for promotion (Khandelwal & Mahdi, 1986).

(a) **Floating Gas Holder Type -** This design was first developed in India in 1954 by the Khadi Village Industry Commission (KVIC), Bombay adapted it from promotion in 1962. Therefore, it is also known as KVIC type Gobar gas plant (Khandelwal & Mahdi, 1986). A floating gas holder type biogas unit consists of digester, gas holder, inlet and outlet assembly and water removal device as shown in Fig. 1 below. The diameter and the depth of the digester vary according to the capacity. There are two models of floating gas holder plant, they are the **horizontal model** and the **vertical model**. The horizontal model is recommended for rocky areas with the high water tables (about 1.5m or less). The vertical model is suitable for non-rocky areas with low water tables (water nearby wells about 3m or more). For both of these models, the average daily production capacity ranges from 1 – 85m^3. Floating gas holder type requires less excavation work compared to fixed-dome type, it is easy to locate defects and the release of gas is at constant pressure.

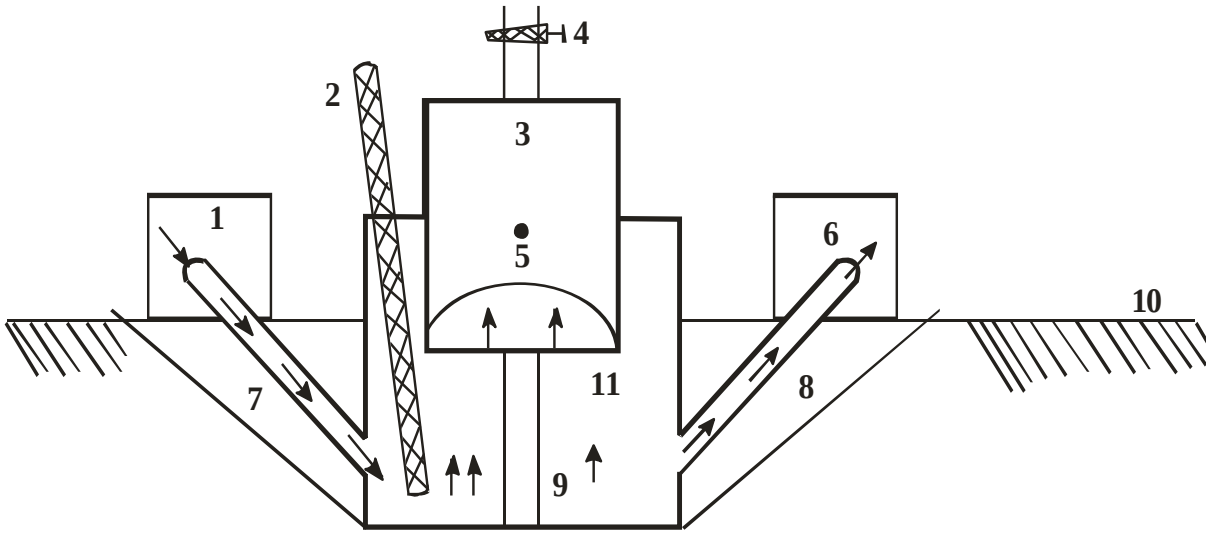


Fig. 1: Floating gas holder type

Key:

1. Slurry Inlet
2. Stirrer
3. Gas holder
4. Gas tap
5. Metallic central guide frame
6. Digested slurry outlet
7. Undigested slurry conveying pipe
8. Digested slurry conveying pipe
9. Partition Wall
10. Slab cover
11. Digester

On the other hand, floating gas holder type is expensive to maintain and requires high capital investment, and the life's span of the gas holder is relatively lower than the life span of the gas holder in the fixed-dome type (Khandelwal & Mahdi, 1986).

Mode of operation - When the gas outlet is opened, the gas so collected is pushed out into the pipeline by the weight of a drum, which is kept upside down on the digester as gasholder. The constant pressure of the gas is normally 8 – 10 centimeter water column. The drum moves up when the gas produced is collected in it and moves down when the gas is removed. This up and down movements of the drum is guided by a central guide pipe fitted in a frame which is fixed to the digester's wall (Khandelwal & Mahdi, 1986).

(b) **Fixed- Dome Type -** This was first developed by the State Planning Institute, Lucknow, in India, in 1978. It is an improved version of the Chinese fixed – dome biogas plant. The fixed dome type biogas unit is entirely a masonry structure. It dispense with the use of steel gas holder both the digester and the gas holder form an underground combined units as shown in Fig 2 below. The dimension of the inlets and outlets are bigger than those of the floating gas holder biogas unit, with the other parts common to both of the designs. The diameter and the height ratio of the digester is fixed at 1.75:1.

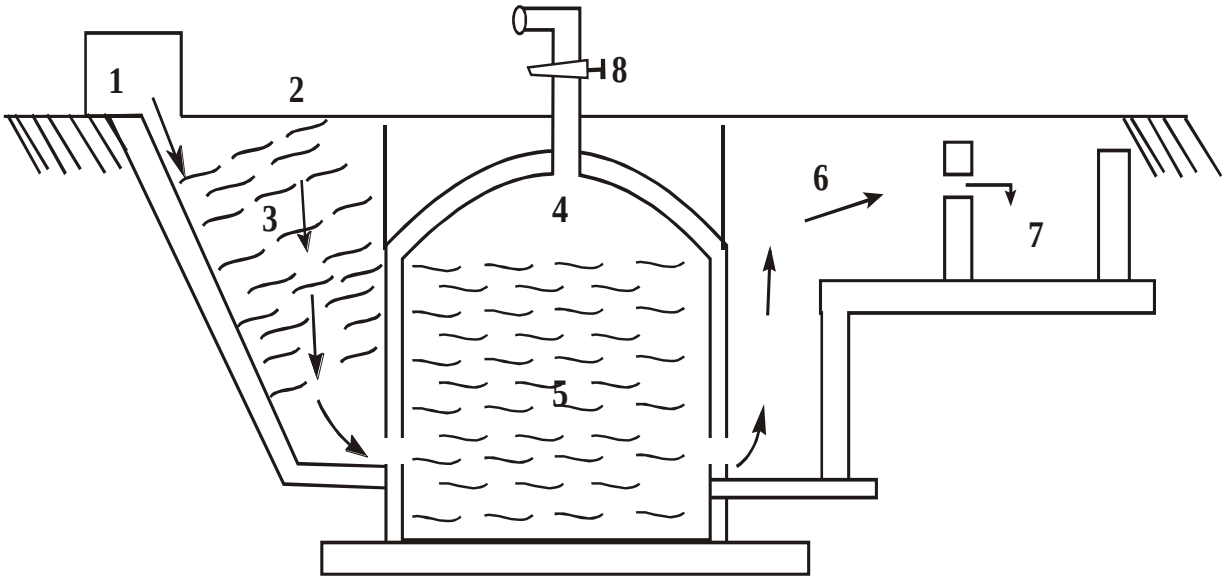


Fig. 2: Fixed - dome type

Key

1. Mixing chamber
2. Slab cover
3. Slurry inlet
4. Dume (as gas holder)
5. Digester
6. Digested slurry outlet
7. Digested slurry overflow
8. Gas tap

The volume of the dome is 60% of the plant capacity (Khandelwal & Mahdi, 1986). Although, in the fixed-dome type, thecost of maintenance and the capital investment are low, it requires no steel gas holder, both the digester and the gas holder have high life span, the effect of low temperature is less, and the space above the plant can be used for other purposes as the plant is underground structure. On the other hand, the release of gas is at variable pressure, it requires high excavation work, location of defect in the dome is very difficult and the construction of the plant is also difficult in high water table areas.

Mode of operation – When the gas is produced, it rises up and gets collected in the slurry. The pressure of the gas pushes the slurry down and causes its diffusion into the outlet chamber. The gas is liberated at a variable pressure ranging from 0 – 90cm water column. The volume of gas stored in the plant at any given time is equal to the volume of the slurry dispatched in the inlet and the outlet chambers (Khandelwal & Mahdi, 1986).

(c) **Ganesh Model -** This type of model has been developed in the district of Rampur Utter Paradesh in India. It is similar to the floating gasholder type design with the exception that the digester portion is made up of an angled iron frame wrapped with polythene sheet instead of a masonry structure. The cost of installation of the digester is about 30 – 40% less than that of floating gasholder type. It is easy to transport

materials required for the fabrication of the digester and less time is taken to install it (Khandelwal & Mahdi, 1986).

The merits and the demerits of each design need to be considered while selecting a model to be constructed. It also depends on certain important factors.

It is worth nothing that biogas production occurs simultaneously with the formation of bioliquid during fermentation.

2.11.2 **Small Scale Batch Type**

This is a laboratory scale set up, constructed for experimental purposes. The digesters used in this type are very small in size when compared with the ones used in large-scale continuous type and therefore can be used as household plants. Though in small scale batch type, more gas is generated per unit volume of slurry; the process takes twice as long as in continuous process. It is preferred for the fermentation of more fibrous materials than large scale continuous process (Itodo, Lucas & Kucha, 1992).

2.11.3 **Management of Biogas Pit**

In order to maintain the normal fermentation process and fulfill the user's need throughout the year, one must manage the pit properly. Practice has shown that there is a great difference between conscientious and careless management. When conscientiously managed pits of small capacity will produce much gas and ensure continuous supply. On the other hand, pits of large capacity, poorly managed, may produce less gas

than smaller pits or may not produce gas at all (Checchi, Traverso, Medici & Fazzine, 1990). In the proper management of biogas pits, the following practices should be noted:

(a) **Mixing of materials:** By stirring the liquid (slurry) intermittently one can ensure contact between the methane producing microbes and the fermentation materials which in turn will produce maximum gas. When pits are not stirred, the fermentation materials will settle into three layers: **The top layer** is scum with high content of fresh materials, very few microbes in both number and variety and much acid is produced here; **the middle layer** is a clearly fermented materials containing very little solids and also few microbes; and **the bottom layer** is sediment and residue rich in many kinds of microbes, but low in fresh materials because it is under a high hydrostatic pressure, the gas produced is dissolved in the fermenting liquid and is not easily released (Khandelwa & Mahdi, 1986).

(b) **Pile composting of materials** – In order to increase the rate of fermentation fibrous materials, the materials should be piled and composted before feeding into digesters, especially straw, grass, weed and maize stalks – must thus be treated because some of them have waxy layer on the surface. Otherwise, not only the materials are hard and the process is lengthy for the materials to rot, but once fed into the pit, they float up to the surface and tend not to mix evenly with the other materials. To pile and compost, the materials are cut into short pieces and

piled up in layers, each layer about 50cm thick. It is best to sprinkle on some materials with a 2-5% lime or ash content, and then also pour on some human or animal manure or waste water then cake over with clay (Khandewal & Mahdi, 1986).

(c) **Rational supply of materials** – In order to maintain a plentiful supply of nourishment for microbes and to ensure continual production of gas, one must keep on supplying fresh materials at reasonable intervals for fermentation as well as water and some of the old materials should be replaced.

2.11.4 Factors to be Considered while Selecting of a Model

The following factors are of paramount importance in the selection of the type of a model to be constructed for large scale biogas production:

Technical factor – The floating gas holder type can be constructed with moderated skill by village masons who are generally conversant with the construction of water wells, whereas, the building of fixed dome type unit involves greater skill and care. It is necessary to acquire know how through training for constructing a leak-proof masonry structure.

Provision of a device to break scum formed on the slurry is also necessary. In the floating gas holder type, the scum can be broken by rotating the holder half way for 5 – 10 minutes once a day. A bamboo/iron rod has to be inserted through the outlet opening to stir the slurry in a fixed dome type which is a cumbersome practice as pointed out by Chowdhury and Fulford (1992).

Equally important is the removal of digested slurry. In a floating gas holder plant type whenever fresh material is added, the digested slurry comes out automatically due to gravitational force. However, in a fixed - dome type plant, often, slurry does not overflow and then it has to be removed by buckets.

Availability of construction materials should also be considered. Good quality cement and bricks should be ensured for fixed - dome type plant while a gas holder fabricated from specified mild steel sheet in a workshop is also needed for a floating gas holder type plant.

Climatic factor - The rate of biogas and bioliquid production is highly dependent on the atmospheric temperature as the plants do not have provision for heating and automatic temperature control (Cheremisionff, 1980).

Geographical factor – Generally, any model can be built where a digester pit can be excavated to about 3m without blasting and removing underground water. However, the horizontal floating gas holder type plant is designed for high water areas, i.e. less than 2m for most part of the year. In such areas, the fixed- dome type should have a reinforced cement concrete foundation.

A biogas and boliquid unit should not be located within a radius of 15m from a drinking well or tube well. This will ensure that no contamination of water with the dung or slurry due to seepage that takes place.

Economic factor – The cost of installation of the floating gas holder type plant is about 20 – 30% higher than hat of a fixed-dome type plant. The cost of the Ganesh model is slightly less than that of fixed - dome type. However, the cost would vary according to the prevailing market price of the materials at a given time.

It is obvious that the plant selected should be cheap provided other factors are also favourable. The fixed-dome type is certainly cheaper than any other model as locally available materials like stone slabs and lime can be used for the construction of the digested portion of the plant as reported by Chein and Hashimoto (1978).

The cost of maintenance should also be as low as possible. Steel gas holder requires painting at least once a year thereby increasing the maintenance cost of floating gas holder type plant.

2.11.5 **Basic Components of a Biogas and Bioliquid Pit**

One of the determinations of the production of biogas is the quality of the pits. These pits must be absolutely hermetically sealed so that the whole pit is watertight and the gas sections are airtight. This requires conscientious work and a strict scientific attitude throughout the process of construction. Each and every pit built should meet the technical specifications as any slackening of attention to quality in the building of these pits will interfere with normal gas production, affect the durability of the pits, and may even require far more work to remedy defects. It might, in the end, waste labour, building materials and time. So before the pit is

built, there should be exhaustive study and discussion of its size, the model to be used, the location and the materials. After thorough investigation, exact plans should be drawn up. The use of gas should be matched with the scientific disposal and treatment of excreta for fertilizer. To build toilets and pigsties above biogas pits economizes on land and permits direct connection of the excreta through with the biogas pit that the human and animal excreta can flow into it automatically. This also saves on labour, and increases the efficiency of excreta disposal. Additionally, in winter, this also serves as insulation, helping to maintain the temperature in the biogas pit and thereby ensuring normal production of gas. In order to achieve these aforementioned benefits and ensure safety, the following considerations have to be considered as suggested by Khandelwal and Mahdi (1986):

(a) **The material inlet -** This is where the materials to be fermented enter into the fermentation compartment. This inlet should be large to allow easy introduction of materials. It is normally a slanting tube or trough. The lower end should be opened into the fermentation compartment at about mid-height. The inlet should also be linked to the excreta troughs of toilets and pigsties. The inlet should incline enough to ensure the natural flow of these materials into the fermentation compartment.

(b) **The sludge outlet -** This is where the sludge from the fermentation process is extracted. Its size should depend on the volume of the

pit: there should be an adequate distance between inlet and outlet to prevent freshly incoming materials from going into the outlet.

(c) **The separation wall -** In the rectangular pit, this separation wall creates a gas storage tank. For the round pit, the separation wall is the wall above the mouths of the inlet and outlet. The depth of the wall is normally calculated downwards from the top of the tank so that it comes to about half the total depth of the pit. If the inlet mouth is too low, the sludge accumulated at the bottom of the pit may cause blockages of the inlet and outlet. Also, if the separation wall is built too low, it will impede air circulation, and pose a danger of suffocation for the people who clear and maintain the pit. If the separation wall is too high, it diminishes the gas storing capacity of the tank, especially at times when fertilizer is needed. If one extracts a little, too much fertilizer and the liquid contents fall below the separation wall, this will cause gas to escape from the tank.

(d) **The Fermentation compartment and the gas storage tank**

These two sections are actually one entity. They connect the inlet and outlet and form the area where the gas is produced and stored. The middle and lower sections are the fermentation compartment, the upper is the gas storage tank, with the cover above it. When the fermentation material is let into the fermentation compartment, gas is produced through the action of micro-organisms and fermentation breakdown and it rises to the

upper section and into the gas storage tank. This compartment and the tank are the principle sections of the pit, and should be strictly sealed to be completely water and airtight.

(e) **Water pressure tank -** The water pressure tank is built above the gas storage tank, with the cover to the pit forming both ceiling of the gas tank and the bottom of the water tank. Around the perimeter of the cover, a ridge about 40cm high could be built, with a hole about 5cm in diameter going through it just above the inlet.

As the gas rises into the gas storage tank, the liquid below presses down; this raises the level of the liquid in the outlet. When it surpasses the height of the cover, the liquid flows through the hole into the water pressure tank: when the gas pressure decreases it flows back out of the water tank into the pit. As the gas is being produced, the liquid level rises; when the gas is being consumed, the liquid level fails, so that by the automatic changes in the water pressure above, the gas within the tank will be maintained at a constant pressure. Through practical experience in many regions, builders took to increasing the volume of the outlet and also increasing the high of the inlet and outlet above the cover until this served the function of a water pressure tank, which subsequently did not have to be built separately. Furthermore, this allowed them to pack in earth on top of the cover, which helped to increase the

pressure exerted by the cover board and also helped maintain the temperature within the pit.

(f) **The gas outlet pipe -** The gas outlet is set into the gas tank cover. At the bottom, it opens into the gas storage tank, level with the bottom of the cover. At the upper end, it may be connected to a plastic or rubber hose tubing to pipe the gas to where it will be used. The connecting pipe may be made of steel, hard plastic, or clay; it is usually 1 – 1.5m long depending on the amount of earth above the cover. The diameter of the pipe should be determined by the diameter of the hose that one wants to fit on to it.

(g) **Mixer** - The mixer is normally made of wooden sticks. It is used to stir the fermenting liquid and to break through the crust or scum formed on the surface of the liquid, so as to let the gas come through normally. According to accepted practice, it is not necessary to fix a mixer into small pits built for individual families. For any large pit of volume exceeding 100cm^3 a mixer should be employed so as to guarantee the normal production of gas.

(h) **Fermentation chamber the pit -** At present, two main shapes of pit are being used in the countryside round and rectangular. In regions where stone is available, it is convenient to build a circular pit out of stone slabs (flat stones having one dimension much smaller than the other two, forming thin slabs that can be used for

surfacing); or stones of irregular shape; or a rectangular and circular pits can be built out of triple concrete.

Triple concrete is a traditional Chinese building material, and in Sichaun it is normally made from lime, sand (which may contain small stones or pebbles), and clay, or lime, crushed clinkers and clay, mixed in specific proportions with water dry and liquid triple concrete differ chiefly in their water content. Dry triple concrete contains roughly 17 – 22% water and must be pounded into place during construction. The liquid version contains more water and is spread onto existing surfaces; its use in the province is not widespread.

2.11.6 **Plant Operation, Maintenance and Precautions**

A biogas and bioliquid plant should be tested for any water seepage and gas leaks before it is put in use. When the plant is in use, frequent checking and maintenance are very essential. Necessary actions should be taken as soon as possible when any problem develop. Only then will the plant be utilized efficiently provided that necessary precautions are taken.

(a) **Floating gas holder type**

Operation – The following operational strategies are of great importance as pointed out by Khandelwal and Mahdi (1986):

(1) **Testing** – Before the plant is filled with the slurry, the digester, gas holder and gas pipeline should be tested for gas leaks. Examine

the digester wall all over by tapping with a small stick and listen for any hollow sound indicating unfilled space in the wall. The hollow space should be chiseled and repaired. The steel gas holder should be tested for gas leaks by keeping water in it overnight. It can be put to a smoke test also by burning a cloth dipped in kerosene inside the holder and watching for the smoke coming out of any of the joints.

The site of leakage should be welded again. Only the tested and painted gas holder should be placed on the digester. However, for testing the gas leaks in the gas holder, after placing on the digester, or when in use, weights should be put on the holder to increase the pressure. The soapy water should be applied to any suspected leaks, especially to spots or rust or welding joints.

(2). **Starting the Plants** – This includes the following:

(a) Slurry preparation – Mix dung with water in a 1:1 or 1:1.25 proportion. This will bring the concentrating of total solids in the slurry to 7.9% because fresh dung contains about 20% total solids and 80% moisture. It can be found through experiments that 7.9% total solids in the slurry give optimum gas production.

It should be ensured that foreign materials like earth, sand, gravel, saw dust, soap, detergent, etc. do not enter the plant. A sieve e.g. chicken-wire can be fitted over the inlet pipe to prevent straw, lumps of dung, stones, etc. from entering the digester.

Dung and water can be mixed manually or with a piece of wood or by means of mixing device until there are no lumps.

(b) Filling the digester – The slurry should be added as quickly as possible. Dung can be collected for up to about 10 days prior to the first filling. However, the dung so collected should not be allowed to dry up and become hard as it can not be mixed easily with water to form slurry. The inlet pipe should be blocked with a piece of wood or a stone wrapped in gunny bag. The plug should be removed from the inlet pipe and care should be taken to see that the plug does not slip down the inlet pipe. While filling the digester with a petition wall for the first time, care must be taken to fill both the compartments equally or else the partition wall will collapse because of uneven pressure. This is done by putting the slurry in equal quantity simultaneously through inlet and outlet pipes. The slurry should be put in until it starts to overflow through the outlet.

(c) Pitting the gas holder and pipeline – The gas holder is lifted into the position until it is in alignment with the central guide. A tripod stand, pulley and chain could be used for lifting the gas holder and placing it in the digester. The main gas valve should be opened to allow air to escape until the gas holder rests on the ledge. The valve is then closed. The hose pipe should be fitted to the gas holder and tightened with pipe clips. All gas cocks for

burners, lamps and moisture traps should be checked to see that they are closed.

(d) Gas production and use – Initially, it takes about 7 to 20 days for the gas holder to become full with gas. The first full quantity of gas should be released and should not be used as it contains air and burning it can cause an explosion. It may also not burn at all because of too high percentage of carbon IV oxide. Air should be purged from the gas pipeline by allowing the gas to flow for an interval prior to use. The burner and lamp are now attached to the gas cock by using pressure rubber tubes. The air adjuster of the burner is kept fully closed a match is lit and applied to the holes in the burner and the gas is then turned on slowly. The gas should burn with a blue flame.

(3) Operating the plant – This includes the following

(a) **Feeding the digester**– The quantity of dung recommended for a particular size of plant should be added daily into the plant. Initial feeding of slurry should only commence after the production of inflammable gas has started and the gas holder and pipeline have been purged with gas. Addition of extra dung will not increase gas production rather, it may reduce gas production because the slurry will be out before it has given most of its gas. Care should be taken to see that materials like sand, gravel, straw, etc do not enter the plant.

(b) **Stirring and breaking the scum layer** – The gas holder will be turned half way in one direction, then back in the opposite direction. This should be done after cooking when the gas holder has a low level of slurry. The process should be done for 3 minutes each in the morning and evening

(c) **Removing water from the pipeline** – Water should be removed from the moisture trap once in about 5 – 7 days in summer and about 10 – 15 days in winter as evaporation of water is higher in the summer months.

Maintenance and general care of the plant - The maintenance and the general care of the plant are broadly divided into 3 categories. The categories are daily, weekly and annually.

The daily maintenance and care includes:

(i) Checking of the mixing ratio of dung with water, which should be 1:1.

(ii) Do not allow gravel, sand etc to enter the plant

(iii) Fill the plant with slurry regularly, preferably at midday

(iv) Rotate the gas holder daily for sometime to break the scum

(v) Light a match first before opening the gas cock of the burner and lamp

The weekly maintenance includes:

(i) Clean the sides of the gas holder with water when it is full with gas and has risen to the maximum level

(ii) Clean the gas burner with soap and water

(iii) Drain off moisture condensate by opening the moisture trap

The annual maintenance includes:

(i) Repaint the gas holder with coat of oxide primer and 2 coats of black bitumen paint. It can be done by allowing the holder to fill up with gas completely until bubbles come out from the side. All dung should be clean with water. All rust should be thoroughly scrapped off using a steel scraper and the surface should be clean with a wire brush. The holder should be washed with water and dried. The holder should then be painted.

(ii) The gas should not be used for 24 hours to allow the paint to dry

(iii) Test the pipeline with aqueous soap solution to ensure that no leaks have developed. Leaks, if any, should be rectified.

(iv) Check the hose pipe for tear and wear and, if necessary, replace it.

Precautions

The following precautions are of paramount importance as suggested by Khandelwal and Mahdi (1986):

(i) Never inhale biogas as it is harmful to human beings. Potentially lethal situations occur most commonly: (a) when a worker enters inside the fixed dome biogas plant for cleaning; and (b) when the gas holder has been removed and slurry is agitated for breaking the scum layer.

(ii) Never allow children and animals to go near the gas plant when the gas holder has been removed and the digester is open. There

would be the danger of children and animals falling into the digester full of slurry, thereby causing death.

(iii) Care must be taken if there is a gas leak. If there is a serious leak, there will be a smell of unburnt gas. The doors and windows should be opened to let the gas dissipate and to let in fresh air. There should be no smoking, no candles, no fires, no matches, no lamps or other open flames until the smell of gas has gone. The main gas valve should be closed. Leaks should be detected and repaired promptly.

(b). **Fixed dome plant**

Operation – The following operational strategies are of paramount importance as pointed out by Khandelwal and Mahdi (1986):

1. **Testing** – Examine the walls with the help of a small stick by tapping all over the wall and listening to any hollow sound indicating unfilled space in the wall. In tense places, the plastered layer should be removed and replaced by a new, fresh layer.

The gas storage dome structure should be examined by fixing a u-shaped safety valve made of glass tube at the gas outlet pipe and then filling the digester with water or inflating with air using a manually operated air pump to make the column of water in the safety valve rise to at least 90cm. After 24 hours, the water column should be checked for a drop in level. Under normal circumstances, the drop in water level should be about 2 – 3 cm.

This positive pressure test should be repeated 2 or 3 times. However, the estimated pressure, i.e. 100cm, which the dome cover can stand, should never be exceeded. If the gas escapes, the leak must be located by pouring some water on all suspected locations outside the gas chamber and around the gas vent pipe joints. Before the leak is repaired, the main gate valve should be opened and the hose pipe removed so that internal pressure of the plant equals atmospheric pressure. If the leak is located at the joint of the gas vent pipe and the dome, the area should be chiseled and the pipe cemented anew.

Cracks or leaks in the dome should be chiseled and wide open, the edges roughened and filled in with cement and sand mix (ratio 1:1) and troweled smooth.

When the gas leak has not been clearly located, the gas chamber should be washed, brushed and coated again with pure cement or a cement and sand mix (ratio 1:1 or 1:2). After the coat has dried, the main gate valve may be closed.

2. **Starting the plant** – This includes the following operational activities:

   (a) **Slurry preparation** – This should be as in floating gas holder type plant (but strictly in the ratio 1:1).

(b) **Feeding the digester** – Fill the plant with a correct mixture of dung slurry (dung and water in the ratio 1:1 through the inlet chamber.

The gas pipe should be disconnected or safety valve, if any, should be opened during filling so as to avoid build-up of any pressure in the dome. The digester should no be filled to more than 75 – 80% of its volume, under any circumstances thus allowing some volume for the storage gas. The quantity of slurry recommended for the particular size of plant should be added daily. Addition of slurry should only commence after the production of inflammable gas has started i.e. after about 20 days of initial filling of plant up to the recommended level.

The only stirring that can be done is by moving a bamboo pole up and down in the inlet and outlet openings. This may help in breaking of scum if done at least once a day.

Maintenance and general care of the plant

The maintenance and care are divided into 3 categories. They are: Daily; monthly; and annual.

The daily maintenance includes:

Addition of dung to the plant, keep the ratio of dung 1:1

Cleaning of the gas burner

The monthly maintenance is achieved by checking the pipeline for leaks with a soap solution.

Annual maintenance includes the following:

(i) Check for gas and water leaks and repair them

(ii) Check gas pipeline and renew, if necessary

(iii) Check hose pipe of wear and tear, and if necessary, replace it

(iv) At intervals of some years or when the plan shows the signs of leakage, it should be completely emptied to renew the sealing and plastering of the inside portion of the plant. Necessary precautions have to be taken while performing this task.

Precautions

(i) Never allow gas to build up in the dome over 1,000mm water column; otherwise the dome will be damaged.

(ii) Never inhale the gas (biogas).

Never allow any person to enter plant when there is slurry in order to avoid accidental fell which may cause even death always keep the opening of the outlet and inlet chambers firmly closed by putting stone or concrete covers on them, otherwise small animals or children can fall into the slurry accidentally and die due to suffocation.

2.12 STORAGE OF BIOGAS AND BIOLIQUID

Biogas – The main component of biogas is methane (CH_4) which has a critical pressure of 471KPa at 82.3^{o}C, and this makes CH_4 difficult to be

liquefied as pointed out by Dangoggo (1984). Yaro (1993) also reported that the gas (CH_4) can be stored in balloons or neoprene bags. CH_4 can also be compressed into cylinders after the removal of CO_2. The removal of CO_2 from biogas can be achieved by absorbing the biogas on monoethanolamine (MEA) or alternatively by bubbling the gas (biogas) through sodium hydroxide or potassium hydroxide. It could also be achieved by bubbling through lime water.

Bioliquid – The bioliquid could be stored in the extracting solvent (absolute methanol) after extraction in an airtight condition to avoid solvent loss, because the presence of solvent (in excess) helps to prevent further microbial attack of the bioliquid.

2.13 BENEFITS OF BIOGAS AND BIOLIQUID TECHNOLOGY

The technology and uses of biogas and bioliquid have recently attracted attention all over the world towards ecological benefits through the utilization of plants and animal wastes for fuel and conservation of natural resources of energy. Therefore, in the developing nations, like Nigeria, application of biogas and bioliquid technology would be of great importance. The following are some of the benefits that could be derived from biogas technology:

(a) **Solving fuel problem -** Biogas is a clean and efficient fuel for cooking and lighting. $1m^3$ of biogas can cook 3 meals for a family of 5 – 6; 1 m^3 of biogas can keep one biogas lamp of a luminosity equivalent to a 60 Watts electricity light burning for 6 – 7 hours; 1 m^3 of biogas can keep

a 1 hp internal combustion engine working for 2 hours – roughly equivalent to 0.6 – 0.7 kg of petrol; $1m^3$ of biogas can drive a 3 tone lorry for 2.8km; and 1 m^3 of biogas can also generate 1.25 Kwh of electricity (Ariane, 1985).

It should be noted that the fuel value of a biogas depends on its CH_4 content. A biogas containing 65% CH_4 and 35% CO_2 has a fuel value of $24mJ/m^3$ while pure CH_4 has a fuel value of 37 mJ/m^3, which is half the heating value per volume of ethane gas, a third of propane, and 50% more than coal gas (Dangoggo, 1984). Dangoggo also pointed out that $1m^3$ of biogas is equivalent to 20kg of firewood, $0.6dm^3$ of Kerosene $0.5dm^3$ of petrol or $0.4dm^3$ of diesel.

On the other hand, agricultural residues and dung cakes used as cooking fuel in rural area is a wasteful practice as hardly 9 – 12% of the fuel value is harnessed as pointed out by Khandelwal and Mahdi (1986).

Moreover, collection and storage of these materials is highly problematic particularly during rainy season.

(b) **Improving health and sanitation -** Developing a biogas programme is an effective way to deal with excreta and improve the hygiene and standard of health in the countryside. One way to eliminate Schistosome eggs, hookworm and other parasites is to compost all manure. Throwing all human and animal excreta into biogas pits solve the problem of waste disposal. After fermentation, the digested slurry contained, on average, over 95% fewer parasite eggs, and in the

fermentation of excreta, the number of schistosome eggs and young hookworm eggs and larvae detected was reduced by 99% as pointed out by Ariane (1985). The transfer of fungal and other pathogens is drastically reduced due to fermentation in biogas pit, and the digested slurry remains free from foul smell, and mosquitoes and flies do not breed in the digested slurry as reported by Ahmad (2000).

(c) **Stimulating agricultural production -** The development of biogas is an important way to stimulate agricultural production; by the way it greatly augments the quantity and quality of organic fertilizer. Manure produced through the process of biogas production has a comparative advantage over ordinary manure in terms of both quality and quantity. Yaro (2003) reported that biogas manure (digested slurry) contains a high percentage of other plant nutrients than undigested slurry and the use of digested slurry as manure improves soil fertility, because about 70 – 75% of the original weight of cattle dung is conserved in biogas unit while in open compost pit 50% or more is lost. Biogas manure known as digested slurry contains a high percentage of other plant nutrients also. This can be seen in Table 2 below.

Digested slurry has been found to be useful for raising fish. Common carp fry and Fingerlings feed on a mixture of rice bran and digested slurry (1:3) and mustard oil care and rice bran (1:1) on equal nitrogen basis showed faster growth with the bran slurry mixture (Khandelwal & Mahdi, 1986).

(d) **Environmental protection -** The utilization of petroleum products and natural gas as fuel releases gaseous products (e.g. CO_2 and SO_2), which are pollutants. Felling down of trees for firewood causes desert encroachment and erosion, and reduces the rate of transpiration, which in turn leads to low relative humidity and subsequently to poor rain. On the other hand, the coming of biogas technology, an environmentally friendly process, a lot of biodegradable wastes could be converted to a useful fuel and germs-free bio-fertilizer (digested slurry); a large number of trees could be saved, which in turn prevents erosion and desert encroachment; and the release of gaseous products into the atmosphere could be minimized by using biogas to replace petroleum products and natural gas, because biogas is smoke-free.

Table 2: Comparison of Plant Nutrients Content between Digested and Undigested Slurries

Plant nutrients	Digested slurry (%)	Undigested slurry (%)
Nitrogen (N)	1.5 – 2.0	0.5 – 1.0
Phosphorus (P)	1.0	0.5 – 0.8
Potash (K_2O)	1.0	0.5 – 0.8

Source: Khandelwal and Mahdi (1986)

(e) Other Benefits - These include direct monetary returns, less work and various qualitative benefits. The monetary returns come from savings on kerosene, diesel, fuel, bottled gas, firewood and so on while the qualitative benefits include easier and cleaner cooking and good soil structure due to fertilization with digested sludge (Bryant, 1979).

In general, where biogas and bioliquid have been developed properly, there has been effective control of parasitic diseases and schistosomiasis; the rural environment has been transformed; agricultural production has been promoted and the general standard of health has been successfully raised.

It is worth noting that the production of biogas takes place concurrently with that of bioliquid, therefore, all the benefits associated with the biogas technology, so also with the bioliquid technology.

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

The materials used for the research were banana leaves, water and yeast.

3.2 CHEMICALS/REAGENTS

The chemicals/reagents used for the research were of good purity. The chemicals were obtained from the British Drug House (BDH), May and Baker (M&B), Fluka – Granite (F-G), Hopkins and Williams (H&W) and Philips- Harris (P-H). The Table below gives the names, reagent grades, manufacturers and percentage purities of some of the chemicals used:

Table 3: Chemicals/Reagents

S/N	Chemicals	Reagents grade	Manufa-cturer	% Purity
1.	Absolute methanol	-	BDH	-
2.	Ammonium molybdate	-	-	-
3.	Arsenic heptohydrate	-	-	-
4.	Benzene	-	-	-
5.	Boric acid	LR	M & B	79.7
6.	Bromocresol green	-	H & W	-
7.	Chloroform	-	-	-
8.	Copper II tetraoxosulphate VI	-	-	-
9.	Diethyl ether	-	-	-
10.	Disodium hydrogen phosphate	-	-	-
11.	Ethanoic acid	AR	P-H	99.5
12.	Ethanol	AR	M & B	95
13.	Glucose	-	-	-
14.	Hexane	-	-	-
15.	Hydrochloric acid	GPR	BDH	36
16.	Iron II tetraoxo-sulphate VI hepta-hydrate	AR	BDH	-
17.	L-cysteine hydrochloride monohydrate	-	-	-
18.	Lead ethanoate	-	M & B	-
19.	Methanal (formaldehyde)	-	-	-

20	Mercury II oxide	-	BDH	-
21	Mehyl red (screened)	-	-	-
22	Pepsin	-	-	-
23	Potassium heptaoxochromate VI	AR	BDH	
24	Potassium tetraoxosulphate VI	AR	M & B	99
25	Selenium IV oxide	-	-	-
26	Sodium chloride	-	M & B	99.9
27	Sodium dihydrogen phosphate monohydrate	-	-	-
28	Sodium hydroxide	LR	BDH	
29	Sodium-potassium tertrate	-	-	-
30	Sodium tetraoxosulphate VI	-	-	-
31	Sodium trioxocarbonate IV	-	-	-
32	Sulphonated barium diphenyl amine	AR	FG	-
33	Tetraoxosulphate VI acid	AR	M & B	98
34	Trioxonitrate V acid	AR	M & B	-
35	Urea	-	-	-

Key:

AR = Analytical Reagent

GPR = General Purpose Reagent

LR = Laboratory Reagent

3.3 APPARATUS / INSTRUMENTS

Table 4: Apparatus / Instruments

S/N	Apparatus/Instruments	Manufacturer
1.	Asbestos	-
2.	Beakers ($100cm^3$ & $250cm^3$)	Pyrex
3.	Boiling tube	Pyrex
4.	Buckner Filter flask	Pyrex
5.	Burette	-
6.	Chromatographic column	-
7.	Colorimeter	-
8.	Conical flasks ($250cm^3$ & $500cm^3$)	Pyrex
9.	Flat bottomed flasks ($250cm^3$ & $500cm^3$)	Pyrex
10.	Fume cupboard	-
11.	Funnel	-
12.	GC- mass spectrometer	Agilent technologist
13.	Heating mantle	-
14.	Hot Plate	-
15.	Kjeldahl apparatus	Heraeus Wittman, West Germany
16.	Liebig condenser	-
16.	Measuring cylinders ($100cm^3$, $250cm^3$, & $1dm^3$)	Pyrex
17	Merkham still apparatus	-
18	Mesh	-

19	Muffle furnace	Dubuque Lowia, USA
20	Oven (OV – 330)	Gallen hamp, England
21	Pipette	-
22	pH metre	Cole permer Inst., U.S.A
23	Plastic cork	-
24	Porcelain crucible	-
25	PVC tube	-
26	Refrigerator	-
27	Round bottomed flasks ($10cm^3$ & $100cm^3$)	Pyrex
28	Rotary evaporator	-
29	Soxhlet extraction set	-
30	Spatula	-
31	Stand and Clamp	-
32	Stirrer	-
33	Thermometer	-
34	Thimble	-
35	Volumetric flasks ($100cm^3$, $250cm^3$ & $500cm^3$)	Pyrex
36	Washing Bottle	-
37	Water Bath	-
38	Water Trough	-
39	Weighing balance (B3002DR)	Mettler Toledo, Switzerland

3.4 **SAMPLE COLLECTION AND PREPARATION**

The sample was collected from a group of banana trees planted by Kano Agricultural and Rural Development Authority (KNARDA) in Bichi town, Kano State. The leaves were fresh and mature at the time of collection.

The sample collected was oven-dried at 37^{o}C for four (4) hours and ground using pestle and mortar, and then sieved to a mesh size of $<250\mu$m.

3.5 PREPARATION OF REAGENTS AND SOLUTIONS

All the reagents and solutions used were prepared according to the known standard methods of preparation using appropriate solvents. Below were some of the reagents/solutions.

(a) **30% Leadethanoate solution:** 30.0g of $(CH_3COO)_2$ Pb was dissolved and diluted to 100cm^3 with distilled water.

(b) **3M Ethanoic acid solution:** 85.25cm^3 of concentrated ethanoic acid was diluted to 500cm^3 with distilled water.

(c) **0.1M Sodium hydroxide solution:** 4.0g of NaOH pellets was dissolved and diluted to 1dm^3 with distilled water.

(d) **10M Sodium hydroxide solution:** 200.0g of NaOH pellets was dissolved and diluted to 500cm^3 with distilled water.

(e) **10% Sodium hydroxide solution:** 10.0g of NaOH pellets was weighed, dissolved and diluted to 100cm^3 with distilled water.

(f) **Mixed boric acid indicator:** 8.0g of Boric acid was dissolved in 380cm^3 of distilled water and heated on a hot plate and heated to

60^{o}c. The solution was cooled and 8.0cm^3 of the mixed indicator solution (prepared separately by dissolving 0.099g of bromocresol green and 0.066g of screened methyl red in 100cm^3 of 95% ethanol) was added. Then 0.1M NaOH was added through a burette until the solution became purple. The resulting solution was then diluted to 400cm^3 with distilled water and mixed thoroughly.

(g) **0.01M Hydrochloric acid solution:** 0.85cm^3 of concentrated hydrochloric acid was diluted to 1000cm^3 with distilled water.

(h) **Mercury catalyst tablet:** 190.0g of K_2SO_4 was accurately weighed and mixed with 5.0g of (HgO) and subsequently ground in a mortar.

(i) **0.16% Barium diphenylamine sulphonate (as Ferroin indicator):** 16.0g of $Ba(C_6H_5)_2$ $NHSO_3$ was dissolved and diluted to 1dm^3 with distilled water.

(j) **1M Potassium heptachromate VI solution:** 147.0g of $k_2Cr_2O_7$ was dissolved and diluted to 500cm^3 with distilled water.

(k) **0.5M Iron II tetraoxosulphate VI Solution:** 139.0g of $Fe_2SO_4.7H_2O$ was dissolved in distilled water, and 15cm^3 of concentrated tetraoxosulphate VI acid was added and cooled. The resulting solution was diluted to 1dm^3 with distilled water.

3.6 CONSTRUCTION OF REACTOR (OR DIGESTER)

A hole was bored on a plastic cork, which was used to cover a Buckner filter flask (B.F.F.). The outlet of the B.F.F. was sealed by blowing with fire. A polyvinylchloride (PVC) tube of 50cm length and 0.8cm internal diameter was inserted into the hole bored on

the plastic cork and glued with superglue, with one end of the PVC free. (i.e. unattached). All the reactors (digesters) used in this research were prepared as such.

3.7 DETERMINATION OF MOISTURE CONTENT OF THE SUBSTRATES

3 porcelain crucibles were pre-heated at 105^0C and cooled in a desiccator to ensure that they were moisture free, and weighed, W_1 each. 5.0g of the prepared (unfermented) sample was thinly spread in each of the porcelain crucibles and weighed rapidly, W_2 each. The porcelain crucibles and their contents were heated in an oven at 105^oC for 3 hours and allowed to cool in a desiccator to a constant weight, W_3 each. The heating and cooling of the loaded porcelain crucibles was repeated 3 times, until a constant weight, W_3 was obtained. Average weight was calculated at the end of each step.

The percentage by weight of the moisture content of the sample was evaluated as follows:-

$$\text{Moisture (\%)} = \frac{W_2 - W_3}{5.0\text{g}} \times 100 \qquad \text{---(21)}$$

where:

W_2 = Average weight of the porcelain crucibles and their contents before heating.

W_3 = Average weight of the porcelain crucibles and their contents after heating.

The same procedure was followed for the fermented substrate.

3.8 DETERMINATION OF ASH CONTENT OF THE SUBSTRATES

3 porcelain crucibles were pre-heated in a muffle furnace to 600^0C. The pre-heated porcelain crucibles were allowed to cool and weighed, W_1 each. 5.0g of the prepared sample was placed in each of the pre-heated porcelain crucibles and each was weighed, W_2. The porcelain crucibles were then placed in a cold muffle furnace, and the temperature was allowed to rise gradually to 600^0C. The porcelain crucibles and their contents were maintained at this temperature (i.e. 600^0C) for 8 hours. The heating was stopped and allowed the loaded porcelain crucibles to cool there in the muffle furnace, after which they were transferred to desiccator and weighted, W_3 each. Average weight was computed at the end of each step.

The percentage by weight of the ash content of the sample was calculated as follows:

$$\text{Ash (\%)} = \frac{W_3 - W_1}{5.0\text{g}} \times 100 \quad \text{---(22)}$$

Where:

W_3 = Average weight of the porcelain crucibles and their contents after heating.

W_1 = Average weight of the porcelain crucibles alone.

The same procedure was followed for the fermented substrate.

3.9 DETERMINATION OF ORGANIC MATTER CONTENT OF THE SUBSTRATES

This was estimated by subtracting the average weights of the moistures and ashes earlier obtained in sections 3.7 and 3.8 respectively from the weights of the prepared sample used for moisture or ash content determination before heating (i.e. 5.0g).

The percentage organic matter was evaluated as follows:

Organic matter (%) = 100% - [moisture (%)+ash (%)] ---- (23)

The same procedure was followed for fermented substrate.

3.10 DETERMINATION OF CARBON CONTENT OF THE SUBSTRATES BY WALKLEY-BLACK METHOD.

For the determination of carbon content of the unfermented substrate, 0.5g of the prepared sample was accurately weighed and dissolved in 250cm^3 distilled water in a flask. 10cm^3 of 1M $K_2Cr_2O_7$ was added and swirled gently. 20cm^3 of concentrated H_2SO_4 was also added using pipette and the flask was swirled until the sample and the reagents were thoroughly mixed. The flask was then swirled vigorously for 1 minute and allowed to stand on a sheet of asbestos for 30 minutes and 100cm^3 of distilled water was subsequently added. 3 – 4 drops of Ferrion indicator (i.e. 0.16% Barium diphenylamine sulphonate) was added and titrated with 0.5M-hydrated $FeSO_4$ solution. The end point was determined when the solution mixture changed from light green to dark green and

eventually to red colour on drop-wise addition of 0.5M hydrated $FeSO_4$ solution. Triplicate sets were made, and the average titre value of 0.5M hydrated $FeSO_4$ solution used was calculated.

Blank titration was also carried out using 0.5g filter paper, and the titre value obtained was recorded and subtracted from the titre value of 0.5M hydrated $FeSO_4$ using the sample.

The carbon content of the sample was evaluated as follows:

$$\text{Carbon (\%)} = \frac{[MvK_2Cr_2O_7 - M'(TFeSO_4 - TB)]\ 0.003(F)}{\text{Weight of the Sample (i.e. 0.5g)}} \times 100 \quad \text{---(24)}$$

Where:

Mv = Molarity x Volume of $K_2Cr_2O_7$ solution used.

M' = Molarity of hydrated $FeSO_4$ solution used

$TFeSO_4$ = Titre value of $FeSO_4$ with sample

TB = Titre value of $FeSO_4$ for the blank titration

$TFeSO_4$ - TB = V = Volume of $FeSO_4$ used

F = Correlation factor 1.33

The same procedure was followed for the fermented substrate.

3.11 DETERMINATION OF NITROGEN CONTENT OF THE SUBSTRATES BY KJELDAHL METHOD

In order to determine the total nitrogen content of the unfermented substrate, 0.5g of the prepared sample was accurately weighed and transferred into a $100cm^3$ Kjeldahl flask and $10cm^3$ of Conc. H_2SO_4 was subsequently added. The flask was shaken for 15 minutes and allowed to stand for 30 minutes. 1.0g of

K_2SO_4–HgO mixture (as mercury catalyst) was also added. The flask and its content were heated continuously at 100^oC using heating mantle in a fume cupboard, till the water was removed and frothing ceased. The heating was continued until the digest was cleared. The flask was allowed to cool and 60cm^3 distilled water was slowly added. 10cm^3 of the digest (aliquot) was pipetted and placed into sample chamber of the merkhan still apparatus, 20cm^3 of 40% NaOH was added to the aliquot. 20cm^3 of 2% H_3BO_3 indicator was pipetted and placed in a receiving flask, which was placed under a condenser, and distillation commenced. The condenser was kept at low temperature and sufficient cold water was allowed to flow through and the heat was regulated to minimize frothing and prevent suck-back. Finally, the nitrogen contained in the distillate was determined by titration using 0.01M standardized HCl, the end point was determined by the colour change from green to pink. The process was repeated 3 times, and the average titre value of 0.01M HCl was calculated.

The same process was applied for the blank sample using 0.5g filter paper.

The nitrogen content of the substrate was evaluated as follows:

$$N(\%) = \frac{T - B \times MHCl \times 0.014 \times V_1}{\text{Weight of sample (i.e. 0.5g)} \times V_2} \times 100 \quad \text{----(25)}$$

where:

T = Titre value of 0.01M HCl used

B = Titre value for the blank sample

MHCl = Molarity of standardized HCl = 0.01M

0.014 = Weight by volume of nitrogen atom/dm^3
= $14g/1000cm^3$

V_1 = Volume of distilled water added for digestion (i.e. $60cm^3$)

V_2 = Volume of digest water (aliquot) pipetted = $10cm^3$

The reactions are illustrated by the equations below:-

$N_{2\,(g)} + 3H_2O_{(l)} + H_2SO_{4(conc.)} \rightarrow (NH_4)_2\,SO_{4(aq)} + {}^3/_2O_{2(g)}$ ---------- (26)

$(NH_4)2SO_{4\,(aq)} + 2NaOH_{(aq)} \rightarrow 2NH_{3(g)} + Na_2SO_{4(aq)} + 2H_2O_{(l)}$ --------- (27)

$NH_{3(g)} + H_3BO_{3(aq)} \rightarrow NH_4^+{}_{(aq)} + H_2BO_3^-{}_{(aq)}$ ----------(28)

$NH_4^+{}_{(aq)} + H_2BO_3^-{}_{(aq)} + HCl_{(aq)} \rightarrow H_3BO_{3(aq)} + NH_4Cl_{(aq)}$ -------- (29)

The same procedure was followed for the fermented substrate.

3.12 DETERMINATION OF CARBON TO NITROGEN RATIO OF THE SUBSTRATES

The Carbon to Nitrogen ratio of the unfermented sample was evaluated by calculating the ratio of Carbon (%) content to nitrogen (%) content determined in sections 3.10 and 3.11, respectively. The expression was as follows: -

$$c/N = \frac{\text{Carbon (\%)}}{\text{Nitrogen (\%)}} \quad \text{---------- (30)}$$

The same procedure was followed for the fermented sample.

3.13 DETERMINATION OF CRUDE PROTEIN CONTENT OF UNFERMENTED AND FERMENTED SUBSTRATES

The protein content of the unfermented substrate was obtained from the nitrogen (%) content of the unfermented substrate determined in section 3.11 above. The percentage protein content was evaluated as follows: protein (%) = Nigroten (%) x 5.7 …… (31).

The same procedure was employed for the fermented substrate.

3.14 DETERMINATOIN OF LIGNIN CONTENT OF UNFERMENTED AND FERMENTED SUBSTRATES

The unfermented sample was ether extracted and dried in an oven; 0.5g of the sample was placed in a stoppered conical flask and 40cm^3 of 2% solution of pepsin in 0.1M HCl was added and subsequently digested for 12 hours at 40^oC with frequent shaking during the first 4 hours. The filtrate was filtered off through a blotting silk and the residue was successively washed with hot water, hot ethanol, hot benzene and hot diethyl ether, after which it was transferred into a 100cm^3 beaker and gently heated to remove the diethylether. The residue obtained was then placed in a beaker and moistened with 4cm^3 of 40% formaldehyde 4cm^3 of 72% H_2SO_4 was subsequently added and allowed to penetrate the residue for 2 minutes. 6cm^3 of concentrated H_2SO_4 was also added and thoroughly stirred. The beaker was immersed in water bath maintained at 70^oC. 30cm^3 of granulating reagent (made up of 1:6 ratio by volume of chloroform and ethanoic acid) was added. The resulting mixture was

poured into 500cm^3 of distilled water and subsequently boiled in a fume cupboard for 15 minutes to remove the chloroform and the clear solution containing the lignin was filtered on a gooch crucible, washed with 200cm^{3}of 5% HCl and dried in an oven maintained at 110^{o}C. The dried residue was weighed and transferred into a porcelain crucible and ashed in a muffle furnace at 600^{o}c for 6 hours. The lignin was determined as loss in weight on ignition. Triplicate determinations were carried out, and the average weight was calculated.

The percentage lignin content was evaluated as follows:

$$\text{Lignin (\%)} = \frac{\left(\text{weight of residue before ashing}\right) - (\text{weight of ash}) \times 100}{\text{weight of residue before ashing}} \quad \ldots\ldots\ldots(32)$$

The same procedure was followed for the fermented substrate.

3.15 DETERMINATION OF CRUDE FIBRE CONTENT OF UNFERMENTED AND FERMENTED SUBSTRATES

The crude fibre determination of the unfermented substrate was achieved via the following steps:

STEP ONE: Preparation Of Digestion Mixture

The digestion mixture was prepared by mixing thoroughly 225cm^3 of glacial ethanoic acid, 250cm^3 of distilled water, 25cm^3 of concentrated trioxonitrate V acid and 10g trichloro ethanoic acid in a 1dm^3 volumetric flask.

STEP TWO: Determination

2.0g of the unfermented substrate was accurately weighted and quantitatively transferred to a 250cm^3 quick fit conical flask. 100cm^3 of digestion mixture (prepared in step one) was added and the mixture was refluxed for 45 minutes with constant shaking. It was then filtered through ashless filter paper. The residue was weighted and then ashed in a muffle furnace at 600^{o}C for 6 hours. The loss in weighed on ignition was expressed as % crude fibre content. Triplicate determinations were carried out, and the average weight was calculated.

The percentage crude fiber was evaluated as follows:

$$\text{Crude fiber (\%)} = \frac{\left(\text{weight of residue before ashing}\right) - (\text{weight of ash}) \times 100}{\text{weight of the sample}} \quad \ldots(33)$$

The same procedure was followed for the fermented substrate.

3.16 DETERMINATION OF CRUDE FAT OF UNFERMENTED AND FERMENTED SUBSRATES

5.0g of the unfermented sample was placed in a fat free extraction thimble and accurately weighed. The thimbles with the sample were then placed in a soxhlet extractor and extracted using petroleum ether (60 – 80^{o}C) for 8 hours on a heating mantle. The thimble was then dried in an oven maintained at 105^{o}C for 24 hours. The loss in weight of the sample was expressed as the crude fat content of the substrate. Triplicate determinations were carried out, and the average weight was calculated.

The percentage crude fat was evaluated as follows:

$$\text{Crude fat (\%)} = \left(\text{weight of sample before extraction}\right) - \left(\text{weight of sample after extraction}\right) \times 100 \quad \ldots(34)$$

weight of sample before extraction.

The same procedure was employed for the fermented substrate.

3.17 DETERMINATION OF TOTAL CARBOHYDRATE OF SUBSTRATES BY L-CYSTEINE TETRAOXOSULPHATE (VI) ACID METHOD

The following steps were followed during this analysis:

STEP ONE*: *Preparation of reagent [L-Cystene Tetraoxosulphate VI acid, $HSCH_2CH(NH_2)CO_2H.HCL.H_2O$]

The reagent was prepared by dissolving L-cysteine hydrochloride monohydrate (70mg) in 100cm^3 of 86% analar H_2SO_4.

STEP TWO*: *Acid hydrolysis of the sample

0.05g of moisture free unfermented sample was moistened with 2cm^3 of 72% H_2SO_4 in a boiling tube and allowed to stand at room temperature for 3 hours. The boiling tube was sealed and the sample was hydrolyzed at 100^oC for 6 hours in an oven. The tube was cooled, cut open and the solution was filtered through sintered glass crucible No. 4, rinsed several times with distilled water and the solution was made to 100cm^3 in a volumetric flask.

STEP THREE: Determination

1cm^3 of the unfermented sample solution (prepared in step two) was added to 5cm^3 of the prepared reagent in an ice-cooled test tube. The test tube was subsequently heated in boiling water bath for 3 minutes. The test ube was removed from the water bath and cooled to room temperature and the absorbance of the sample was read at 430nm

using a corning 252 colorimeter and recorded. Triplicate determinations were carried out, and the average absorbance was calculated.

A standard glucose solution in the concentration range 0.1 – 1.0mg/dm^3 was prepared and the corresponding absorbance for each concentration was read at 430nm using 252 colorimeter and recorded.

A plot of absorbance against concentration for the absorbance obtained for the respective concentrations of the standard glucose solution

was made. From the plot, the corresponding concentration of the sample was deduced by tracing the position at which the average absorbance of the sample intercepted the curve of the plot.

The percentage total carbohydrate was estimated as follows:

$$\text{Total carbohydrate (\%)} = \frac{\left(\begin{array}{c}\text{Conc. of the sample} \\ \text{from the plot}\end{array}\right)}{\left(\begin{array}{c}\text{Conc. of the sample} \\ \text{Prepared}\end{array}\right)} \times 100 \quad \ldots\ldots\ldots \quad (35)$$

The same procedure was followed for the fermented substrate.

(See appendix 10a for the details).

3.18 DETERMINATION OF REDUCING SUGAR OF SUBSTRATES BY NELSON'S METHOD.

STEP ONE*: *Preparation of reagents

Reagent A: This was prepared by dissolving 25.0g anhydrous sodium trioxocarbonate IV, 25.0g sodium – potassium tartrate, 20.0g sodium hydrogencarbonate and 200.0g anhydrous sodium tetraoxosulphate VI in distilled water and made to 1dm^3.

Reagent B: This was prepared by dissolving Copper II tetraoxosulphate VI (30.0g) in 450cm^3 distilled water, and 4 drops of concentrated tetraoxosulphate VI acid was added.

Reagent C: This was prepared by dissolving ammonium molybdate (25.0g) in 45cm^3 of distilled water, and 21cm^3 of concentrated tetraoxosulphate VI acid was added. A solution of arsenate heptahydrate (3.0g in 25cm^3 of distilled water) was prepared and subsequently added to

the ammonium molybdate solution. The solution was made to 500cm^3 and incubated at 37^{o}C for 24 hours.

Reagent D: This was prepared by mixing 1.0cm^3 of Reagent B with 25.0cm^3 of reagent A.

STEP TWO*: *Determination Procedure – 1.0cm^3 of the unfermented sample solution prepared by acid hydrolysis in section 3.17 above was added to 1.0cm^3 of reagent D and heated at 100^{o}C for 20 minutes. The solution was cooled. 1.0cm^3 of reagent C was added, mixed thoroughly using vortex mixer, and diluted to 25.0cm^3 in a Nelson's tube. The absorbance was read at 520nm using a corning 252-colorimeter. Triplicate determinations were carried out, and the average absorbance was calculated.

A standard analar glucose solution in the concentration range (0.1–1.0mg/dm^3) was prepared and the corresponding absorbance for each concentration was read at 520nm using 252 colorimeter and recorded.

A plot of absorbance against concentration for each of the absorbance obtained for the respective concentrations of standard analar glucose solution was made. From the plot, the corresponding concentration of the sample was deduced by tracing the position at which the average absorbance of the sample intercepted the curve of the plot.

The percentage concentration was expressed as follows:

$$\text{Reducing sugar (\%)} = \left(\frac{\text{conc. Of sample from the plot}}{\text{Conc. of sample prepared}}\right) \times 100 \quad \ldots. (36)$$

The same procedure was followed for the fermented sample.

(See appendix 10b).

3.19 PREPARATION OF SLURRY

The slurry was prepared as follows: 4.0g of the prepared sample was accurately weighed and dissolved in 25cm^3 distilled water containing 0.18g yeast (the best slurry conditions determined in the preliminary analysis).

For the preparation of the slurry using buffer solution, the Henderson-Hasselbalch equation was applied. The equation is

$$pH = pKa + \log \frac{[Base]}{[acid]} \quad \text{----------(37)}$$

The equation was applied and determined the volumes of the solutions of the buffer salts (acid and base) that were mixed and formed the buffer solution of required volume, molar concentration and pH. It should be noted that for a buffer solution of any required pH, the molar

concentration of acid must always be equal to that of base (i.e. $M_A = M_B$). (See appendix 2 for the details). The slurry was prepared by dissolving 4.0g substrate in 25cm^3 buffer solution containing 0.18g yeast.

The molar concentrations of the buffer solution used were 0.1M, 0.2M, 0.3M, 0.4M, and 0.5M, while the pH of the buffer solution used were 6.8, 7.0, 7.2, 7.3, 7.4, and 7.6. The molar concentrations were used to investigate the effect of buffering while the different pH values were used to study the effect of pH.

In order to investigate the effect of urea (as nutritive additive) on biogas and bioliquid production, 0.010M, 0.015M, 0.020M, 0.025M and 0.030M urea solutions were prepared and used for making the slurry.

In order to investigate the combined effect of a mixture of buffer capacity and urea, the slurry was prepared by dissolving the substrate in a buffer solution of a known pH and molar concentration, which contained calculated amount of urea. The pH and the molar concentration of the buffer solution used, were respectively deduced from the effect of pH and the effect of buffering earlier investigated, in which the pH and the molar concentrations at which high biogas production was obtained were considered. The masses of urea added into the buffer solution were: 0.015g, 0.023g, 0.030g, 0.038g and 0.045g. These masses were respectively obtained from 0.01M, 0.015M, 0.020M, 0.025M and 0.030M using the following relation:

$$^1/V = X_M \quad \text{---------- (38)}$$

where

1 = mole of urea,

X_M = required molar concentration of urea (i.e. 0.01M, 0.015M, 0.020M, 0.025M and 0.030M).

V = Volume of urea solution of the required molar concentration with which the molar mass of 1 mole urea (i.e. 60g) was divided and obtained the required mass of urea that was dissolved in 1000cm^3 of buffer solution, which was equated and evaluated the masses of urea that was dissolved in 25cm^3. (See appendix 3 for more details).

Proportionately increased quantities of slurries were equally prepared based on 4g/25cm^3 and 0.18g yeast, depending upon the quantities of biogas and bioliquid needed irrespective of the type of solvents, concentrations and P^H employed. (See appendix 4 for further details).

3.20 DETERMINATION OF SUITABLE WATER CONTENT FOR MAXIMUM BIOGAS PRODUCTION

For the generation and collection of biogas, slurry was prepared and placed in a digester. The digester was made airtight and placed in a water bath maintained at 33^{o}C. [The temperature selection was made based on the various literature reports such as Ekwenchi et al (1989); Epenyong et al (1994); Airehrour(1994)]. The unattached end of the PVC of the digester (as described in section 3.6) was channeled to an empty Buckner Filter Flask (B.F.F.), which was used to prevent suck-back of water. The outlet of this empty B.F.F was connected to another B.F.F

containing water, with its outlet connected to another empty B.F.F., which was used to prevent suck back of brine (28% NaCl) from the trough. A PVC tube was attached to the outlet of the second empty B.F.F., which was positioned under an inverted measuring cylinder filled with brine in a water trough filled with brine such that the free end of the PVC tube was directed upright in the measuring cylinder as shown in Fig. 3 of section 3.29. The downward displacement of brine in the measuring cylinder served as the measure of the volume of biogas generated. All the connections were made using PVC tubes of equal length and internal diameter with the one described in Section 3.6. Triplicate sets were made and the average volume of biogas generated for each was calculated and recorded.

The digesters were as follows:

Digester A $\Rightarrow$ 4.0g substrate + 0.06g yeast + 15cm^3 H_2O

Digester B $\Rightarrow$ 4.0g substrate + 0.06g yeast + 20cm^3 H_2O

Digester C $\Rightarrow$ 4.0g substrate + 0.06g yeast + 25cm^3 H_2O

Digester D $\Rightarrow$ 4.0g substrate + 0.06g yeast + 30cm^3 H_2O

Digester E $\Rightarrow$ 4.0g substrate + 0.06g yeast + 35cm^3 H_2O

3.21 DETERMINATION OF OPTIMUM YEAST CONCENTRATION FOR MAXIMUM BIOGAS PRODUCTION

The sets used for the generation and collection of biogas were as in section 3.20, only that the concentrations of the slurries in this case were equal in all of the digesters (i.e. 4.0/25cm^3) containing different

quantities of yeast. Triplicate sets were made, and the average volume of biogas generated was calculated for each.

The digesters were as follows:

Control digester $\Rightarrow$ 4.0g substrate +25cm^3 H_2O + 0.06g yeast

Digester A $\Rightarrow$ 4.0g substrate +25cm^3 H_2O + 0.08g yeast

Digester B $\Rightarrow$ 4.0g substrate +25cm^3 H_2O + 0.10g yeast

Digester C $\Rightarrow$ 4.0g substrate +25cm^3 H_2O + 0.12g yeast

Digester D $\Rightarrow$ 4.0g substrate +25cm^3 H_2O + 0.14g yeast

Digester E $\Rightarrow$ 4.0g substrate +25cm^3 H_2O + 0.18g yeast

Digester F $\Rightarrow$ 4.0g substrate +25cm^3 H_2O + 0.20g yeast

NB – The choice of 25cm^3 H_2O was based on the slurry condition that generated more biogas in section 3.20.

3.22 VERIFICATION OF OPTIMUM TEMPERATURE CONDITION SELECTED FOR MAXIMUM BIOGAS PRODUCTION

In order to verify the temperature condition selected, set-ups were made as in section 3.20, but the digesters were maintained at different temperatures. The temperatures used were room temperature (25^{o}C), 33^{o}C, 40^{o}C and 45^{o}C. All the digesters contained equal concentration of slurries (i.e. 4g/25cm^3 + 0.18g yeast each). The digesters were labelled A, B, C, D and E. Triplicate sets were made, and the average volume of biogas generated was calculated for each.

NB: 33^{o}C served as control temperature, and the choice of 0.18g yeast was based on the yeast concentration that generated more biogas in section 3.21.

3.23 EFFECT OF SLURRY MASS CHANGE ON BIOGAS PRODUCTION AT FIXED CONCENTRATION OF YEAST.

The set ups used were also as in section 3.20, but the concentrations of the slurries differed. The volume of water added to each digester was proportionately calculated according to the mass of the substrate used. This

was obtained based on 4g/25cm^3. Yeast (0.18g) was also added to each digester. (See appendix 4b for the details).

The digesters used were as follows:

Control digester $\Rightarrow$ 4.0g substrate +25cm^3 H_2O + 0.18g yeast

Digester A $\Rightarrow$ 6.0g substrate +37.5cm^3 H_2O + 0.18g yeast

Digester B $\Rightarrow$ 8.0g substrate +50.0cm^3 H_2O + 0.18g yeast

Digester C 10.0g substrate +62.5cm^3 H_2O + 0.18g yeast

Digester D $\Rightarrow$ 12.0g substrate +75.0cm^3 H_2O + 0.18g yeast

Digester E $\Rightarrow$ 14.0g substrate +87.5cm^3 H_2O + 0.18g yeast

Triplicate sets were made, and the average volume of biogas generated was calculated for each.

3.24 EFFECT OF SLURRY MASS CHANGE ON BIOGAS PRODUCTION AT VARYING CONCENTRATION OF YEAST.

The sets used were also as in section 3.20, with the same concentrations of the slurries but different quantities of yeast were added differed. The volume of water added to each of the masses of the substrate used was obtained as in section 3.23. The quantities of yeast added were obtained based on 0.18g yeast per 25cm^3 H_2O. (See appendixes 4b and c for the details).

The specification of the digesters were as follows:

Control digester $\Rightarrow$ 4.0g substrate + 25cm^3 H_2O + 0.18g yeast

Digester A $\Rightarrow$ 6.0g substrate + 37.5cm^3 H_2O + 0.27g yeast

Digester B $\Rightarrow$ 8.0g substrate + 50.0cm^3 H_2O + 0.36g yeast

Digester C $\Rightarrow$ 10.0g substrate + 62.5cm^3 H_2O + 0.45g yeast

Digester D $\Rightarrow$ 12.0g substrate + 75.0cm^3 H_2O + 0.54g yeast

Digester E $\Rightarrow$ 14.0g substrate + 87.5cm^3 H_2O + 0.63g yeast

Triplicate sets were made and the average volumes of biogas generated were calculated for each.

3.25 EFFECT OF BUFFERING ON BIOGAS PRODUCTION USING BUFFER SOLUTION

The sets used were as in section 3.20, but in the case, buffer solutions of pH 7.3 with different molar concentrations were used instead of water. 0.18g yeast was added to each of the digesters.

The digesters were as follows:

Control digester $\Rightarrow$ 4.0g substrate + 25cm^3 H_2O +0.18g yeast

Digester A $\Rightarrow$ 4.0g substrate+25cm^3 of 0.1M buffer solution + 0.18g yeast

Digester B $\Rightarrow$ 4.0g substrate + 25cm^3 of 0.2M buffer solution + 0.18g yeast

Digester C $\Rightarrow$ 4.0g substrate + 25cm^3 of 0.3M buffer solution + 0.18g yeast

Digester D $\Rightarrow$ 4.0g substrate + 25cm^3 of 0.4M buffer solution + 0.18g yeast

Digester E ⇒ 4.0g substrate + 25cm^3 of 0.5M buffer solution + 0.18g yeast

Triplicate sets were made and the average volume of biogas generated was calculated for each.

3.26 EFFECT OF pH OF THE SLURRY ON BIOGAS PRODUCTION

The sets used were also as in section 3.20, but in both the control and the experimental digesters 0.2M buffer solutions of different pH were used. The quantities of yeast added were equal in all the digesters (0.18g).

NB: 0.2M of buffer solution was used because it was the molar concentration of buffer solution from which high biogas was generated.

The digesters were as follows:

Control Digester ⇒ 4g substrate 0.18g yeast + 25cm^3 of 0.2M buffer solution of pH 7.3

Digester A ⇒ 4g substrate 0.18g yeast+25cm^3 of 0.2M buffer solution of pH 6.8

Digester B ⇒ 4g substrate 0.18g yeast+25cm^3 of 0.2M buffer solution of pH 7.0

Digester C ⇒ 4g substrate 0.18g yeast+25cm^3 of 0.2M buffer solution of pH 7.2

Digester D ⇒ 4g substrate 0.18g yeast+25cm^3 of 0.2M buffer solution of pH 7.4

Digester E ⇒ 4g substrate 0.18g yeast+25cm^3 of 0.2M buffer solution of pH 7.6

Triplicate sets were made and the average volume of biogas generating was calculated for each.

3.27 EFFECT OF UREA ON BIOGAS PRODUCTION

The set ups were as in section 3.20, but the slurries except that of the control where water was used, were urea solutions of different molar concentrations. The quantities of yeast added were equal in all the digesters (i.e. 0.18g).

The digesters used were as follows:

Control digesters $\Rightarrow$ 4.0g substrate 25cm3 H_2O + 0.18g yeast

Digester A $\Rightarrow$ 4.0g substrate + 25cm^3 of 0.01M urea + 0.18g yeast

Digester B $\Rightarrow$ 4.0g substrate + 25cm^3 of 0.015M urea + 0.18g yeast

Digester C $\Rightarrow$ 4.0g substrate + 25cm^3 of 0.02M urea + 0.18g yeast

Digester D $\Rightarrow$ 4.0g substrate + 25cm^3 of 0.025M urea + 0.18g yeast

Digester E $\Rightarrow$ 4.0g substrate + 25cm^3 of 0.03M urea + 0.18g yeast

Triplicate sets were made, and the average volume of biogas generated was calculated for each.

3.28 COMBINED EFECT OF BUFFERING AND ADDITION OF UREA ON BIOGAS GENERATION

The set ups were as in section 3.20. The digesters used (control digester inclusive), each contained slurry made of 4.0g substrate and 0.2M buffer solution of pH 7.3. Different quantities of urea were added to the experimental digesters, but no urea was added to the control digester 0.18g yeast was also added to each of the digesters (control inclusive).

Triplicate sets were made, and the average volume of biogas generated for each was calculated:

The digesters were of the following specification:

Control digester $\Rightarrow$ 4.0gsubstrate +25cm^3 of 0.2m buffer solution
+ 0.18g yeast without urea

Digester A $\Rightarrow$ 4.0g substrate + 25cm^3 of 0.2m buffer solution
+ 0.18g yeast + 0.01g urea

Digester B $\Rightarrow$ 4.0g substrate + 25cm^3 of 0.2m buffer solution
+ 0.18g yeast + 0.023g urea

Digester C $\Rightarrow$ 4.0g substrate + 25cm^3 of 0.2m buffer solution
+ 0.18g yeast + 0.03g urea

Digester D $\Rightarrow$ 4.0g substrate + 25cm^3 of 0.2m buffer solution
+ 0.18g yeast + 0.038g urea

Digester E $\Rightarrow$ 4.0g substrate + 25cm^3 of 0.2m buffer solution
+ 0.18g yeast + 0.045g urea

NB: The choice of 0.2M buffer solution of pH 7.3 was based on the molar concentration and pH of the buffer solution that yielded high volume of biogas.

3.29 BIOGAS GENERATION AND COMPOSITIONAL ANALYSIS

For the collection of biogas, CH_4, CO_2 and H_2S separately, the following set-ups were used: -

SET A: For the collection of biogas (i.e. CH_4, CO_2 and H_2S), the slurry prepared in section 3.19 was placed in the digester constructed in Section 3.6. The digester was airtight and placed in a water bath maintained at 33^{o}c. The unattached end of the PVC tube of the digester was channeled to an empty B.F.F, which was used to prevent suck-back of water. The outlet of this empty B.F.F. was connected to another B.F.F. containing water, with its outlet connected to another empty B.F.F., which was used to prevent suck-back of brine (28% NaCl) from the trough. A PVC tube

was attached to the outlet of the second empty B.F.F., which was positioned under an inverted measuring cylinder filled with brine (28% NaCl) in a water trough filled with water such that the free end of the PVC tube was directed upright in the measuring cylinder as shown in Fig. 3 below. The downward displacement of brine in the measuring cylinder served as the measure of the volume biogas generated. All the connections were done using PVC tubes of the same size with the one described in section 3.6. The procedure was repeated 3 times, and the average daily volume of the biogas generated was recorded.

Fig. 3- Set A: Direct Collection of Biogas

*In the set up above, stand and clamp was used to support the gas – holder

SET B: A parallel set-up was arranged for the removal of CO_2 from the biogas generated and collection of CH_4 and H_2S. In this case, the biogas generated after passing through an empty B.F.F. was then passed through 1M NaOH solution in another B.F.F. to absorb CO_2 according to the equation:

$2NaOH\ (aq) + CO_2\ (g) \rightarrow Na_2CO_3\ (aq) + H_2O\ (l)$ ----------(39)

The outlet of the B.F.F. containing 1M NaOH solution was connected to another empty B.F.F. and the remaining gases (i.e. CH_4 and H_2S) after passing through the second empty B.F.F. were finally collected over water as shown in Fig. 4 below. Triplicate sets were made, and the average daily volume of biogas generated was recorded.

SET C: In order to remove H_2S from the biogas generated as well as to collect CH_4 and CO_2, similar set up with Set B was made, only that in this case, the absorbent used was 30% lead ethanoate in 3M ethanoic acid to

absorbs H_2S as shown in Fig.5 below. The equation for the reaction was as follows:-

$(CH_3COO)\ 2Pb_{(aq)} + H_2S_{(g)} \rightarrow 2CH_3COOH_{(aq)} + PbS_{(s)}$ -------- (40)

Triplicate sets were made, and the average daily volume of biogas generated was recorded.

Fig. 4- Set B: Absorption Technique for the Removal of CO_2
*In the set up above, stand and clamp was used to support the gas-holder

Fig. 5- Set C: Absorption Technique for the Removal of H_2S
*In the set up above, stand and clamp was used to support the gas - holder

The volume of water used in set A and the volumes of absorbents used in sets B and C were equal, this is to ensure that the physical conditions remain the same in all the set ups.

The volume of CH_4 in the biogas was evaluated as follows:

$$VCH_4 = V_{Biogas} - [VCO_2 + VH_2S] \quad \text{-----(41)}$$

V_{Biogas} = Volume of biogas generated in Set A.

VCO_2 = Volume of biogas generated in Set A – Volume of biogas generated in Set B.

VH_2S = Volume of biogas generated in Set A – Volume of biogas generated in Set C.

where, V_{Biogas}, VCH_4, VCO_2 and VH_2S are the respective volumes of biogas, CH_4, CO_2 and H_2S.

The percentage composition of the biogas generated was evaluated as follows:

$$CH_4(\%) = \frac{VCH_4}{V_{Biogas}} \times 100 \quad \text{----- (42)}$$

$$CO_2(\%) = \frac{VCO_2}{V_{Biogas}} \times 100 \quad \text{----- (43)}$$

$$H_2S(\%) = \frac{VH_2S}{V_{Biogas}} \times 100 \quad \text{----- (44)}$$

The concentrations of the slurries in all set-ups were equal i.e., 40g substrate/250cm^3 H_2O + 1.8g yeast. The slurries were proportionately prepared based on 4g substrate/25 cm^3 H_2O and 0.18g yeast. (See appendixes 4b and c for more details).

3.30 **EFFECT OF BUFFERING ON THE COMPOSITION OF BIOGAS**

The set ups and the concentrations of the slurries were as in section 3.29, only that in making the slurry, 0.2M buffer solution of pH 7.3 was used instead of water. Triplicate sets were used, and the average volume of biogas generated was calculated for each.

***NB**: 0.2M Buffer solution of pH 7.3 was used for the same reason as in section 3.28.*

3.31 EFFECT OF ADDITION OF UREA ON BIOGAS COMPOSITION

The set ups and the concentrations of the slurries were as in section 3.29, only that, the slurries were prepared using 0.015M urea solution. Triplicate sets were made, and the average volume of biogas generated was calculated for each.

NB: The choice 0.015M urea solution was made because it was the molar concentration of urea at which high biogas was generated.

3.32 COMBINED EFFECT OF BUFFERING AND OF UREA (AS A NUTRITIVE ADDITIVE) ON BIOGAS COMPOSITION

The set ups and the concentrations of the slurries were as in section 3.29, only that the slurries were prepared using 0.2M buffer solution of pH 7.3 containing 0.38g urea per 250cm^3. Triplicate sets were made, and the average volume of biogas generated was calculated for each.

NB: 0.2M buffer solution of pH 7.3 and the quantity of urea added 0.38g were used because it was the condition at which high biogas generation was observed, where 4g substrate/ 25cm^3 of 0.2M buffer solution of pH 7.3 containing 0.38g urea were used.

3.33 EFEECT OF LIPID (OIL) ON BIOGAS PRODUCTION.

The set ups were as in section 3.20. The concentration of the slurry used in each digester was 4g/25cm^3 H_2O in which different quantities of oil was added using micro-syringe. 0.18g yeast was also added to each digester. Triplicate sets were made, and the average volume of biogas generated was calculated for each.

The digesters were as follows:

Control digester $\Rightarrow$ 4.0g substrate + 25cm^3 H_2O + 0.18g yeast without oil

Digester A $\Rightarrow$ 4.0g substrate + 25cm^3 H_2O + 0.18g yeast + 0.05g oil

Digester B $\Rightarrow$ 4.0g substrate + 25cm^3 H_2O + 0.18g yeast + 0.07g oil

Digester C $\Rightarrow$ 4.0g substrate + 25cm^3 H_2O + 0.18g yeast + 0.09g oil

Digester D $\Rightarrow$ 4.0g substrate + 25cm^3 H_2O + 0.18g yeast + 0.10g oil

Digester E $\Rightarrow$ 4.0g substrate + 25cm^3 H_2O + 0.18g yeast + 0.15g oil

(See appendix 8a for the details about the weight of oil used)

3.34 EFFECT OF SUGAR ON BIOGAS PRODUCTION

The set ups and the concentrations of the slurries were as in section 3.33, only that different quantities of sugar were added to the digesters instead of oil. Triplicate sets were made and the average volume of biogas generated was calculated for each.

The digesters were as follows:

Control Digester$\Rightarrow$ 4.0g substrate+25cm^3 H_2O+0.18g yeast without Sugar

Digester A $\Rightarrow$ 4.0g substrate+25cm^3 H_2O+0.18g yeast +0.02g sugar

Digester B $\Rightarrow$ 4.0g substrate+25cm^3 H_2O+0.18g yeast +0.03g sugar

Digester C $\Rightarrow$ 4.0g substrate+25cm^3 H_2O+0.18g yeast +0.04g sugar

Digester D ⇒ 4.0g substrate+25cm^3 H_2O+0.18g yeast +0.05g sugar

Digester E ⇒ 4.0g substrate+25cm^3 H_2O+0.18g yeast +0.06g sugar

3.35 COMBINED EFFECT OF OIL AND SUGAR ON BIOGAS PRODUCTION.

The set ups and the condition of the slurries were as in section 3.33, only that oil, sugar and a mixture of oil and sugar, were respectively added to digester A, B and C. Triplicate sets were made, and the average volume of biogas generated was calculated for each.

The digesters were as follows:

Control digester ⇒ 4.0g substrate+25cm^3 H_2O + 0.18g yeast

Digester A ⇒ 4.0g substrate+25cm^3 H_2O + 0.18g yeast + 0.09g oil

Digester B ⇒ 4.0g substrate+25cm^3 H_2O + 0.18g yeast + 0.06g sugar

Digester C ⇒ 4.0g substrate + 25cm^3 H_2O + 0.18g yeast + 0.09g oil + 0.06g sugar

3.36 **EFFECT OF PROTEIN ON BIOGAS PRODUCTION**

The set ups and the concentrations of the slurries were as in section 3.33, only that different quantities of blood meal (as source of protein) were added to the digesters instead of oil. Triplicate sets were made, and the average volume of biogas generated was calculated for each.

The digesters were as follows:

Control digester ⇒ 4.0g substrate+25cm^3 H_2O + 0.18g yeast without blood meal

Digester A ⇒ 4.0g substrate+25cm^3 H_2O + 0.18g yeast + 0.01g blood meal

Digester B $\Rightarrow$ 4.0g substrate+25cm^3 H_2O + 0.18g yeast + 0.02g blood meal

Digester C $\Rightarrow$ 4.0g substrate+25cm^3 H_2O + 0.18g yeast + 0.03g blood meal

Digester D $\Rightarrow$ 4.0g substrate+25cm^3 H_2O+ 0.18g yeast + 0.04g blood meal

Digester E $\Rightarrow$ 4.0g substrate+25cm^3 H_2O + 0.18g yeast + 0.05g blood meal

3.37 EFFECT OF CARBOXYLIC ACID ON BIOGAS PRODUCTION

The set ups and the concentrations of the slurries were as in section 3.33, only that different quantities of ethanoic acid were added to the digesters instead of oil. Triplicate sets were made, and the average volume of biogas generated was calculated for each.

The digesters were as follows:

Control Digester $\Rightarrow$ 4.0g substrate + 25cm^3 H_2O + 0.18g yeast without CH_3COOH

Digester A $\Rightarrow$ 4.0g substrate + 25cm^3 H_2O +0.18g yeast + 0.01g CH_3COOH

Digester B $\Rightarrow$ 4.0g substrate + 25cm^3 H_2O +0.18g yeast + 0.02g CH_3COOH

Digester C $\Rightarrow$ 4.0g substrate + 25cm^3 H_2O +0.18g yeast + 0.03g CH_3COOH

Digester D $\Rightarrow$ 4.0g substrate + 25cm^3 H_2O +0.18g yeast + 0.04g CH_3COOH

Digester E $\Rightarrow$ 4.0g substrate + 25cm^3 H_2O +0.18g yeast + 0.05g CH_3COOH

(See appendix 8b for the details about the weight of the ethanoic acid used).

3.38 **EFFECT OF ADDITION OF YEAST ON BIOGAS PRODUCTION POTENTIAL OF FERMENTED SLURRY**

The set ups were as in section 3.20. The slurry used in each of the digesters was prepared by mixing 4.0g of fermented substrate with 25cm^3H_2O, and 0.18g yeast was added. Triplicate sets were made, and the average volume of biogas generated was calculated for each.

The digesters were as follows:

Control digester $\Rightarrow$ 4.0g unfermented substrate + 25cm^3 H_2O + 0.18g yeast

Exp. digesters $\Rightarrow$ 4.0g fermented substrate + 25cm^3 H_2O + 0.18g yeast

3.39 **BIOGAS PRODUCTION POTENTIAL OF EXTRACTED SUBSTRATE**

The set ups and the concentrations of the slurries were as in section 3.38, but in this case, extracted substrate was used instead of fermented substrate. Triplicate sets were made and the average volume of biogas generated was calculated for each.

The digesters were as follows:

Control digesters $\Rightarrow$ 4.0g unextracted substrate + 25cm^3 H_2O +0.18g yeast

Exp. digesters $\Rightarrow$ 4.0g extracted substrate + 25cm^3 H_2O + 0.18g yeast

3.40 COMPARISON OF BIOGAS GENERATION OF BANANA LEAVES AND MAIZE COB, MAIZE STALK, WATER HYACINTH, ELEPHANT GRASS AND COW DUNG.

The set ups were as in section 3.20, but in this case, the substrates were of different kinds. The substrates used were: Banana leaves (as control), maize cob, maize stalk, water hyacinth, elephant grass and cow dung, and $25cm^3$ H_2O was added to each. With the exception of the digester containing cow dung, 0.18g yeast was added to each of the digesters.

The digesters were as follows:

Control digester $\Rightarrow$ 4.0g banana leaves + $25cm^3$ H_2O +0.18g yeast

Digester A $\Rightarrow$ 4.0g maize cob + $25cm^3$ H_2O + 0.18g yeast

Digester B $\Rightarrow$ 4.0g maize stalk + $25cm^3$ H_2O + 0.18g yeast

Digester C $\Rightarrow$ 4.0g water hyacinth + $25cm^3$ H_2O + 0.18g yeast

Digester D $\Rightarrow$ 4.0g elephant grass + $25cm^3$ H_2O + 0.18g yeast

Digester E $\Rightarrow$ 4.0g cow dung+ $25cm^3$ H_2O without yeast

Triplicate sets of each were made, and the average volume of biogas generated was calculated.

3.41 PRODUCTION AND COLLECTION OF BIOLIQUID (MALTENES AND ASPHALTENES) FROM FERMENTED AND UNFERMENTED SLURRIES

After the generation of biogas from the organic slurry, the fermented slurry was drained and used for bioliquid extraction. This was achieved by soxhlet extraction using absolute methanol at 60^oC. The bioliquid was recovered from the solvent (absolute methanol) after extraction by evaporation using rotary evaporator, after which it was

concentrated to a constant weight using hot plate at 37^{o}C in a fume cupboard. The same procedure was followed for the collection of bioliquid from unfermented substrate.

3.42 PRECIPITATION OF ASPHALTENES

1.0g of the concentrated bioliquid from the fermented slurry was dissolved using a mixture of 1cm^3 methanol and 40cm^3 hexane in a beaker and subsequently placed in a refrigerator for 24hours. The maltenes (deasphaltened oils), which is composed of saturates, monoaromatics, diaromatics, polyaromatics, and small quantities of resins and polars (hydrocarbons containing any of O, S, N or halogens) being the soluble

component of the bioliquid remained in the solution while the asphaltenes (usually composed of resins and polars) being the insoluble component settled down at the bottom of the beaker. The solution was carefully decanted and both the maltenes (solution) and the asphaltenes (precipitate) were separately dried.

The same procedure was followed for the precipitation of asphaltenes from unfermented slurry extract (bioliquid).

3.43 COLUMN CHROMATOGRAPHIC SEPARATION OF MALTENES (DEASPHALTENED OILS) OF FERMENTED AND UNFERMENTED SLURRIES

For the separation of the components of the maltenes obtained from the fermented slurry into saturates, monoaromatics, diaromatics, polyaromatics and resins/polars. A glass column of 40cm length and 0.85cm internal diameter with a bed volume of 22.72cm^3 was packed with

silica gel of particles size 0.063 – 0.014nm (activated at 100^{o}C overnight in an oven) and alumina of particles size 3.74µm in the ratio 1:2 silica gel to alumina.

The sample to packing material (sorbent) ratio was 1:70 for silica gel and 1:140 for alumina, with a column maximum load capacity of 72.5mg. (See appendices 9a, b and c for the details).

The column was carefully packed with silica gel, which was relatively denser than alumina followed by alumina, which was relatively less dense than silica gel to allow for uniform sample size densities and avoid void volumes.

The packed column was washed with 30cm^3 of hexane. The flow rate of the hexane through the column was 0.33cm^3/minutes. This slow flow rate was due to the micro-bore nature of the column (0.85cm internal diameter) and the fine structure of the packing materials. The cleaning (washing) of the column was done with utmost care to avoid air bubbles from entering the column, which could result, into further lowering of the flow rate.

After washing the packed column, the sample was then applied as a concentrated solution of hexane. The sample loaded in the packed column

was eluted in five (5) separate fractions with the following solvents of 30cm^3 each: For the first fraction (saturates), hexane was used as eluent; for the second fraction (monoaromatics), 5% benzene/hexane was used

as eluent; for the third fraction (diaromatics), 15% benzene/hexane was used as eluent; for the fourth fraction (polyaromatics) 20% benzene, 20% diethyl ether and 60% methanol was used as eluent; and for the fifth fraction (resins/polars) methanol was used as eluent.

Each of the fractions was collected in a known weight 100cm^3 beaker, and dried in a fume cupboard and weighed. The weight difference between the weight of the beaker together with the fraction and that of the beaker alone was calculated as the weight of each fraction.

The same procedure was followed for the maltenes collected from the unfermented slurry.

3.44 UREA ADDUCTION OF N-ALKANES FROM THE SATURATES FRACTIONS OF MALTENES OF THE FERMENTED BIOLIQUID

In order to preferentially separate the straight chain alkanes (n-alkanes) from the mixture of n-alkanes, iso-alkanes and cyclics of the saturates fraction obtained in section 3.43 above, urea adduction process was employed.

The fraction (38.00mg saturates) was first dissolved in n-hexane, after which a saturated solution of urea in acetone was gently added. A white crystalline precipitate, which contained the n-alkanes was formed on addition of saturated urea solution. When no more crystals were formed with the addition of saturated urea solution, the addition of the saturated urea solution was stopped because all the interstitial spaces of the crystalline structure of the urea were filled up with the adducted n-alkanes. The crystals formed were filtered off, washed with hexane and dissolved in distilled water. The dissolved crystals were extracted several times with

n-hexane. The n-hexane –extract, which contained the n-alkanes was air –dried in a clean weighed dry 50ml beaker to a constant weight.

3.45 THIOUREA ADDUCTION OF ISO-ALKANES AND THE SUBSEQUENT SEPARATION OF CYCLICS

The urea non-adduct, which was left in the aqueous solution after extraction with be n-hexane in section 3.44 above was air – dried and dissolved in benzene. A saturated solution of thiourea in methanol was prepared for the adduction of cycloalkanes (cyclics).The urea non-adduct in benzene was mixed with the thiourea solution in methanol in ratio of 1.1 (V/V) and left at room temperature and crystals were formed. The crystals formed were filtered off (as a residue), washed several times with benzene and dissolved in distilled water.The dissolved residue was then extracted with benzene. The benzene– extract was evaporated to dryness in a clean 50ml beaker and weighed as cyclics. The filtrate was also evaporated to dryness in another clean 50ml beaker and weighed as iso-alkanes.

4.46 GAS CHROMATOGRAPHIC IDENTIFICATION OF GASEOUS DEGRADATION PRODUCTS (BIOGAS) USING FLAME IONISATION DETECTOR (FID) AND TEHRMAL CONDUCTIVITY DETECTOR (ICD)

In order to identify the individual components of the $C_1 – C_4$ of the hydrocarbons in the biogas generated, a gas – liquid chromatograph (glc) fitted with the pora - park VZ7 (dimension 8m x 3mm) column made of stainless steel was used. The column was kept at 150^0c, the flame ionization detector (FID), which was maintained at 220^0c was used. Helium was used as carrier gas (flow rate $50m^3$ /min and pressure $5kg/cm^2$). The sample size was 8µl, which was injected into glc at 100^0c injection temperature. The component was identified using its glc retention

time, which was compared with the glc retention time of liquefied petroleum gas (LPG) obtained from crude oil distillation, which served as a standard retention time.

A thermal conductivity detector (TCD) was used for the identification of $C0_2$. The column was programmed between 40^0c – 100^0c at a heating rate of 30^0c / minute after an initial holding period of 3.5 minutes. The carrier gas, the flow rate and the pressure of the carrier gas, the sample size and the injection temperature were the same as for FID.

4.47 GC–MASS SPECTROMETRIC ANALYSIS OF THE HEAVER HYDROCARBONS IN THE LIQUID DEGRADATION PRODUCTS

The GC- Mass Spectrometre used was 6890N network GC system coupled with the 5973 network mass selective system. The temperature range of the oven of the column was 150^0c – 300^0c (oven temperature), auxiliary temperature (i.e the maximum temperature set for the analysis) was 320^0c, the column was capillary in shape with an internal diameter of 0.25mm, the length of the column was 30cm and the column maximum capacity was 10µl. The carrier gas used was helium.

The sample was first dissolved in n-haxane and subsequently 6µl was injected into the gas chromatograph at an injection temperature of 200^0c for component separation by volatilization. The volatilized components from the gas chromatography were after separation of the carrier gas, passed to the ionization chamber of the mass spectrometer, where they were bombarded, with high energy and ionized. The ions formed were then accelerated in an electric field and separated according to their mass to charge ratio (m/e) by a magnetic field, and the relative abundance of the ions were counted by the detector. The output of the

detector were amplified and recorded by the recorder. The mass spectrum was shown as a plot of relative abundance versus m/e and printed out. The structure of the compounds in the sample were elucidated from the labeled peaks of the mass spectrum and the corresponding fragmentation patterns of the labeled peaks. See appendixes 11a -11q for more details.

It should be noted that fragment ions were also present and their relative abundance were also detected and shown on the same spectra.

CHAPTER FOUR

RESULTS

4.1 DESCRIPTION OF THE TABLES OF RESULTS

Table 5 shows the average volumes of biogas generated (cm^3) daily for the determination of suitable water content. From the table, it could be seen that digesters A, B,C, D and E in which the respective volumes of water used in making the slurries were $15cm^3$, $20cm^3$, $25cm^3$, $30cm^3$ and $35cm^3$ generated $36.00cm^3$, $45.00cm^3$, $56.50cm^3$, $36.50cm^3$ and $8.00cm^3$, respectively.

Table 5: Determination of Suitable Water Content

Average volumes of biogas generated (cm^3) daily

Time	**Digesters of Equal Capacities**				
(Day)	**A**	**B**	**C**	**D**	**E**
01	21.00	24.00	26.50	21.00	2.00
02	9.00	13.00	16.00	10.50	0.00
03	4.00	4.00	12.00	0.00	4.00
04	2.00	4.00	0.00	0.00	2.00
05	0.00	0.00	0.00	0.00	0.00
06	0.00	0.00	0.00	0.00	0.00
07	0.00	0.00	0.00	0.00	0.00
Total	**36.00**	**45.00**	**56.50**	**36.50**	**8.00**

Table 6 shows the average volume of biogas generated in cm^3 daily for the determination of the optimum concentration of yeast needed for the maximum biogas generation from a slurry whose concentration was 4g/25 cm^3. The Table shows that digester E in which 0.18g yeast was added yielded more biogas than all the other digesters investigated (i.e, control digester, A, B, C, D, E and F) in which 0.06g,0.08g, 0.10g,0.12g, 0.14g and 0.20g yeast were respectively added, despite the fact that all the digesters used for the determination were of equal slurry concentration.

Table 6: Determination of Optimal Yeast Concentration

Average volumes of biogas generated (cm^3) daily

Time	**Digesters of Equal Capacities**						
(Day)	**Control**	**A**	**B**	**C**	**D**	**E**	**F**
01	26.50	38.00	39.00	39.00	42.00	45.00	36.00
02	16.00	9.00	14.00	18.00	24.00	22.00	12.50
03	12.00	6.00	10.00	11.50	8.00	14.00	6.00
04	2.00	6.00	6.00	6.00	2.00	0.00	0.00
05	0.00	0.00	0.00	0.900	0.00	0.00	0.00
06	0.00	0.00	0.00	0.00	0.00	0.00	0.00
07	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total	**56.50**	**59.00**	**69.00**	**74.50**	**76.00**	**81.00**	**54.50**

Table 7 shows that the optimal temperature condition for maximum biogas generation was 33^0C, because it was the temperature at which highest cumulative volume of biogas was generated when compared with the volumes of biogas generated at room temperature,40^0C and 45^oC. independently.

Table 7 – Determination of Optimal Temperature Condition.

Cumulative average volumes of biogas generated (cm^3)

Digesters of equal capacities	**Temperatures**			
	Room Temperature	33^OC	40^OC	45^OC
A	43.80	81.50	68.00	40.50
B	43.00	82.00	68.50	38.00
C	43.50	80.90	68.50	40.00
D	44.00	81.00	69.20	37.00
E	42.90	82.00	66.90	41.00

Table 8 shows that digester B in which 8g/50cm^3 slurry was used yielded the highest volume of biogas when compared with the respective volumes of biogas generated in the control digester, A,C,D and e in which the concentrations of the slurries were 49/25cm^3, 6g/37.5cm^3, 10g/62.5cm^3, 12g/75cm^3 and 14g/87.5cm^3, respectively. This shows that digester B in which 8g/50cm^3 slurry was used, was the best.

Table 8: Effect of Slurry Mass Change on Biogas Generation at Fixed Concentration of Yeast

Average volumes of biogas generated (cm^3) daily

Time	Digesters of Equal Capacities					
(Day)	Control	A	B	C	D	E
01	45.00	82.50	86.80	74.00	68.50	49.70
02	22.00	43.00	51.60	44.00	38.00	29.00
03	14.00	24.00	34.00	30.00	26.50	20.00
04	0.00	12.00	18.00	8.00	10.50	8.00
05	0.00	0.00	6.00	0.00	0.00	0.00
06	0.00	0.00	0.00	0.00	0.00	0.0
07	0.00	0.00	0.00	0.00	0.00	0.00
08	0.00	0.00	0.00	0.00	0.00	0.00
Total	**81.00**	**161.50**	**196.40**	**156.00**	**143.50**	**106.70**

Table 9 shows that the generation of biogas increases with increase in the amount of slurry and the amount of yeast added provided that the concentration of the slurry and the amount yeast added are optimum.

Table 9: Effect of Slurry Mass Change on Biogas Generation at Varying Concentration of Yeast

Average Volumes of Biogas Generated (cm^3) daily

Time	Digesters of Equal Capacities					
(Day)	Control	A	B	C	D	E
01	45.00	76.00	93.60	101.00	108.00	114.00
02	22.00	49.00	58.00	60.50	72.00	80.50
03	14.00	32.00	36.00	42.00	47.50	52.00
04	0.00	18.00	21.00	30.00	33.00	38.50
05	0.00	0.00	0.00	8.00	12.00	16.00
06	0.00	0.00	0.00	0.00	0.00	0.00
07	0.00	0.00	0.00	0.00	0.00	0.00
08	0.00	0.00	0.00	0.00	.00	0.00
Total	**81.00**	**175.00**	**208.60**	**241.50**	**272.50**	**301.00**

Table 10 shows that digester B in which 0.2M buffer solution was used generated highest volume of biogas when compared with the volumes of biogas generated in the control digester, A, C, D and E in which the molar concentrations were 0.0M, 0.1M, 0.3M, 0.4M and 0.5M, respectively.

Table 10: Effect of Buffering on Biogas Generation Using Buffer Solution of pH 7.3 but different Molar Concentrations

Average volumes of biogas generated (cm^3) daily

Time	Digesters of Equal Capacities					
(Day)	Control	A	B	C	D	E
01	45.00	46.00	55.00	40.00	29.00	18.00
02	22.00	24.00	36.00	20.00	12.00	6.00
03	14.00	9.00	13.00	6.00	2.00	0.00
04	3.00	8.00	6.00	0.00	0.00	0.00
05	0.00	0.00	0.00	0.00	0.00	0.00
06	0.00	0.00	0.00	0.00	0.00	0.00
07	0.00	0.00	0.00	0.00	0.00	0.00
Total	**81.00**	**88.00**	**110.00**	**66.00**	**43.00**	**24.00**

Table 11 shows the effect of pH on biogas generation. In spite of the fact that the molar concentrations of the buffer solution used were the same (i.e. 0.2M) in all the digesters investigated, the control digester in which the pH of the buffer solution was 7.3 generated more biogas than all the other pH values investigated(ie,6.8,7.0,7.2,7.4 and 7.6) in digester A,B,C,D and E, respectivily. Thus, 7.3 was found to be the best P^H for maximum biogas generation.

Table 11: Effect of pH on Biogas Generation using 0.2M Buffer Solution of different pH

Average volumes of biogas generated (cm^3) daily

Time	Digesters of Equal Capacities					
(Day)	Control	A	B	C	D	E
01	55.00	47.00	49.00	58.50	47.00	35.80
02	36.00	14.00	23.00	22.00	28.00	20.00
03	13.00	3.00	7.00	6.00	8.00	8.00
04	6.00	0.00	4.00	3.00	4.00	2.00
05	0.00	0.00	0.00	0.00	0.00	0.00
06	0.00	0.00	0.00	0.00	0.00	0.00
07	0.00	0.00	0.00	0.00	0.00	0.00
Total	**110.00**	**64.00**	**83.00**	**89.50**	**87.00**	**65.80**

The effect of urea on biogas yield is shown in Table 12. The Table shows that digester B in which 0.015M urea was added generated more biogas than the control digester, A,C,D and E in which 0.0M,0.01M, 0.02M, 0.025M and 0.03M urea, respectively.

Table 12: Effect of Urea on Biogas Production

Average volumes of biogas generated (cm^3) daily

Time	Digesters of Equal Capacities					
(Day)	Control	A	B	C	D	E
01	45.00	60.00	63.00	60.00	54.00	46.00
02	22.00	29.00	32.00	29.50	26.00	18.00
03	14.00	7.50	4.00	5.00	2.00	4.00
04	0.00	0.00	0.00	0.00	0.00	0.00
05	0.00	0.00	0.00	0.00	0.00	0.00
06	0.00	0.00	0.00	0.00	0.00	0.00
07	0.00	0.00	0.00	0.00	0.00	0.00
Total	**81.00**	**96.50**	**99.00**	**94.50**	**82.00**	**68.00**

The result of the combined effect of a buffer solution and urea as a nutritive additive on biogas generation is shown in Table 13. The Table shows that digester D in which a mixture of 0.2M buffer solution of pH 7.3 and 0.038g urea was used yielded more biogas than the control digester, A, B, C and E in which 0.0g, 0.01g, 0.023g, 0.03g and 0.045g urea, where respectively added, despite the fact that the pH and the molar concentrations of the buffer solution used were the same.

Table 13: Combined Effect of a Mixture of 0.2M Buffer Solution of pH 7.3 and Urea on Biogas Production

Average volumes of biogas generated (cm^3) daily

Time	Digesters of Equal Capacities					
(Day)	Control	A	B	C	D	E
01	55.00	53.00	61.00	65.00	70.00	52.00
02	36.00	32.00	33.00	36.00	41.00	24.00
03	13.00	14.50	15.00	18.00	12.00	13.00
04	6.00	12.00	6.00	7.00	6.00	5.00
05	0.00	0.00	0.00	0.00	0.00	0.00
06	0.00	0.00	0.00	0.00	0.00	0.00
07	0.00	0.00	0.00	0.00	0.00	0.00
Total	**110.00**	**111.50**	**115.00**	**126.00**	**129.00**	**94.00**

Table 14 shows the compositional analysis of the biogas generate using water. From Table, it could be seen that digester A in which biogas (CH_4,CO_2 and H_2S) were collected, generated 835.00cm^3; digester B in which CH_4 and H_2S were collected, generated 575.50cm^3; and digester C in which CH_4 and CO_2 were collected, generated 834.00 cm^3 . Thus, the amount of biogas generated was 835.00 cm^3, the amount of CO_2 generated was 835.00 cm^3 – 575.50 cm^3, the amount of H_2S generated was 835.00 cm^3 – 834.0 cm^3 and the amount of CH_4 generated was 835.00 cm^3 - (the amounts of CO_2 + H_2S generated).

Table 14 : Compositional Analysis of Biogas Generated

Average volumes of biogas generated (cm^3) daily

Time	Digesters of equal capacities		
(Day)	A	B	C
01	308.00	193.00	306.00
02	128.00	98.00	130.00
03	102.00	62.00	120.00
04	122.00	51.00	100.00
05	85.00	85.00	84.00
06	48.00	49.00	47.00
07	30.00	0.00	18.00
08	12.00	24.50	16.00
09	0.00	0.00	0.00
10	0.00	13.00	0.00
11	0.00	0.00	12.00
12	0.00	0.00	0.00
13	0.00	0.00	0.00
14	0.00	0.00	0.00
Total	**835.00**	**575.50**	**834.00**

The effect of buffering on the composition of the biogas generated is shown in Table 15. The Table shows that the use of buffer solution in making slurry instead of using water enhances the generation of biogas, even though, it causes the CH_4 content of the biogas to decrease while the CO_2 and H_2S contents to increase. The Table also shows that the control digester in which water was used in making the slurry, generated 835.00cm^3 of biogas, CH_4, CO_2 and H_2S. The Table also shows that from volume of biogas generated in digester A (1,058.00cm^3), the volume of CO_2 generated in digester B was estimated as 1,058.00cm^3 – 696.00cm^3, the volume of H_2S generated in digester C was estimated as 1,058.00cm^3 – 1,055.50.00cm^3 and the volume of CH_4 generated was estimated as 1,058.00cm^3 – (the volumes of CO_2 + H_2S generated).

Table 15 : Effect of Buffering on the Composition of Biogas

Average volumes of biogas generated (cm^3) daily

Time	Digesters of Equal Capacities			
(day)	Control	A	B	C
01	308.00	382.00	242.00	380.50
02	128.00	143.00	98.00	141.00
03	102.00	120.00	96.50	118.50
04	122.00	112.00	68.00	96.00
05	85.00	102.00	22.00	98.00
06	48.00	98.00	59.00	92.50
07	30.00	62.00	58.00	66.00
08	12.00	39.00	40.50	42.00
09	0.00	0.00	0.00	0.00
10	0.00	0.00	12.00	0.00
11	0.00	0.00	0.00	21.00
12	0.00	0.00	0.00	0.00
13	0.00	0.00	0.00	0.00
14	0.00	0.00	0.00	0.00
Total	**835.00**	**1,058.00**	**696.00**	**1,055.50**

Table 16 shows the effect of urea on the composition of the biogas produced. The Table shows that the use of urea solution in making the slurry instead of water enhances biogas generation. The Table also shows that the control digester generated 835.00cm^3 of biogas (i.e CO_2, H2S and CH_4), the volume of biogas generated in digester A was 1,042.00cm^3, the volume of CO_2 generated in digester B was estimated as 1,042.00cm^3 – 669.00cm^3, the volume of H_2S generated in digester C was estimated as 1,042.00cm^3 – 1,038.50cm^3 and the volume of CH_4 generated was estimated as 1,042.00cm^3 – (the volumes of CO_2 + H_2S generated).

Table 16: Effect of Urea on the Composition of Biogas.

Average volume of biogas generated (cm^3) daily

Time	Digesters of Equal Capacities			
(day)	Control	A	B	C
01	308.00	373.00	212.00	369.50
02	128.00	138.50	108.00	132.00
03	102.00	107.00	86.00	109.00
04	122.00	121.00	73.00	119.00
05	85.00	96.00	49.00	94.00
06	48.00	67.00	38.00	69.00
07	30.00	52.00	0.00	47.00
08	12.00	35.00	42.00	43.00
09	0.00	30.50	41.00	0.00
10	0.00	0.00	0.00	34.00
11	0.00	22.00	20.00	22.00
12	0.00	0.00	0.00	0.00
13	0.00	0.00	0.00	0.00
14	0.00	0.00	0.00	0.00
Total	**835.00**	**1,042.00**	**669.00**	**1,038.50**

The combined effect of buffer solution and urea as a nutritive additive on the composition of the biogas generated is shown in Table 17. The Table shows that the use of a mixture of buffer solution and urea in making the slurry enhances biogas yield. Table also shows that the control digester generated 1,058.00cm^3 of biogas (i.e CO_2, H_2S and CH_4), the volume of biogas (i.e CO_2, H_2S and CH_4) generated in digester A was 1,159.00cm^3, the volume of CO_2 generated in digester B was estimated as 1,159.00cm^3 – 700.00cm^3, the volume of H_2S generated in digester C was estimated as 1,159.00cm^3 – 1,154.50cm^3 and the volume of CH_4 generated was estimated as 1,159.00cm^3 – (the volumes of CO_2 + H_2S generated).

Table 17: Combined Effect of 0.2M Buffer Solution (pH 7.3) and Urea on Biogas Composition

Average volume of biogas generated (cm^3) daily

Time	Digesters of Equal Capacities			
(day)	Control	A	B	C
01	382.00	398.50	248.00	389.00
02	143.00	156.00	102.50	143.50
03	120.00	122.50	98.00	124.00
04	98.00	104.00	45.00	106.00
05	102.00	99.00	66.00	94.50
06	98.00	84.00	31.50	82.50
07	62.00	72.00	0.00	76.00
08	39.00	61.50	38.00	68.00
09	0.00	42.00	43.00	42.00
10	0.00	0.00	28.00	29.00
11	0.00	20.00	0.00	0.00
12	0.00	0.00	0.00	0.00
13	0.00	0.00	0.00	0.00
14	0.00	0.00	0.00	0.00
Total	**1,058.00**	**1,159.50**	**700.00**	**1,154.50**

Table 18 shows the compositional comparison of the biogas generated at different operational conditions. The results revealed that though, the composition of the biogas generated was the same (CH_4,$C0_2$ and H_2S), but the percentages of the constituents gases differed at different operational conditions.

Table 18: Compositional Comparison of the Biogas Generated at Different Operational Conditions.

Condition of the slurry	Percentage Composition		
	CH_4	$C0_2$	H_2S
Contained water	68.78	31.10	0.12
Contained buffer solution	65.54	34.22	0.24
Contained urea	63.86	35.80	0.34
Contained buffer solution & urea	59.94	39.63	0.43

Table 19 shows the percentage increase of CH_4, $C0_2$ and H_2S of the biogas generated under different operational conditions. The results revealed that the percentage of CH_4 in the biogas generated decreased while the percentages of $C0_2$ and H_2S increased, when buffer solution, urea and a mixture of urea and buffer solution were independently added the slurry instead of water alone.

Table 19: Percentage Increase of CH_4, CO_2 and H_2S at Different Operational Conditions

Condition / ***Composition***	***Using buffer solution***	***Addition of urea***	***Using buffer solution & addition of urea***
CH_4(%)	20.71	15.84	20.97
CO_2 (%)	39.50	43.74	55.50
H_2S(%)	150.00	250.00	400.00

***See appendixes 6a, b and c for more details**

Table 20 shows the effect of addition of oil on biogas production. The result revealed that addition of oil to slurry enhanced biogas generation, more particularly at optimum concentration as shown by digester C in which 0.09g oil was added to 4g/25cm^3 slurry generated more biogas than the control digester, A, B, D and E in which 0.00g, 0.05g, 0.07g, 0.10g and 0.15g oil, were respectively added.

Table 20: Effect of Lipids (Oil) on Biogas Production

Average volumes of biogas generated (cm^3) daily.

Time	**Digesters of equal capacities**					
(Day)	Control	A	B	C	D	E
01	45.00	49.00	58.00	53.00	49.00	40.00
02	22.00	38.00	38.00	42.00	48.00	58.00
03	14.00	24.00	28.50	29.00	21.00	17.00
04	0.00	16.00	14.50	18.00	13.00	10.50
05	0.00	14.00	16.00	15.00	20.00	10.00
06	0.00	0.00	11.00	13.00	10.00	12.00
07	0.00	0.00	0.00	0.00	0.00	0.00
08	0.00	0.00	0.00	0.00	0.00	0.00
09	0.00	0.00	0.00	0.00	0.00	0.00
Total	**81.00**	**141.00**	**166.00**	**170.00**	**161.00**	**147.50**

Table 21 shows the effect of addition of sugar to slurry on biogas production. From the result, it could be seen that the production of biogas in the control digester, A, B, C, D and E in which 0.00g, 0.02g, 0.03g, 0.04g, 0.05g and 0.06g sugar, were respectively added increased with the increase in the quality of sugar added.

Table 21: Effect of Sugar on Biogas Production

Average volumes of Biogas Generated (cm^3) daily

Time	Digesters of equal capacities					
(Day)	Control	A	B	C	D	E
01	45.00	46.00	48.00	53.00	57.00	62.00
02	22.00	41.00	53.00	42.00	49.00	50.00
03	14.00	32.00	34.50	29.00	44.00	50.00
04	0.00	14.00	23.50	25.00	30.50	39.50
05	0.00	3.00	12.00	15.00	8.00	25.00
06	0.00	0.00	5.50	14.00	0.00	10.00
07	0.00	0.00	0.00	6.00	0.00	0.00
08	0.00	0.00	0.00	0.00	0.00	0.00
09	0.00	0.00	0.00	0.00	0.00	0.00
Total	**81.00**	**136.00**	**176.50**	**184.00**	**186.50**	**236.50**

Table 22 shows that addition of a mixture of oil and sugar enhances biogas generation. From the Table, it could be seen that digester C in which a mixture of Oil and sugar was added to the slurry generated more biogas than digesters A and B in which oil and sugar, were respectively added. The Table also shows that the control digester in which neither oil nor sugar was added generated far less biogas than digesters A and B.

Table: 22 Combined Effect of Oil and Sugar on Biogas Production

Average volume of biogas generated (cm^3) daily

Time	Digesters of Equal Capacities			
(day)	Control	A	B	C
01	45.00	53.00	62.00	110.00
02	22.00	42.00	50.00	95.50
03	14.00	29.00	50.00	43.00
04	0.00	18.00	39.50	12.00
05	0.00	15.00	25.00	0.00
06	0.00	13.00	10.00	0.00
07	0.00	0.00	0.00	0.00
08	0.00	0.50	0.00	0.00
09	0.00	0.00	0.00	0.00
Total	**81.00**	**170.00**	**236.50**	**405.50**

Table 23: Effect of Protein on Biogas Production.

The result for the effect of protein on biogas production is shown in Table 23. The result shows that addition of protein to the slurry enhances biogas yield at optimum concentration. The Table also shows that digester C in which 0.03g blood meal (as a source of protein) was added, generated more biogas than the control digester, A, B, D and E in which 0.00g, 0.01g, 0.02g, 0.04g and 0.05g blood meal, were respectively added.

Table 23: Effect of Protein on Biogas Production

Average volume of biogas generated (cm^3) daily

Time	Digesters of equal capacities					
(Day)	Control	A	B	C	D	E
01	45.00	46.00	42.00	46.00	45.00	46.00
02	22.00	42.00	40.50	44.00	40.00	37.00
03	14.00	31.00	36.00	33.00	32.00	22.00
04	0.00	11.00	12.00	14.00	12.00	7.00
05	0.00	0.00	2.00	8.00	2.00	2.00
06	0.00	0.00	0.00	0.00	0.00	0.00
07	0.00	0.00	0.00	0.00	0.00	0.00
08	0.00	0.00	0.00	0.00	0.00	0.00
Total	**81.00**	**130.00**	**132.50**	**145.00**	**131.00**	**114.00**

Table 24 shows the effect of Carboxylic acid (ethanoic acid) on biogas production. The Table shows that addition of carboxylic acid enhanced biogas production at optimum concentration. It also reveals that digester B in which 0.02g ethanoic acid was added gave high production when compared with the control digester A, C, D and E in which 0.00g, 0.01g, 0.03g, 0.04g and 0.05g ethanoic acid, were respectively added.

Table 24: Effect of Carboxylic acid on Biogas Production

Average volumes of Biogas generated (cm^3) daily

Time	Digesters of equal capacities					
(Day)	Control	A	B	C	D	E
01	45.00	41.00	38.00	27.00	20.00	19.00
02	22.00	22.50	37.00	42.00	37.00	27.00
03	14.00	23.00	17.00	14.00	17.00	17.00
04	0.00	27.00	30.00	24.00	20.00	26.00
05	0.00	19.00	20.50	10.00	17.00	18.00
06	0.00	10.00	17.00	8.00	6.00	4.00
07	0.00	2.50	5.00	2.00	0.00	2.0
08	0.00	0.00	0.00	0.00	0.00	0.00
09	0.00	0.00	0.00	0.00	0.00	0.00
10	0.00	0.00	0.00	0.00	0.00	0.00
Total	**81.00**	**145.00**	**164.50**	**127.00**	**117.00**	**113.00**

The effect of addition of yeast on the biogas generation potential of fermented slurry is shown in Table 25. The Table shows that addition of yeast to fermented slurry (i.e. after first fermentation) led to generation of biogas with prolonged production period. It also shows that quantities of biogas generated in the subsequent fermentations (i.e the second and third) were not as much as in the first fermentation.

Table 25: Effect of addition of Yeast on Biogas Production Potential of Fermented Slurry

Average volumes of Biogas generated (cm^3) daily

Time (Day)	Digesters of equal capacities		
	Control Digester (1^{st} Fermentation)	2^{nd} fermentation	3^{rd} fermentation
01	45.00	18.00	16.50
02	22.00	14.50	10.00
03	14.0	10.00	10.00
04	0.00	8.00	6.50
05	0.00	10.00	0.00
06	0.00	0.00	0.00
07	0.00	0.00	0.00
08	0.00	0.00	0.00
Total	**81.00**	**60.50**	**43.00**

Table 26 shows the biogas generation potential of extracted substrate. The Table shows that the extracted substrate yielded far less gaseous degradation product (biogas) than the unextracted one.

Table 26: Biogas Generation Potential of Extracted Substrate

Average volumes of Biogas generated (cm^3) daily

Time (Day)	Digesters of equal capacities	
	Control Digesters (Unextracted substrate)	Exp. Digester (Extracted Substrate)
01	45.00	20.50
02	22.00	15.00
03	14.00	10.00
04	0.00	6.50
05	0.00	0.00
06	0.00	0.00
07	0.00	0.00
Total	81.00	52.00

Table 27 gives the comparison of biogas generation of banana leaves and Maize cob, Maize stalk, Water hyacinth, Elephant grass and Cow dung. The results shows that with the exception of the digester E in which Cow dung without yeast was used, all the other digesters (i.e A, B, C and D) in which Maize cob, Maize stalk, water hyacinth and elephant grass, were respectively used generated more biogas than the control digester in which banana leaves used, in spite of the fact that all the digesters were of equal slurry concentration.

Table 27: Comparison of Biogas Generation of Banana leaves and Maize cob, Maize stalk, Water hyacinth, Elephant grass and Cow dung.

Average volumes of Biogas generated (cm^3) daily

Time	Digesters of equal capacities					
(Day)	Control	A	B	C	D	E
01	45.00	110.00	70.00	64.00	72.50	0.00
02	22.00	59.00	20.50	92.50	90.0	0.00
03	14.00	48.00	0.00	52.50	55.00	0.00
04	0.00	49.00	10.00	40.00	94.00	10.00
05	0.00	47.00	15.00	75.00	62.00	12.00
06	0.00	35.00	10.00	50.00	41.50	8.50
07	0.00	22.50	10.00	20.00	30.00	0.00
08	0.00	0.00	0.00	0.00	20.50	0.00
09	0.00	0.00	0.00	0.00	0.00	0.00
10	0.00	0.00	0.00	0.00	0.00	0.00
11	0.00	0.00	0.00	0.00	0.00	0.00
Total	**81.00**	**370.50**	**135.50**	**394.00**	**465.50**	**30.50**

The comparison of some physical parameters of fermented and unfermented substrates is shown in Table 28. The Table shows that the moisture and ash contents of the fermented substrate were relatively higher than those of the unfermented; the organic matter, N and C contents of the fermented substrate were relatively lower than those of the unfermented one; and C/N of the fermented substrate was higher than that of the unfermented substrate.

Table 28: Comparison of some physical Parameters of Fermented and Unfermented Substrates.

Physical Parameters (%)	Unfermented Substrate	Fermented Substrate
Moisture	4.73	8.64
Ash	12.13	22.15
Organic Matter	83.14	69.21
Nitrogen	0.24	o.084
Carbon	5.41	2.77
C/N	22.5:1	33:1

Table 29 shows the fungal degradation products of the 40g banana leaves used. The Table shows that the amount of bioliquid generated was relatively higher than that of the biogas. It also shows that only 5.571g of the 40g banana leaves used was consumed during the process.

Table29: Fungal Degradation products of 40g Banana leaves.

Degradation Product	
Weight of Organic Matter Consumed (g)	5.571
Weight of Biogas Generated (g)	1.551
Weight of Bioliquid Generated (g)	4.020
% of Organic Matter Consumed	13.93
% of Biogas Generated	3.88
% of Bioliquid Generated	10.05

(See appendixes 7 d, e and f for more details)

Table 30 shows the chemical compositions of the banana leaves used. The Table shows that the percentage of carbohydrate was relatively higher than the percentage of each of the other components while crude protein was the least components. It also shows that the greater percentage degradation of the total carbohydrate was due to the reducing sugar.

Table 30: Chemical Composition of Banana leaves

Composition (%)	Unfermented Substrate	Fermented substrate	% Degradation
Crude Protein	1.34	0.48	0.86
Crude Fibre	21.88	20.73	1.15
Crude Fat	9.71	6.00	3.71
Lignin	16.00	15.65	0.35
Total Carbohydrate	52.00	36.60	15.40
Reducing Sugar	22.00	8.40	13.60
Non-Reducing Sugar	30.00	28.20	1.80

** The reducing sugar obtained was from the total carbohydrate.*

Table 31 gives the amount of bioliquid extracted and its components. The Table shows that the asphaltenes content of the unfermented slurry was higher than its maltenes content while the asphaltenes content of the fermented slurry was lower than its maltenes content. It also shows that the bioliquid generated from the unfermented slurry was relatively higher than that of the fermented one while the amount of unfermented slurry consumed during the process was relatively lower than the amount of fermented slurry consumed.

Table 31: Bioliquid Extraction and Components Separation

Parameters (g)	Unfermented Slurry	Fermented Slurry
Weight of Substrate before Extraction	40.00	40.00
Weight of Substrate after Extraction	34.44	34.32
Amount of Substrate Consumed	5.56	5.68
Amount of Bioliquid Generated	4.020	3.97
Amount of Maltenes Produced	1.934	2.114
Amount of Asphaltenes Produced	2.086	1.856

Table 32 shows the components of the maltenes collected from both the fermented and unfermented slurries. The Table shows that the saturates content of the fermented slurry was relatively higher than those of the unfermented slurry; the resins and polars contents of the fermented slurry were relatively lower than those of the unfermented slurry; and aromatics contents of the unfermented slurry were relatively higher than those of the fermented slurry.

Table 32: Separation of Maltenes Components by Column Chromatography

Fractions (mg)	Unfermented Slurry	Fermented Slurry
Saturates	0.50	38.00
Monoaromatics	15.20	9.20
Diaromatics	11.90	6.70
Polyaromatics	31.20	11.30
Resins and Polars	6.60	3.80

Table 33 shows the amounts of n-alkanes, iso-alkanes and cyclics obtained from the analysis of 38.00mg saturates fraction of the fermented bioliquid. The analysis revealed that the amount of n-alkanes in the saturates was greater than the sum of the iso-alkanes and cyclics.

Table 33: Separation of N-alkanes, Iso-alkanes and Cyclics from 38.00mg Saturates Fraction Through Urea and Thiourea Adduction.

Component	Amount (mg)
N-alkanes	22.64
Iso – alkanes	10.36
Cyclics	2.57

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Table 34 gives the compositions of the gaseous degradation products (biogas) and liquefied petroleum gas (LPG) obtained from crude oil distillation. The GC – analysis of the biogas generated in this work and that of LPG carried out using flame ionization detector (FID) and thermal conductivity detector (TCD). The analysis revealed that the biogas was composed of CH_4 and $C0_2$ while the LPG was composed of C_1-C_8 n-alkanes.

Table 34: GC – Analysis of Biogas and LPG

Component	RT	Area	Height	Moles %	Factor
* $C0_2$	0.503	9921	213	0.309	1.000
* CH_4	2.230	3201799	429396	99.691	1.000
CH_4	2.246	56534	8618	1.145	1.000
C_2H_6	2.423	257741	37917	5.222	1.000
C_3H_8	2.730	878316	109247	17.796	1.000
C_4H_{10}	3.193	625767	69407	12.679	1.000
C_5H_{12}	3.530	1515640	128395	30.710	1.000
C_6H_{14}	4.833	945645	66118	19.161	1.000
C_7H_{16}	5.480	654173	39516	13.255	1.000
C_8H_{18}	6.736	1541	143	0.031	1.000

* Components identified in biogas

Table 35 shows the series of relatively heavier n-alkanes present in the bioliquid generated. The result showed that the molecular difference between each of the 2 successive n-alkanes identified was CH_2 with relative molecular weight of 14.

Table 35: GC – Mass Spectrometric Analysis of N- alkanes in the Bioliquid

S/N	* Relative molecular weight of n-alkane	Retention time (min)	No. of C-atom	Elucidated molecular formula	Name
1.	268	19.987	C_{19}	$C_{19}H_{40}$	Nonadecane
2.	282	20.989	C_{20}	$C_{20}H_{42}$	Eicosane
3.	296	21.921	C_{21}	$C_{21}H_{44}$	Heneicosane
4.	310	22.847	C_{22}	$C_{22}H_{46}$	Docosane
5.	324	23.696	C_{23}	$C_{23}H_{48}$	Tricosane
6.	338	24.539	C_{24}	$C_{24}H_{50}$	Tetracosane
7.	352	25.346	C_{25}	$C_{25}H_{52}$	Pentacosane
8.	366	26.117	C_{26}	$C_{26}H_{54}$	Hexacosane
9.	380	26.841	C_{27}	$C_{27}H_{56}$	Heptacosane
10.	394	27.607	C_{28}	$C_{28}H_{58}$	Octacosane
11.	408	28.373	C_{29}	$C_{29}H_{60}$	Nonacosane
12.	422	29.346	C_{30}	$C_{30}H_{62}$	Triacotane
13.	436	30.391	C_{31}	$C_{31}H_{64}$	Hentriacotane
14.	450	31.643	C_{32}	$C_{32}H_{66}$	Dotriacotane
15.	464	33.139	C_{33}	$C_{33}H_{68}$	Tritriacotane
16.	478	34.913	C_{34}	$C_{34}H_{70}$	Tetratriacotane
17.	492	37.092	C_{35}	$C_{35}H_{72}$	Pentatriacotane

* Relative molecular weights determined from fragmentation patterns of n-alkanes.

Table 36 shows the iso-alkanes identified in the bioliquid analysed. The analysis showed that the iso-alkanes identified were of relative molecular weights within the range of 254-280, which corresponded to the relative molecular weights of the hydrocarbons within the range of $C_{18}H_{38}$ – $C_{27}H_{56,}$ with molecular difference between 2 successive identified iso-alkanes of $CH_{2.}$

Table 36: GC – Mass Spectrometric Analysis of Iso-alkanes in the Bioliquid.

S/N	* Relative molecular weight of iso-alkanes	Retention time (min)	No. of C-atom	Elucidated Molecular formula
1.	254	18.987	C_{18}	$C_{18}H_{38}$
2.	268	20.038	C_{19}	$C_{19}H_{40}$
3.	282	20.893	C_{20}	$C_{20}H_{42}$
4.	296	21.973	C_{21}	$C_{21}H_{44}$
5.	310	22.952	C_{22}	$C_{22}H_{46}$
6.	324	24.074	C_{23}	$C_{23}H_{48}$
7.	338	25.433	C_{24}	$C_{24}H_{50}$
8.	325	27.089	C_{25}	$C_{25}H_{52}$
9.	366	29.226	C_{26}	$C_{26}H_{54}$
10.	380	31.944	C_{27}	$C_{27}H_{56}$

296 ***** is the relative molecular weight that was not shown on the fragmentation pattern (Fig. 30)

Table 37 gives the cyclic hydrocarbons present in the bioliquid analyzed. The analysis revealed that the cyclic hydrocarbons identified were of relative molecular weights within the range of 324 – 394, which corresponded to the relative molecular weights of $C_{23}H_{48}$ – $C_{28}H_{58,}$ with molecular difference between 2 successive cyclic hydrocarbons identified of CH_2.

Table 37: GC- Mass Spectrometric Analysis of Cyclic Hydrocarbons in the Bioliquid

S/N	*Relative molecular weight of cyclics	Retention time (min)	No. of C-atom	Elucidated molecular formula
1.	324	20.074	C_{23}	$C_{23}H_{48}$
2.	338	25.439	C_{24}	$C_{24}H_{50}$
3.	352	27.137	C_{25}	$C_{25}H_{52}$
4.	366	29.226	C_{26}	$C_{26}H_{54}$
5.	380	31.944	C_{27}	$C_{27}H_{56}$
6.	394	35.440	C_{28}	$C_{28}H_{58}$

***** Relative molecular weights determined from fragmentations of the cyclic hydrocarbons

5.2. **CONCLUSION**

All the physio-chemical parameters employed in this research enhanced both the generation of biogas and bioliquid at optimum operational conditions. Though, addition of urea, addition of buffer solution as well as the use of a mixture of urea and buffer solution each showed adverse effect on the quality of the biogas generated by decreasing slightly the CH_4 content and increasing slightly the CO_2 and H_2S contents. But of all the adverse effects of some of the nutritive additives used, the composition of the biogas generated was in accordance with the composition needed for biogas of good fuel value.

The bioliquid generated in this work was composed of n-alkanes, iso-alkanes and cyclic hydrocarbons, which could serve as source of fuel (biofuel) with quality similar to those kerosene and fuel oil, and could also serve as a source of lubricants and waxes.

5.2.1 **Recommendations**

From the analyses carried out and the results obtained in this research, it would be of paramount importance if the following recommendations would be taken into consideration:-

i. For the generation of biogas rich in CH_4 with low environmental hazards, banana leaves could be used as substrate.

ii. The substrate (banana leaves) could be used for the generation of biogas of good fuel value.

iii. The substrate should not be used for large scale biogas production.

iv. The substrate could be used for large scale bioliquid production.

v. The use of buffer solutions; the use of urea as a nutritive additive; and the use of a mixture of buffer solution and urea for the generation of biogas of good fuel value and low environmental hazards should be avoided.

vi. For rapid and high production of biogas and bioliquid, the use of nutritive additives: sugar, oil, ethanoic acid and blood meal should be adopted.

vii. The substrate could be used in animal feeds for energy supply, because it contains a lot of reducing sugar.

5.3 SUGGESTIONS ON THE AREAS OF FURTHER WORK

The analyses carried out in this research were only restricted to a limited scope of interest. In order to have a thorough knowledge of biogas and bioliquid, the following investigations have to be carried out:

i. Determination of paraffinicity and aromaticity of bioliquid.

ii. Comparative analysis of the organic components of the gaseous degradation product (biogas) from different substrates.

iii. Qualitative analysis of polyaromatics compounds in bioliquid.

iv. Quantitative analysis of nalkanes in bioliquid.

v. Analysis of the fuel value of biogas generated from different sources

vi. Isolation of resins and polars from asphaltenes in their pure forms.

vii. Determination of the element(s) associated with the aromatic compound of the polar component of asphaltenes.

viii. Comparative analysis of the maltenes and asphaltenes contents of bioliquid of different sources.[269]

ix. Studies of the influence of micro-organisms other than yeast on the compositions and quantities of biogas and biloliquid from banana leaves.

5.4 SUMMARY OF THE FINDINGS

The percentage of the organic matter consumed due to fungal degradation was found to be 13.93% of which 3.88% was biogas and 10.05% was bioliquid.

The weights of the gaseous degradation product (biogas) and liquid degradation product (bioliquid) generated from 40.0g banana leaves were 1.551g and 4.020g, respectively. And it was found that the greater part of the degradation was due to the protein and total carbohydrate (especially the reducing sugar) contents of the substrate while the least degraded components were the fibre and lignin.

The quantity of the bioliquid generated from the unfermented slurry was higher than the one generated from the fermented slurry; the quantity of the maltenes generated from the fermented slurry was higher than the one generated from the unfermented slurry; and the quantity of asphaltenes in the unfermented slurry was higher than that in the fermented slurry.

In the fermented slurry, the saturates contents of the maltenes were found to be higher than all the other components while resins and polars were the least. On other hand, in the unfermented slurry, polyaromatics were the highest while saturates were the least.

The gaseous degradation products (biogas) was composed of CH_4 and CO_2, and the components of the liquid degradation products (bioliquid) identified were n-alkanes, iso-alkanes and cyclic hydrocarbons in the order n-alkanes> iso-alkanes> cyclic hydrocarbons.

5.5 CONTRIBUTION TO KNOWLEDGE

The research has greatly contributed to knowledge in the following areas:

i. ***Renewable energy:-*** From the composition of the biogas generated, the research revealed that banana leaves (as an agricultural waste) can be used to produce gaseous fuel (biogas) of good fuel value.

ii. ***Environmental Science:-*** The emphasis given in the research on the use of wastes instead of firewood by rural populace for fuel, could help to protect the land environment from erosion and desertification as well as air pollution, provided that the technology of biogas and bioliquid production is employed. The research also emphasized on the process of converting wastes into useful products, which also serves as a measure of controlling wastes, improving health and encouraging environmental sanitation.

iii. ***Agricultural Science:-*** The research highlighted the advantage of using digested slurry in improving soil fertility and enhancing aquaculture production. It also highlighted the importance of agricultural wastes (banana leaves inclusive) for energy generation needed for crops processing as well as a source of energy for

animals (when used as animal feeds) because of its high reducing sugar content.

iv. ***Organic Synthesis:-*** The research revealed that important organic products such as saturates, aromatics, resins and polars could be generated and isolated from banana leaves.

v. ***Biological Science:-*** The research also revealed the conditions under which a fungus (yeast) can be made inactive, which could also be applied to other fungi to control their infections by deactivation.

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APPENDIXES

APPENDIX I

PREPARATION OF BUFFER SOLUTION OF pH 7.3 USING A BUFFER SALT, $NaH_2PO_4.H_2O/Na_2HPO_4$ OF Ka 7×10^{-8} (acid) (base)

To find the pK_a of the buffer salt, the relation

pka = $-\log K_a$ was used, but Ka = 7×10^{-8}

$\Rightarrow pK_a = -\log 7 \times 10^{-8}$

$= - (-7.1549)$

$= 7.1549 \approx 7.155$

NB – For buffer capacity, $pK_a = \pm 1$ was considered.

In order to determine the volume of buffer acid (V_A) and buffer base (V_B) needed to prepare a buffer solution of pH 7.3, the Henderson – Hasselbalch equation was used. The equation is as follows:

$$pH = pK_a + \log_{10} \frac{[\text{Conjugate Base}]}{[\text{Acid}]} \quad \ldots\ldots\ldots\ldots\ldots \text{(a)}$$

$$\Rightarrow 7.3 = 7.155 \log_{10} \frac{[HPO_4^{2-}]}{[H_2PO_4^-]}$$

$$\therefore \log_{10} \frac{[HPO_4^{2-}]}{[H_2PO_4^-]} = 7.3 - 7.155$$

$$\log_{10} \frac{[HPO_4^{2-}]}{[H_2PO_4^-]} = 0.145$$

$$\frac{[HPO_4^{2-}]}{[H_2PO_4^-]} = 10^{0.145}$$

$$\frac{[HPO_4^{2-}]}{[H_2PO_4^-]} = 1.40 \ldots\ldots\ldots\ldots\ldots\ldots \text{(b)}$$

But $\frac{[HPO_4^{2-}]}{[H_2PO_4^-]}$ = Mole ratio $\frac{\text{base}}{\text{acid}} = \frac{M_BV_B}{M_AV_A}$ ..... (c)

$$\Rightarrow \frac{[HPO_4^{2-}]}{[H_2PO_4^-]} = \frac{M_BV_B}{M_AV_A} \ldots\ldots\ldots\ldots \text{(d)}$$

$$\frac{M_BV_B}{M_AV_A} = 1.40 \ldots\ldots\ldots\ldots \text{(e)}$$

But for a buffer solution, the molar concentration of acid must always be equal to the molar concentration of base (i.e. $M_A = M_B$). For this, equation (e) became

$$\frac{V_B}{V_A} = 1.40 \ldots\ldots\ldots\ldots \text{(f)}$$

$$\Rightarrow V_B = 1.40 \text{ X } V_A$$

$$\therefore V_B = 1.4V_A \ldots\ldots\ldots\ldots \text{(g)}$$

From section 3.20, the best slurry condition established (i.e. the suitable water content determined) was 4.g/25cm^3. Therefore, 25cm^3 is the suitable volume to be prepared

$$\Rightarrow V_A + V_B = 25cm^3 \ldots\ldots\ldots\ldots \text{(h)}$$

By substituting the value of V_B from (g) into (h), (h) becomes:

$$V_A + 1.4V_A = 25cm^3$$

$$\Rightarrow 2.4\ V_A = 25cm^3 \ldots\ldots\ldots\ldots \text{(i)}$$

$$\therefore V_A = \frac{25cm^3}{2.4} \approx 10.42cm^3 \ldots\ldots\ldots\ldots \text{(j)}$$

From (h), $V_A + V_B = 25cm^3$

$$\therefore V_B\ (25 - 10.42)\ cm^3$$

$$V_B = 14.58cm^3 \ldots\ldots\ldots\ldots \text{(k)}$$

From j and k respectively, 10.42cm^3 $NaH_2PO_4.H_2O$ was mixed with 14.58cm^3 Na_2HPO_4 and formed 25cm^3 of their buffer solution of pH 7.3. The same procedure was followed for calculating the volumes of acid (VA) and the volumes of base (VB) of the buffer solutions of the other given pH.

APPENDIX 2

PREPARATION OF 1M UREA SOLUTIONS

Since the molar mass of urea [$(NH_2)_2CO$] = 60g, therefore, 1M urea was prepared as follows:

60g urea was weighed and dissolved to 1dm^3 in a volumetric flask with distilled water.

All the required molar concentrations of urea were prepared from the 1M urea solution earlier prepared, using the following relation:

$V_1 \times 1M = V_2 \times XM$

where V_1 = volume of 1M urea solution

V_2 = volume of the required molar concentration of urea to be prepared from 1M urea solution.

X_M = required molar concentration of urea to be prepared from 1M urea solution.

Example: Preparation of 100cm^3 of 0.01M urea solution from 1M urea solution.

$V_1 \times 1M = V_2 \times XM$

$$V_1 = \frac{100cm^3 \times 0.01M}{1M} = 1.0cm^3$$

$\Rightarrow$ 1.0cm^3 of 1M urea solution was added to 99cm^3 distilled water and formed 100cm^3 of 0.01M urea solution.

NB – The same procedure was followed for all the required molar concentrations and volumes of urea solutions prepared from 1M urea solution.

APPENDIX 3

PREPARATION OF UREA SOLUTION USING 0.2M BUFFER SOLUTION OF pH 7.3

In order to prepare urea solution using 0.2M buffer solution of pH 7.3, the following equation was used and the quantity of urea that was dissolved in 1000cm^3 of the buffer solution used was evaluated: $^1/_v = X_M$

where 1 $\Rightarrow$ mole of urea,

X_M is the required molar concentration of urea solution

V is the volume of urea solution of the required molar concentration with which the molar mass of 1 mole urea (i.e. 60g) was divided and gave the required mass of urea that was dissolved in 1000cm^3 buffer solution of required molar concentration and known pH.

E.g. the amount (in gram) of the urea obtained from 0.01M urea solution that was dissolved in 1000cm^3 buffer solution of 0.2M buffer solution of pH 7.3 was determined as follows:

Step 1: Determination of the volume of 0.01M urea solution. This was achieved by using the relation:

$^1/_V = X_M$

$^1/_v$ 0.01M

$V = \frac{1}{0.01M} = 100cm^3$

Step 2: Determination of the amount of urea to be dissolved in $1000cm^3$ of 0.2M buffer solution of P^H 7.3. Since 1 mole of urea contains 60g urea, therefore, the mass of urea that was dissolved in $1000cm^3$ of 0.2M buffer solution was obtained by dividing the molar mass of 1mole urea with the volume of 0.01M urea solution (i.e. $100cm^3$) obtained

$\Rightarrow \frac{60g}{100cm^3}$ = 0.6g urea/dm^3 of 0.2M buffer solution of pH 7.3

= 0.6g urea/$1000cm^3$ of 0.2M buffer solution of pH 7.3

= 0.06g urea/$100cm^3$ of 0.2M buffer solution of pH 7.3

Since 0.6g urea $\longrightarrow$ 1000 cm^3

X $\longrightarrow$ $25cm^3$

$\therefore x = \frac{0.6g \text{ urea} \times 25cm^3}{1000cm^3}$

= 0.015g urea

$\Rightarrow$ 0.015g urea/$25cm^3$ of 0.2M buffer solution of pH 7.3

ALTERNATIVE METHOD

To find the mass of 0.01M urea to be dissolved in $1000cm^3$ of 0.2M buffer solution of pH 7.3, the following relation was employed:

No. of moles = $\frac{m}{M.m}$

$\therefore$ m= 0.01 x 60 = 0.6g

Where m = mass of urea

M.m. = molar mass or urea

NB: Similar procedure was followed for the calculating the masses of urea required for 0.015M, 0.02M, 0.025M, and 0.030M urea that was dissolved

in 1000cm^3 of 0.2M buffer solution of pH 7.3 separately. It could also be followed for calculating any mass of urea required in 1000cm3 buffer solution of any given molar concentration and pH.

APPENDIX 4

PROPOTIONATELY INCREASED VOLUMES AND MASSESS

Appendix 4a: Buffer Solution of pH 7.3

From appendix1, 25cm^3 buffer solution → 10.42 cm^3 acid

250cm^3 buffer solution → x acid

$$x = \frac{250cm^3 \times 10.42cm^3}{25cm^3}$$

$$= 104.20cm^3$$

$\therefore V_A = 104.20cm^3$

But $V_A + V_B = 250cm^3$

$\Rightarrow V_B = 250cm^3 - 104.20cm^3$

$= 145.80cm^3$

NB: The same procedure could be followed for calculating any proportionate volume of acid (V_A) and volume of base (V_B).

Appendix 4b: Preparation of Slurry

Since, 4.0g substrate → 25cm^3

Examples (i) for 6g substrate, 4.0g → 25cm^3

6.0g → x

.: $x = \frac{6.0g \times 25\ cm^3}{}$

$$4.0g$$

$$= 37.5cm^3$$

(ii) for 40g substrate,

$$4.0g \rightarrow 25cm^3$$

$$40g \rightarrow x$$

$$\therefore \quad x = \frac{40g \times 25cm^3}{4.0g}$$

$$= 10 \times 25cm^3$$

$$= 250cm^3$$

The same procedure could be followed for any given weight of substrate.

Appendix 4c: Quantity of Yeast used

(a) Based on the volume of the water used. Examples:

i. For $37.5cm^3$ H_2O,

$25cm^3$ $H_2O \rightarrow$ 0.18g yeast

$37.5cm^3$ $H_2O \rightarrow x$

$$\therefore \quad x = \frac{37.5cm^3 \times 0.18g}{25cm^3}$$

$$= 0.27g$$

ii. For $250cm^3$ H_2O,

$25cm^3$ $H_2O \rightarrow$ 0.18g yeast

$250cm^3$ $H_2O \rightarrow$ x yeast

$$\Rightarrow x = \frac{250cm^3 \times 0.18g}{25cm^3}$$

$$\therefore \quad x = 1.8g \text{ yeast}$$

$\Rightarrow$ 1.8g yeast will be added to $250cm^3$ H_2O.

The same procedure was followed for the other volumes of water used.

b) Based on the weight of the substrate used

i. For 6.0g substrate,

4.0g substrate → 0.1 8g yeast

6.0g substrate → x

∴ $x = \frac{6.0g \times 0.18g}{4.0g}$

$= 0.27g \Rightarrow$ 0.27g yeast was added to 6.0g substrate.

ii. For 40g substrate,

4.0g substrate → 0.18g yeast

40g substrate → x yeast

$\Rightarrow x = \frac{40 \times 0.18}{4} = 1.8g$ yeast

$\Rightarrow$ 1.8g yeast was added to 40g substrate.

The same procedure used for the other given weights of substrate.

APPENDIX 5

PERCENTAGE COMPOSITION OF THE BIOGAS GENERATED IN SECTIONS 3.29, 3.30, 3.31 AND 3.32

APPENDIX 5a: Percentage of CH_4, CO_2 and H_2S of the Biogas Generated in Section 3.29.

(i) $\%\ CO_2 = \left(\frac{A - B}{A} \times 100 \right)$

$= \frac{835 - 575.5\ cm^3}{835.0} \times 100$

$$= \left(\frac{259.5}{835.0} \right) cm^3 \times 100$$

$$= 31.07\% \cong 31.1\%$$

(ii) $\% H_2S = \frac{A - C}{A} \times 100$

$$= \left(\frac{835 - 834}{835} \right) cm^3 \times 100$$

$$= \left(\frac{1}{835} \right) cm^3 \times 100$$

$$= 0.12\%$$

(iii) $\% CH_4 = 100\% - [\%CO_2 + \%H_2S]$

$$= 100\% - [31.1\% + 0.12\%]$$

$$= 68.78\%$$

Alternative method

$\%CH_4 = A - C = D$

$$= (835 - 834)\ cm^3 = 1cm^3, D$$

$$B - D = E$$

$$\Rightarrow (575.5 - 1)\ cm^3 = 574.5cm^3, E$$

$\% CH_4 = \frac{E}{A} \times 100$

$$= \left(\frac{574.5}{835} \right) cm^3 \times 100$$

$$= 68.8\%$$

or $\%CH_4 = A - B = D \Rightarrow (835 - 575.5)\ cm^3 = 259.5cm^3$

$C - D = E \quad \Rightarrow (834 - 259.5)\ cm^3 = 574.5cm^3$

$\%CH_4 = {}^{E}/_{A} \times 100\%$

$$= \left(\frac{574.5}{} \right) cm^3 \times 100$$

$$= \frac{835}{} \quad 68.8\%$$

where A, B and C were the total volumes of biogas generated in section 3.29 and shown in Table 14.

APPENDIX 5b: Percentage of CH_4, CO_2 and H_2S of the Biogas Generated in Section 3.30

(i) $\%\ CO_2 = \frac{A - B}{A} \times 100$

$$= \left(\frac{1{,}058 - 696}{1{,}058}\right) cm^3 \times 100$$

$$= \left(\frac{362}{1{,}058}\right) cm^3 \times 100$$

$$= 34.22\%$$

(ii) $\%\ H_2S = \frac{A - C}{A} \times 100 = \left(\frac{1{,}058 - 1{,}055.5}{1{,}058}\right) cm^3 \times 100$

$$= \left(\frac{2.5}{1{,}058}\right) cm^3 \times 100$$

$$= 0.24\%$$

(iii) $\%\ CH_4 = 100\% - [\%CO_2 + \%H_2S]$

$$= 100\% - [34.22\% + 0.24\%]$$

$$= 100\% - 34.46\%$$

$$= 65.54\%$$

ALTERNATIVE METHOD

$CH_4 = A - C \Rightarrow (1058 - 1055.5)\ cm^3 = 2.5\ cm^3$, D

$= B - D \Rightarrow (696 - 2.5)\ cm^3 = 693.5\ cm^3$, E

$\%\ CH_4 = \frac{E}{A} \times 100$

$$= \left(\frac{693.5}{108.5}\right) cm^3 \times 100 = 65.55\%$$

Or CH_4 = $A - B = D \Rightarrow (1{,}058 - 696)\ cm^3 = 362\ cm^3$

$C - D = E \Rightarrow (1{,}055.5 - 362)\ cm^3 = 693.5\ cm^3$

$$\% CH_4 = \frac{E}{A} \times 100$$

$$= \left(\frac{693.5}{1{,}058}\right) cm^3 \times 100$$

$$= 65.55\%$$

where A, B and C were the total volumes of biogas generated in section 3.30 and shown in Table 15

APPENDIX 5c: Percentage of CH_4, CO_2 and H_2S of the Biogas generated in Section 3.31

(i) $$\% CO_2 = \frac{A - B}{A} \times 100$$

$$= \left(\frac{1{,}042 - 669}{1{,}042}\right) cm^3 \times 100$$

$$= 35.8\%$$

(ii) $$\% H_2S = \frac{A - C}{A} \times 100$$

$$= \left(\frac{1{,}042 - 1{,}038.5}{1{,}042}\right) cm^3 \times 100$$

$$= 0.34\%$$

(iii) $$\% CH_4 = 100\% - [\%CO_2 + \%H_2S]$$

$$= 100\% - [35.8\% + 0.34\%]$$

$$= 100\% - 36.14\%$$

$$= 63.86\%$$

ALTERNATIVE METHOD

$CH_4 = A - C \Rightarrow (1042 - 1038.5)\ cm^3 = 3.5\ cm^3$, D

$= B - D \Rightarrow (669 - 3.5)\ cm^3 = 665.5\ cm^3$, E

$$\% CH_4 = \left(\frac{665.5}{1042}\right) cm^3 \times 100$$

$$= 63.87\%$$

Or $CH_4 = A - B = D \Rightarrow (1{,}042 - 669)\ cm^3 = 373 cm^3$

$C - D = E \Rightarrow (1{,}038.5 - 373)\ cm^3 = 665.5\ cm^3$

$$\% CH_4 = \frac{E}{A} \times 100$$

$$= \left(\frac{665.5}{1042}\right) cm^3 \times 100$$

$$= 63.87\%$$

Where A, B and C were the total volumes of biogas generated in section 3.31 and shown in Table 16

Appendix 5d: Percentage of CH_4, CO_2 and H_2S of the Biogas Generated in Section 3.32

(i) $$\% CO_2 = \frac{A - B}{A} \times 100$$

$$= \left(\frac{1{,}159.5 - 700}{1{,}159.5}\right) cm^3 \times 100$$

$$= \left(\frac{459.5}{1{,}159.5}\right) cm^3 \times 100 = 39.63\%$$

(ii) $$\% H_2S = \frac{A - C}{A} \times 100$$

$$= \left(\frac{1{,}159.5 - 1{,}154.5}{1{,}159.5}\right) cm^3 \times 100$$

$$\left(\quad\right)$$

$$= \frac{5}{1{,}159.5}\ cm^3 \times 100$$

$$= 0.43\%$$

(iii) $\%\ CH_4 = 100\% - [\%CO_2 + \%H_2S]$

$$= 100\% - [39.63\% + 0.43\%]$$

$$= 100\% - 40.06\%$$

$$= 59.94\%$$

ALTERNATIVE METHOD

$CH_4 = A - C \Rightarrow (1159.5 - 1154.5)\ cm^3 = 5.0\ cm^3$, D

$= B - D \Rightarrow (700 - 5)\ cm^3 = 695\ cm^3$, E

$$\%\ CH_4 = \frac{E}{A} \times 100$$

$$= \left(\frac{695}{1{,}159.5}\right) cm^3 \times 100$$

$$= 59.94\%$$

Or $CH_4 = A - B = D \Rightarrow (1{,}159.5 - 700)\ cm^3 = 459.5\ cm^3$

$C - D = E \Rightarrow (1{,}154.5 - 459.5)\ cm^3 = 695.5\ cm^3$

$$\%\ CH_4 = \frac{E}{A} \times 100$$

$$= \left(\frac{695}{1159.5}\right) cm^3 \times 100$$

$$= 59.94\%$$

where A, B and C were the total volumes of biogas generated in section 3.32 and shown in Table 17.

APPENDIX 6

PERCENTAGE INCREASE OF CH_4, CO_2 AND H_2S UNDER THE INFLUENCE OF BUFFER SOLUTION AND UREAIN SECTIONS 3.30, 3.31 AND 3.32

Appendix 6a: Percentage increase in CH_4, CO_2 and H_2S of Biogas generated in section 3.30 (i.e effect of Buffer Solution)

(i) % increase in CH_4

From Table 4, the H_2S generated in section 3.29 was

$= A - C$

$= (835 - 834)cm^3$

$= 1cm^3$ ..... D

The CH_4 generated in section 3.29 was

$= B - D \quad \Rightarrow (575.5 - 1)\ cm^3$

$= 574.5\ cm^3$ ..... a

From Table 15, H_2S generated in section 3.30 was

$= A - C$

$= (1{,}058 - 1{,}055.5)cm^3$

$= 2.5cm^3$ ..... E

The CH_4 generated in section 3.30 was

$= B - E \quad = \quad (696 - 2.5)\ cm^3$

$= 693.5cm^3$ ..... b

.: the % increase in CH_4 in section 3.30 $= \frac{b - a}{a} \times 100$

$= \left(\frac{593.5 - 574.5}{574.5}\right) cm^3 \times 100$

$= \left(\frac{119}{574.5}\right) cm^3 \times 100$

= 20.71%

ALTERNATIVE METHOD

From Table 14, the CH_4 generated in section 3.29 was

= A – B = D $\Rightarrow$ (835 – 575.5) cm^3= 59.5 cm^3

C - D= a $\Rightarrow$ (834-259.5) cm^3 = 574.5 cm^3

From Table 15, the CH_4 generated in section 3.30 was

= A – B = D $\Rightarrow$ (1,058 – 696) cm^3 = 362 cm^3

C – D = b $\Rightarrow$ (1,055.5 – 362) cm^3 = 693.5 cm^3

:. the % increase in CH_4 in section 3.30 = $\frac{b - a}{a}$ x 100

$$= \left(\frac{693.5 - 574.5}{574.5} \right) cm^3 \times 100$$

= 20.71%

(ii) % increase in CO_2

From Table 14, the CO_2 generated in section 3.29 was

= A – B

= (835 – 575.5)cm^3

= 259.5 cm^3 ..... a

From Table 15, the CO_2 generated in section 3.30 was

= A – B = (1,058 – 696)cm^3

= 362 cm^3 ..... b

.: the % increase in CO_2in section 3.30 was

$$= \frac{b - a}{a} \times 100$$

$$= \left(\frac{362 - 259.5}{259.5} \right) cm^3 \times 100$$

= 39.5%

(iii) % increase in H_2S

From Table 14, the H_2S generated in section 3.29 was

= A – C

= (835 – 834)cm^3

= 1cm^3 ..... a

From Table 15, the H_2S generated in section 3.30 was

= A – C $\Rightarrow$ (1, 058 – 1,055.5) cm^3

= 2.5cm^3 ..... (b)

.: the % increase in H_2S for section 3.30 was = $\frac{b - a}{a}$ x 100

= $\left(\frac{2.5 - 1}{1}\right)cm^3$ x 100

= $\left(\frac{1.5}{1}\right)cm^3$ x 100

= 150%

Where A, B and C were the total volumes of biogas generated in the respective sections.

Appendix 6b: Percentage increase in CH_4, CO_2 and H_2S of the Biogas Generated in Section 3.31(i.e effect of urea)

(i) % increase in CH_4

From Table 14,

The H_2S generated in section 3.29 was

= A – C

= (835 – 834)cm^3

= 1cm^3 ..... D

The CH_4 generated in section 3.29 was = B – D

= (575.5 – 1)cm^3

= 574.5cm^3 ..... a

From Table 16, the H_2S generated in section 3.31 was

= A – C

$= (1{,}042 - 1{,}038.5)\ cm^3$

$= 3.5$ ..... E

The CH_4 generated in section 3.31 was = B – E

$= (669 - 3.5)\ cm^3$

$= 665.5cm^3$ ..... b

.: the % increase in CH_4 for section 3.31 was $= \frac{b - a}{a} \times 100$

$= \left(\frac{665.5 - 574.5}{574.5}\right) cm^3 \times 100$

$= \left(\frac{91}{574.5}\right) cm^3 \times 100$

$= 15.84\%$

ALTERNATIVELY

The CH_4 for section 3.29 = $=A - B = D \Rightarrow (835\text{-}575.5)\ cm^3 = 259.5\ cm^3$

$C - D = a \Rightarrow (834 - 259.5)\ cm^3 = 574.5\ cm^3$

The CH_4 for section 3.31 $= A - B = D \Rightarrow (1{,}042 - 669)\ cm^3 = 373\ cm^3$

$7C - D = b \Rightarrow (1{,}038.5 - 373)\ cm^3 = 665.5\ cm^3$

.: the % increase in CH_4 for Section 3.31 $= \frac{b - a}{a} \times 100$

$= \left(\frac{665.5 - 574.5}{574.5}\right) cm^3 \times 100$

$= \left(\frac{91}{574.5}\right) cm^3 \times 100$

$= 15.84\%$

(ii) % increase in CO_2

From Table 14, the CO_2 generated in section 3.29 was

$= A - B$

$= (835 - 575.5)cm^3$

$= 295.5cm^3$ ..... a

From Table 16, the CO_2 generated in section 3.31 was

$= A - B$

$= (1{,}042 - 669)cm^3$

$= 373cm^3$ ……. b

.: the % increase in CO_2 for Section 3.31 $= \frac{b - a}{a} \times 100$

$= \left(\frac{373 - 259.5}{259.5}\right) cm^3 \times 100$

$= \left(\frac{113.5}{259.5}\right) cm^3 \times 100$

$= 43.74\%$

(iii) % increase in H_2S

From Table 14, the H_2S generated in section 3.29 was

$= A - C$

$= (835 - 834)cm^3$

$= 1cm^3$ ……. a

From Table 16, the H_2S generated in section 3.31 was

$= A - C$

$= (1{,}042 - 1{,}038.5)cm^3$

$= 3.5cm^3$ ……. b

.: the % increase in H_2S in section 3.31 was

$= \frac{b - a}{a} \times 100$

$= \left(\frac{3.5 - 1}{1}\right) cm^3 \times 100$

$= \left(\frac{2.5}{1}\right) cm^3 \times 100$

$= 250\%$

where A, B and c were the volumes of biogas generated in the respective sections.

Appendix 6c: Percentage Increase in CH_4, CO_2 and H_2S of the Biogas Generated in Section 3.32 (i.e combined effect of urea and buffer solution)

(i) **% increase in CH_4**

From Table 14, the H_2S generated in section 3.29 was

$= A - C$

$= (835 - 834)cm^3$

$= 1cm^3$ ..... D

The CH_4 generated in section 3.29 was

$= B - D$ $= (575.5 - 1)cm^3$

$= 574.5cm^3$ ..... a

From Table 17, the H2S generated in Section 3.32 was

$= A - C$

$= (1,159.5 - 1,154.5)cm^3$

$= 5cm^3$ ..... E

The CH4 generated in section 3.32 was

$= B - E$ $= (700 - 5)cm^3$

$= 695cm^3$ ..... b

.: the % increase in CH_4 for Section 3.32

$$= \frac{b - a}{A} \times 100$$

$$= \left(\frac{695 - 574.5}{574.5}\right) cm^3 \times 100$$

$$= \frac{120.5}{} \; cm^3 \times 100$$

$$\left(\quad \right)$$
$$574.5$$

$$= \quad 20.97\%$$

ALTERNATIVELY

The CH_4 for section 3.29 $= A - B = D \Rightarrow (835\text{-}575.5)\ cm^3 = 259.5\ cm^3$

$C - D = a \Rightarrow \quad (834 - 259.5)\ cm^3 = 574.5\ cm^3$

The CH_4 for section 3.32 $= A\text{-}B = D \Rightarrow (1{,}159.5 - 700)\ cm^3 = 459.5\ cm^3$

$C - D = b \Rightarrow (1{,}154.5 - 459.5)\ cm^3 = 695\ cm^3$

.: the % increase in CH_4 for Section 3.32 was

$$= \quad \frac{b - a}{a} \times 100$$

$$= \quad \left(\frac{695 - 574.5}{574.5} \right) cm^3 \times 100$$

$$= \quad \left(\frac{120.5}{574.5} \right) cm^3 \times 100$$

$$= \quad 20.97\%$$

(ii) **% increase in CO_2**

From Table 14, the CO_2 generated in section 3.29 was

$$= \quad A - B$$

$$= \quad (835 - 575.5) cm^3$$

$$= \quad 295.5 cm^3 \ldots\ldots\ldots\ldots\ldots\ldots \text{ a}$$

From Table 17, the CO_2 generated in section 3.32 was

$$= \quad A - B$$

$$= \quad (1{,}159.5 - 700)\ cm^3$$

$$= \quad 459.5 cm^3 \quad \ldots\ldots\ldots\ldots\ldots \text{ b}$$

.: the % increase in CO_2 for Section 3.32 was

$$= \frac{b - a}{a} \times 100$$

$$= \left(\frac{459.5 - 295.5}{259.5}\right) cm^3 \times 100$$

$$= \left(\frac{164}{295.5}\right) cm^3 \times 100$$

$$= 55.5\%$$

(iii) **The % increase in H_2S**

From Table 14, the H_2S generated in section 3.29 was

$$= A - C$$

$$= (835 - 834)\ cm^3$$

$$= 1cm^3 \ldots\ldots\ldots\ldots\ldots\ldots a$$

From Table 17. the H_2S generated in section 3.32 was

$$= A - C$$

$$= (1{,}159.5 - 1{,}154.5)cm^3$$

$$= 5cm^3 \ldots\ldots\ldots\ldots\ldots\ldots b$$

.: the % increase in H_2S for section 3.32 was

$$= \frac{b - a}{a} \times 100$$

$$= \left(\frac{5 - 1}{1}\right) cm^3 \times 100$$

$$= \left(\frac{4}{1}\right) cm^3 \times 100$$

$$= 400\%$$

where A, B and C were the volumes of biogas generated in the respective sections.

APPENDIX 7

PERCENTAGE FUNGAL DEGRADATION OF 40g BANANA LEAVES INTO BIOGAS AND BIOLIQUID BY 1.8g YEAST AT 33^0C

In order to evaluate the % of biogas and bioliquid generated from 40g banana leaves using 1.8g yeast, the general gas equation was employed. The equation is PV = nRT

Where P = atmospheric pressure = 760mmHg = 1atmp.

V = Volume of biogas generated in the experiment/litre

$$= \frac{Vcm^3}{1000\ cm^3}$$

T = Temperature = 33^0c = 33 + 273K = 306K

R = Universal gas constant = 0.08206 litre atm/mol.K

n = number of moles.

NB: The volume of biogas generated in section 3.32 and shown in Table 17 was considered because it was the volume of biogas generated at optimum operational conditions (i.e. the volume of biogas generated at established favourable conditions).

Volume of CH_4 =A $\Rightarrow$ 1159.5cm^3 = 1.1595 dm^3

Volume of CO_2 =A-B $\Rightarrow$(1159.5 –700)cm^3 = 459.5 cm^3=0.4595 dm^3

Volume of H_2S = A-C $\Rightarrow$(1159.5–1154.4) cm^3 = 5 cm^3 = 0.005 dm^3

Appendix 7a: Weight of CH_4 Generated from 40g Banana leaves at 33^0c

In order to find the weight of CH_4 generated, it is necessary to find its number of moles. The number of moles of CH_4 generated was determined as follows using the general gas equation: PV = nRT

$\therefore n = PV/RT$

$$n = \frac{1 \times 1.1595 \text{ litre atm.}}{0.08206 \text{ lit-atm.} \times 306\text{K/mol. K}}$$

$$= \frac{1 \times 1.1595}{0.08206/\text{ mol} \times 306} = \frac{1.1595 \times \text{mol}}{0.08206 \times 306} = \frac{1.1595 \text{ mol}}{25.1104}$$

$\therefore$ n = 0.0462 mol.

But 1 mol. of CH_4 = 16g

$\therefore$ 0.0462 mole $CH_4 = X_1$

$$X_1 = \frac{0.0462 \text{ mole} \times 16\text{g}}{1 \text{ mole}} = 0.739\text{g } CH_4$$

$\Rightarrow$ 0.739g CH_4 was produced from 40g of banana leaves.

Appendix 7b: Weight of CO_2 Generated from 40g Banana leaves at 33^0c

$$n = PV/RT = \frac{1 \times 0.4595 \text{ litre atm.}}{0.08206 \text{ litre atm.} \times 306\text{K/mole K}}$$

$$\therefore n = \frac{0.4595 \times \text{mole}}{0.08206 \times 306} = \frac{0.4595\text{mole}}{25.1104} = 0.0183\text{mole}$$

But, 1 mole of CO_2 = 44g

$\therefore$ 0.0183 mole of $CO_2 = X_2$

$$\Rightarrow X_2 = \frac{0.0183 \text{ mole} \times 44\text{g}}{1 \text{ mole}} = 0.8052\text{g}$$

$\Rightarrow$ 0.8052g CO_2 was produced from 40g of banana leaves

Appendix 7c: Weight of H_2S Generated from 40g of Banana leaves at 33^0c

$$n = PV/RT = \frac{1 \times 0.005 \text{ litre atm.}}{0.08206 \text{ litre atm.} \times 306 \text{K/mole K}}$$

$$\therefore n = \frac{0.005 \times \text{mole}}{0.08206 \times 306} = \frac{0.005\text{mole}}{25.1104} = 0.000199 \text{ mole}$$

But 1 mole H_2S = 34g

$\therefore$ 0.000199 mole $H_2S = X_3$

$\Rightarrow X_3 = 0.000199 \times 34 = 0.0068g$

$\Rightarrow X_3$ = 0.0068g H_2S was produced from banana leaves

Appendix 7d: Weight and percentage of Biogas Generated from 40g Banana leaves.

From appendices 7a, 7b and 7c respectively, the weight of Biogas

generated = $X_1+X_2+X_3$

= (0.739+0.8052+0.0068)g

= 1.551g

$\therefore$ % biogas generated = $\frac{1.551g}{40g} X100=3.88\%$

Appendix 7e: Percentage of Organic Matter Consumed and Percentage of Bioliquid Generated from 40g Banana leaves

From Table 28, the % organic matter consumed was evaluated as follows:

(% org. matter of the unfermented substrate) - (% org. matter of the fermented substrate)

= 83.14% - 69.21% = 13.93%

Since 3.88% was found to be biogas as found in appendix 7d,

$\therefore$ % bioliquid = % org. matter consumed - % biogas generated

= 13.93% - 3.88% = 10.05%

Appendix 7f: Weight of Bioliquid Generated from 40.0g Banana leaves

Since 40.0g banana leaves produced 1.551g biogas (i.e. 3.88% biogas) and 10.05% bioliquid, therefore, the weight of the bioliquid was evaluated as follows:

1.551g biogas $\rightarrow$ 3.88% biogas

x $\longrightarrow$ 10.05% bioliquid

$$\therefore x = \frac{\text{1.551g biogas x 10.05\% bioliquid}}{\text{3.88\% biogas}}$$

$$x = \frac{\text{1.551 x 10.05 bioliquid}}{3.88}$$

$$= \frac{15.588}{3.88} = 4.020\text{g bioliquid}$$

APPENDIX 8

DETERMINATION OF THE WEIGHTS OF OIL AND ETHANOIC ACID

Appendix 8a: Weight of Oil

The weight of oil was obtained thought the following relation:

$$\text{Density} = \frac{\text{Mass}}{\text{Volume}}$$

but density of oil = 0.8g/cm^3 .

Eample, for 0.05g oil, density = $\frac{0.05\text{g}}{\text{Volume}}$

$$\Rightarrow 0.8\text{g/cm}^3 = \frac{0.05\text{g}}{\text{Volume}}$$

$$\therefore \text{Volume} = \frac{0.05\text{g}}{0.8\text{g/cm}^3}$$

$$= 0.0625\text{cm}^3$$

$$\approx 0.063\text{cm}^3$$

$\Rightarrow 0.063\text{cm}^3$ oil was added to the slurry as 0.05g oil

The same procedure was followed for each of the weight of oil used.

Appendix 8b: Weight of Ethanoic acid

The weight of ethanoic acid was obtained as follows:

$$\text{Density} = \frac{\text{Mass}}{\text{Volume}}$$

but density of ethanoic acid = $1.22 g/cm^3$

Example, for 0.01g of ethanoic acid, $1.22 g/cm^3 = \frac{0.01g}{\text{Volume}}$

$$\therefore \text{Volume} = \frac{0.01g}{1.22 g/cm^3}$$

$$= 0.0082 cm^3$$

$\Rightarrow$ 0.0082 cm^3 ethanoic acid was added to the slurry as 0.01g ethanoic.

The same produce was followed for each weight of ethanoic acidused

APPENDIX 9

PACKING OF CHROMATOGRAPHIC COLUMN

Appendix 9a: Bed Volume of the Column (BV)

Length of the column (L) = 40cm

Internal diameter of the column (D) = 0.85cm

Radius of the column (r) = $D/2 = \frac{0.85cm}{2} = 0.425cm$

Cross-sectional area of the column (A)

$= \pi r^2$, but $\pi = 3.142$

$$A = 3.142 (0.425cm)^2$$

$$= 0.568cm^2$$

but $BV = \pi r^2 L$

$\therefore BV = 0.568cm^2 \times 40cm$

$= 22.72cm^3$

Appendix 9b: Packing Ratio of Silica gel to Alumina in the Column

For one part of the column, the volume to be occupied by silica gel

$$= \frac{BV}{3}$$

$$= \frac{22.72cm^3}{3}$$

$$= 7.57cm^3$$

For one part of the column, the bed volume was divided by 3 because volume = L X L X L.

So, the volume occupied by silica gel = $7.57cm^3$

ut quantity ratio of silica gel to alumina = 1:2

∴ The volume occupied by alumina = $7.57cm^3$ x 2 = $15.15cm^3$

For $1cm^3$ of silica gel, the weight of silica gel = 0.6771g

(i.e. density of silica gel = $0.6771g/cm^3$)

∴ The weight of silica gel packed in the column

= density of silica gel x volume of silica gel

= 0.6771g/cm3 x $7.57cm^3$ = 5.13g

For $1cm^3$ of alumina, the weight of alumina = 0.6684g

(i.e. density of alumina = $0.6684g/cm^3$)

∴ The weight of alumina packed in the column

= density of alumina x volume of alumina

= $0.6684g/cm^3$ x $15.15cm^3$

= 10.13g

Appendix 9c: Sample to Packing Ratio

For silica gel, the sample to packing material ratio = 1:70

but the weight of the silica gel = 5.13g

$\therefore$the weight of the sample = $\frac{5.13g}{70}$

= 0.073g

= 73.0mg

For alumina, the sample to packing material ratio = 1:140,

but the weight of alumina = 10.13g

$\therefore$The weight of the sample = $\frac{10.13g}{140}$ = 0.072g = 72.0mg

Capacity of the column = $\frac{73.0mg + 72.0mg}{2}$

= 72.5mg

APPENDIX 10

Appendix 10a: Determination of Total Carbohydrate

The concentrations and corresponding absorbance of standard glucose solution are given below:

Concentration (mg/dm^3)	Absorbance
0.1	0.122
0.2	0.148
0.3	0.245
0.4	0.390
0.5	0.480
0.6	0.620
0.7	0.725
0.8	0.880
0.9	0.985
1.0	1.120

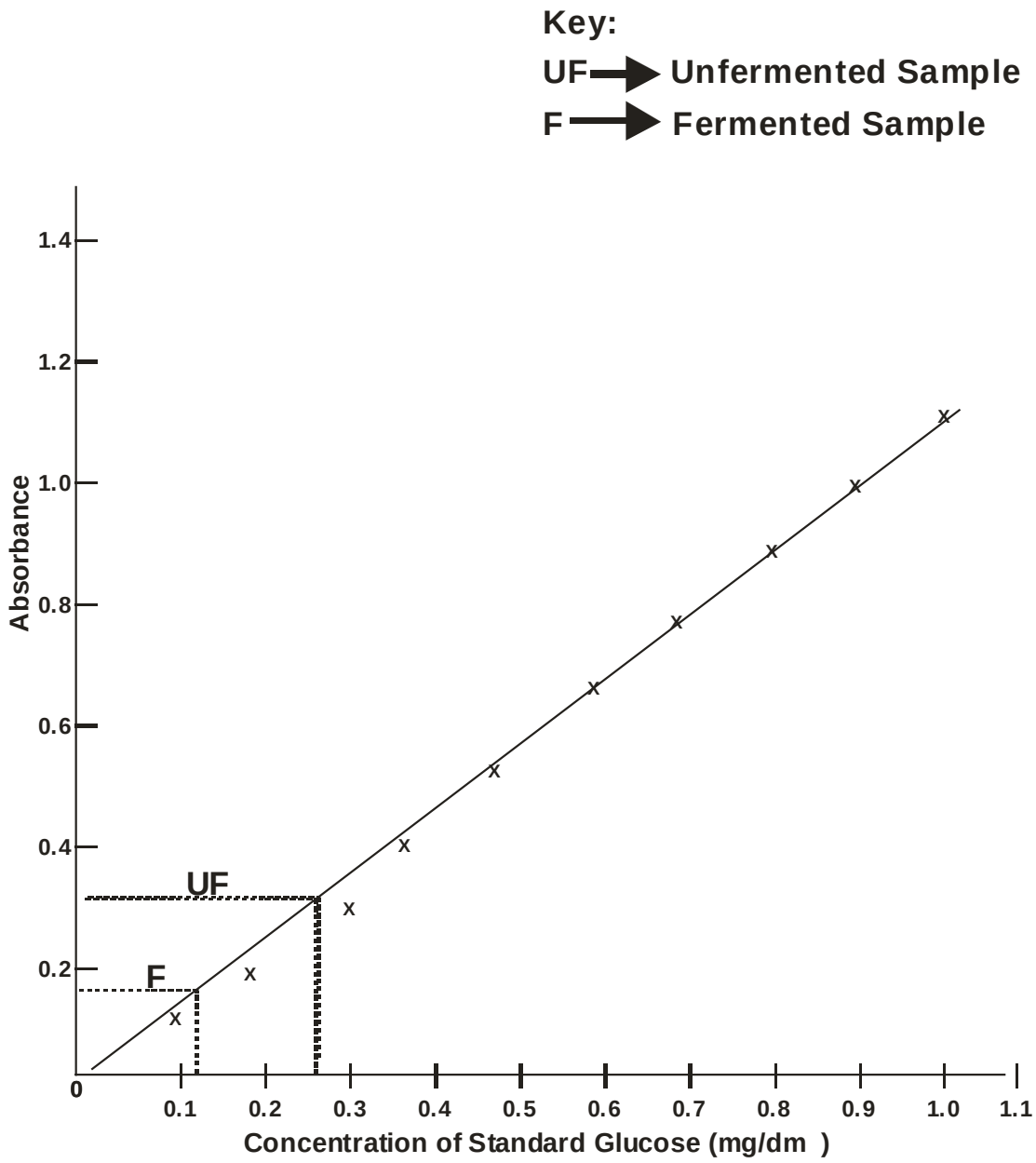

Fig. 6: Calibration Curve for Total Carbohydrate Determination.

Key:

UF → Unfermented Sample

F → Fermented Sample

Average absorbance of the unfermented sample = 0.250

Concentration of the unfermented sample deduced from the plot

$= 0.260 g/dm^3$

Average absorbance of the fermented sample = 0.170

Concentration of the fermented sample deduced from the plot

$= 0.120 mg/dm^3$

% Conc. of Total Carbohydrate of the Unfermented Sample

$$= \frac{\text{Concentration of the sample from the plot}}{\text{Concentration of the sample prepared}} \times 100$$

$$= \frac{0.260 mg/dm^3}{0.5\ mg/dm^3} \times 100 = 52\%$$

% Conc. of Total Carbohydrate of the Fermented Sample

$$= \frac{\text{Concentration of the sample from the plot}}{\text{Concentration of the sample prepared}} \times 100$$

$$= \frac{0.120 g/dm^3}{0.5 g/dm^3} \times 100$$

$$= 24.0\%$$

% Degradation of total carbohydrate =

$$\left(\text{\% conc. of unfermented sample}\right) - \left(\text{\% conc. of fermented sample}\right)$$

$= 52.0\% - 24.0\%$

$= 28\%$

Appendix 10b: Determination of Reducing Sugar.

The concentrations and corresponding absorbance of standard glucose solution are given below:

Conc. (mg/dm^3)	Absorbance
0.1	0.120

0.2	0.168
0.3.	0.260
0.4	0.340
0.5	0.450
0.6	0.520
0.7	0.628
0.8	0.688
0.9	0.800
1.0	1.025

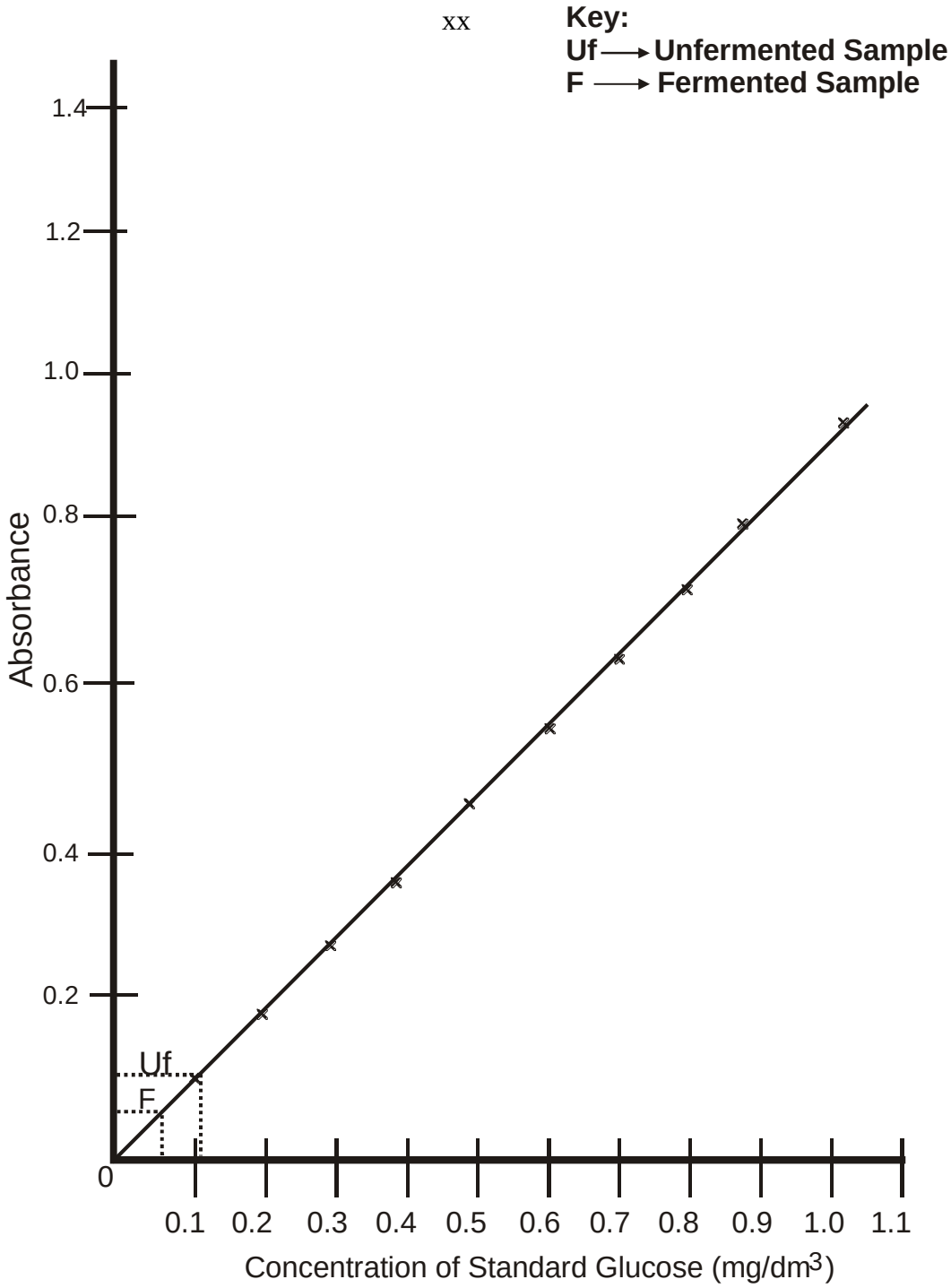

Fig. 7: Calibration Curve for Reducing sugar Determination.

Key:
Uf⟶ Unfermented Sample
F ⟶ Fermented Sample

Average absorbance of the unfermented sample = 0.100

Concentration of the unfermented sample deduced from the plot =0.11mg/dm^3

Average absorbance of the fermented sample = 0.04

Concentration of the fermented sample deduced from the plot = 0.042mg/dm^3

% Conc. Of reducing sugar of the unfermented sample

$$= \frac{\text{Concentration of the sample from the plot}}{\text{Concentration of the sample prepared}} \times 100$$

$$= \frac{0.11\text{mg}/\text{dm}^3}{0.5\text{mg}/\text{dm}^3} \times 100 = 22\%$$

Conc. of reducing sugar of the fermented sample

$$= \frac{\text{Concentration of the sample from the plot}}{\text{Concentration of the sample prepared}} \times 100$$

$$= \frac{0.042\text{mg}/\text{dm}^3}{8.4\%} \times 100$$

% Degradation of Reducing sugar = the (% Conc. of the unfermented sample) _ (% Conc. of fermented sample)

= 22.0% - 8.4%

= 13.6%

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APPENDIX 11
ELUCIDATION OF N-ALKANES FROM THE MASS SPECTRA USING THEIR RESPECTIVE FRAGMENTATION PATTERNS

1. **Appendix 11a:- Scan 2762 (19.980min) from Fig 9**

   Peak 43 → $CH_3\ CH_2\ CH_2$- → Mwt. = 43
   Peaks 43 – 197 → $(CH_2)_{11}$ → Mwt. = 154
   Peaks 197 – 225 → $(CH_2)_2$ → Mwt. = 28
   Peaks 225 – 268 → $CH_3CH_2(CH_2)$ → Mwt. = 43
   Compound Elucidated → $CH_3(CH_2)_{17}CH_3$ → Mwt. = 268
   (Nonadecane)

2. **Appendix 11b:- Scan 2932 (20.989min) from Fig. 10**

   Peak 43 → $CH_3CH_2CH_2$ → Mwt. = 43
   Peaks 43 – 253 → $(CH_2)_{15}$ → Mwt. = 210
   Peaks 253 – 283 → CH_3CH_2 → Mwt. = 29
   Compound Elucidated → $CH_3(CH_2)CH_3$ → Mwt. = 282
   (Eicosane)

3. **Appendix 11c:- Scan 3089 (21.921min) from Fig. 11**

   Peaks 43 → $CH_3CH_2CH_2$ → Mwt. = 43
   Peaks 43 – 57 → CH_2 → Mwt. = 14
   Peaks 57 – 85 → $(CH_2)_2$ → Mwt. = 28
   Peaks 85 – 113 → $(CH_2)_2$ → Mwt. = 28
   Peaks 113 – 197 → $(CH_2)_6$ → Mwt. = 84
   Peaks 197 – 225 → $(CH_2)_2$ → Mwt. = 28
   Peaks 225 – 281 → $(CH_2)_4$ → Mwt. = 56
   Peaks 281 – 296 → CH_3 → Mwt. = 15
   Compound Elucidated → $CH_3(CH_2)_{19}CH_3$ → Mwt. = 296
   (Heneicosane)

4. **Appendix 11d: Scan 3245 (22.847min) from Fig. 12**

   Peak 43 → $CH_3\ CH_2\ CH_2$ → Mwt. = 43
   Peaks 43 – 295 → $(CH_2)_{18}$ → Mwt. = 252
   Peaks 295 – 310 → CH_3 → Mwt. = 15
   Compound Elucidated → $CH_3(CH2)_{20}CH_3$ → Mwt. = 310
   (Docosane)

5. **Appendix 11e: Scan 3388 (21.696min) from Fig. 13**

   Peak 43 → $CH_3CH_2CH_2$ → Mwt. = 43
   Peaks 43 – 57 → CH_2 → Mwt. = 14
   Peaks 57 – 85 → $(CH_2)_2$ → Mwt. = 28
   Peaks 85 – 127 → $(CH_2)_3$ → Mwt. = 42
   Peaks 127 – 179 → $(CH_2)_5$ → Mwt. = 70

Peaks 195 – 239 ⟶ $(CH_2)_3$ ⟶ Mwt. = 42
Peaks 239 – 309 ⟶ $(CH_2)_5$ ⟶ Mwt. = 70
Peaks 309 – 324 ⟶ CH_3 ⟶ Mwt. = 15
Compound Elucidated ⟶ $CH_3(CH_2)_{21}CH_3$ ⟶ Mwt. = 324
(Tetracosane)

6. **Appendix 11f: Scan 3530 (24.539min) from Fig. 14**
Peak 43 ⟶ $CH_3CH_2CH_2$ ⟶ Mwt. = 43
Peaks 43 – 323 ⟶ $(CH_2)_{20}$ ⟶ Mwt. = 280
Peaks 323 – 338 ⟶ CH_3 ⟶ Mwt. = 15
Compound Elucidated ⟶ $CH_3(CH_2)_{22}CH_3$ ⟶ Mwt. = 338
(Tetracosane)

7. **Appendix 11g: Scan 3666 (25.346min) from Fig. 15**
Peak 43 ⟶ $CH_3CH_2CH_2$ ⟶ Mwt. = 43
Peaks 43 – 337 ⟶ $(CH_2)_{21}$ ⟶ Mwt. = 294
Peaks 337 – 252 ⟶ CH_3 ⟶ Mwt. = 15
Compound Elucidated ⟶ $CH_3(CH_2)_{22}CH_3$ ⟶ Mwt. = 352
(Pentacosane)

8. **Appendix 11h: Scan 3796 (26.117min) from Fig. 16**
Peaks 57 – 281 ⟶ $CH_3(CH_2)_{19}$ ⟶ Mwt. = 281
Peaks 281 – 366 ⟶ $CH_3(CH_2)_5$ ⟶ Mwt. = 85
Compound Elucidated ⟶ $CH_3(CH_2)_{24}CH_3$ ⟶ Mwt. = 366
(Hexacosane)

9. **Appendix 11i: Scan 3918 (26.841min) from Fig. 17**
Peaks 57 – 281 ⟶ $CH_3(CH_2)_{19}$ ⟶ Mwt. = 281
Peaks 281 – 323 ⟶ $(CH_2)_3$ ⟶ Mwt. = 42
Peaks 323 – 380 ⟶ $CH_3(CH_2)_3$ ⟶ Mwt. = 57
Compound Elucidated ⟶ $CH_3(CH_2)_{25}CH_3$ ⟶ Mwt. = 380
(Heptacosane)

10. **Appendix 11j: Scan 4047 (27.607min) from Fig. 18**
Peak 43 ⟶ $CH_3CH_2CH_2$ ⟶ Mwt. = 43
Peaks 43 – 365 ⟶ $(CH_2)_{23}$ ⟶ Mwt. = 322
Peaks 365 – 394 ⟶ CH_3CH_2 ⟶ Mwt. = 29
Compound Elucidated ⟶ $CH_3(CH_2)_{26}(CH_3)$ ⟶ 394
(Octacosane)

11. **Appendix 11k: Scan 4176 (28.373min) from Fig 19**
Peak 43 ⟶ $CH_3CH_2CH_2$ ⟶ Mwt. = 43
Peaks 43 – 71 ⟶ $(CH_2)_2$ ⟶ Mwt. = 28
Peaks 71 – 393 ⟶ $(CH_2)_{23}$ ⟶ Mwt. = 322
Peaks 393 – 408 ⟶ CH_3 ⟶ Mwt. = 15
Compound Elucidated ⟶ $CH_3(CH_2)_{27}CH_3$ ⟶ Mwt. = 408
(Nonacosane)

12. **Appendix 11l: Scan 4340 (29. 346min) from Fig. 20**
Peaks 57 – 295 ⟶ $CH_3(CH_2)_{20}$ ⟶ Mwt. = 295

Peaks 295 – 422 ⟶ $CH_3(CH_2)_8$ ⟶ Mwt. = 127
Compound Elucidated ⟶ $CH_3(CH_2)_{28}CH_3$ ⟶ Mwt. = 422
(Triacotane)

13. **Appendix 11m: Scan 5416 (30.319min) from Fig. 21**
Peaks 57 – 295 ⟶ $CH_3(CH_2)_{20}$ ⟶ Mwt. = 295
Peaks 295 – 393 ⟶ $(Ch_2)_7$ ⟶ Mwt. = 98
Peaks 393 – 436 ⟶ $CH_3CH_2CH_2$ ⟶ Mwt. = 43
Compound Elucidated ⟶ $CH_3(CH_2)_{29}CH_3$ ⟶ Mwt. = 436
(Hentracotane)

14. **Appendix 11n: Scan 4727 (31.643min) from Fig. 22**
Peak 43 ⟶ C_3H_7 ⟶ Mwt. = 43
Peaks 43 – 71 ⟶ $(CH_2)_2$ ⟶ Mwt. = 28
Peaks 71 – 393 ⟶ $(CH_2)_{23}$ ⟶ Mwt. = 322
Peaks 393 – 450 ⟶ $CH_3(CH_2)_3$ ⟶ Mwt. = 57
Compound Elucidated ⟶ $C_3H_7(CH_2)_{28}CH_3$ ⟶ Mwt. = 450
(Dotriacotane)

15. **Appendix 11o: Scan 4979 (33.139min) from Fig. 23**
43 ⟶ C_3H_7 ⟶ Mwt. = 43
Peaks 43 – 85 ⟶ $(CH_2)_3$ ⟶ Mwt. = 42
Peaks 85 – 295 ⟶ $(CH_2)_{15}$ ⟶ Mwt. = 210
Peaks 295 – 421 ⟶ $CH_3(CH_2)_9$ ⟶ Mwt. = 126
Peaks 421 – 464 ⟶ $CH_3(CH_2)_2$ ⟶ Mwt. = 43
Compound Elucidated ⟶ $C_3H_7(CH_2)_{29}CH_3$ ⟶ Mwt. = 464
(Tritriacotane)

16. **Appendix 11p: Scan 5278 (34.913min) from Fig. 24**
43 ⟶ C_3H_7 ⟶ Mwt. = 43
Peaks 43 – 85 ⟶ $(CH_2)_3$ ⟶ Mwt. = 42
Peaks 85 – 281 ⟶ $(CH_2)_{14}$ ⟶ Mwt. = 196
Peaks 281 – 463 ⟶ $(CH_2)_{13}$ ⟶ Mwt. = 182
Peaks 463 – 478 ⟶ CH_3 ⟶ Mwt. = 15
Compound Elucidated $C_3H_7(CH_2)_{30}CH_3$ ⟶ Mwt. = 478
(Tetratricotane)

17. **Appendix 11q: Scan 5645 (37.092min) from Fig. 25**
43 ⟶ C_3H_7 ⟶ Mwt. = 43
Peaks 43 – 57 ⟶ CH_2 ⟶ Mwt. = 14
Peaks 57 – 113 ⟶ $(CH_2)_4$ ⟶ Mwt. = 56
Peaks 113 – 281 ⟶ $(CH_2)_{12}$ ⟶ Mwt. = 168
Peaks 281 – 492 ⟶ $CH_3(CH_2)_{14}$ ⟶ Mwt. = 211
Compound Elucidated $C_3H_7(CH_2)_3CH_3$ ⟶ MWt. = 492
(Pentatriacotane)

NB:
The same procedure was followed for the elucidation of iso-alkanes cyclora-alkanes, anomatics and polar compound elucidated.