

**OPTIMIZATION OF BIOGAS PRODUCTION FROM SOME  
AGROWASTES IN A BATCH SYSTEM BIOREACTOR**

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**CERTIFICATION**


I certify that this work "Optimization of biogas production from some agrowastes in a batch system bioreactor" was carried out by Nwaneri, Chioma Blessing ( Reg No: 20134877878) in partial fulfillment of the requirements for the award of Doctor of philosophy (Ph.D) degree in Environmental Microbiology, in the Department of Microbiology, Federal University of Technology Owerri.

  
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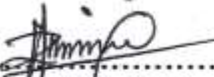
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## **DEDICATION**

This work is dedicated to the blessed memory of my beloved husband, Late Mr J.C. Nwaneri.

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## ABSTRACT

This study was carried out to co-digest and optimize biogas production from some agrowastes in a batch system bioreactor. Six substrates; Palm oil mill effluent (POME), Pig dung, Cow dung, Poultry manure, Cassava peels, and Cabbage waste were used in the investigation. The proximate analysis of each substrate and the microbiological analysis of the co-digested substrates were obtained. The substrates were anaerobically digested as single substrate and co-digested with each other to improve the nutritional composition and biodegradability. Laboratory scale 10L capacity bioreactors were used to carry out the batch system anaerobic digestion of the different substrates for substrate ratios of 1:1, 2:1, 3:1, pH range of 6.8-8.0 and a hydraulic retention time of 45days. Then Box Behnken's design of response surface methodology of three factors; substrate ratio, pH, and hydraulic retention time at three levels; 1:1, 2:1, 3:1(substrate ratios), 5, 7, 9 (pH) and 15days, 30days and 45days (hydraulic retention time) were used in the optimization, considering the substrate with the highest biogas yield. Sodium hydroxide (NaOH) and hydrochloric acid (HCl) were used for adjusting the pH values to the required range. Compositional gas analysis of biogas produced was carried out using Gas Chromatography. The proximate analysis of the substrates showed that the C:N ratios of POME, pig dung, cow dung, poultry manure, cassava peels and cabbage waste were 10.13:1, 5.84:1, 17.50:1, 14.24:1, 46:1 and 16.90:1 respectively. It was also observed that none of the substrates had an optimum C: N ratio hence there was need for co-digestion of the substrates to improve biogas yield. The result also showed that co-digestion of cassava peels with cow dung had the highest biogas yield, flamed on the second day with bright blue flame and had the highest percentage methane content of 78.05%. The results showed the presence of these bacterial isolates; *Escherichia coli*, *Enterobacter* sp, *Salmonella* sp, *Shigella* sp, *Bacillus* sp, *Pseudomonas* sp, *Staphylococcus* sp, *Micrococcus* sp and fungal isolates were *Saccharomyces* sp, *Aspergillus* sp, *Rhizopus* sp, *Penicillium* sp, *Geotrichum* sp. The optimization result showed that the substrate ratio of 3:1, pH 7 and 15days hydraulic retention time had the highest biogas yield, highest methane content of 79.143% and flamed on the second day of the anaerobic digestion. Statistical analysis of the results showed significant difference ( $p \leq 0.05$ ) in biogas production in all the treatments. Further anaerobic digestion of cassava peels with cow dung using the predicted values from the computer aided Box Behnken design response surface methodology analysis showed the same trend with the experimental data. In terms of sustainable biogas production, the outstanding result showed by CP/CD 3:1 at pH 7 proves that the test parameter can be adopted for domestic biogas production.

**Keywords:** Anaerobic digestion, Agrowastes, Optimization, Gas Chromatography, Biogas, Methane content.

# CHAPTER ONE

## INTRODUCTION

### 1.1 Background information

The drastic increase in world population has caused the ever-increasing price and depleting supply of fossil fuel. This fuel is non renewable and has some environmental problems like green house gas emission that causes global warming coupled with the indiscriminate dumping of wastes with the unpleasant sight and this has triggered the search for new natural materials that will be amenable to processing during extraction. Thus this will reduce costs and equally substitute fossil fuel like petroleum products and natural gas which are the main energy reservoirs of the world. These fossil fuels are not fulfilling the world energy demand rather they are creating environmental problems such as green house gases emission, resulting to climate change (NCAR, 2008). Consequently this has resulted to increasing global temperature thereby creating a serious threat to the environment hence the need for renewable sources of energy as a remedy (Solomon & Lora, 2009).

Presently, mankind is producing more waste than ever before, creating unprecedented problems to the environment because people in developed and underdeveloped countries throw away mountains of waste indiscriminately (Demirbas, 2009; Basu & Mettanant, 2009). The wastes with the unpleasant sight and offensive odour emanating from them constitute environmental pollution, however since the advent of biotechnology it has been shown that huge sums of revenue are lying beneath these mountains of trash which can be converted to treasure.

Waste generation is an unavoidable component of our daily life as almost all human activities generate waste on daily basis. These wastes can be grouped based on the sources into (i) Domestic (ii) Municipal (iii) Agricultural and (iv) Industrial. The main contributors to the wastes

produced generally in the environment are the agricultural and municipal sector (Ganiyu & Oloke, 2012).

Agricultural rich country like Nigeria should focus on the energy generation from biomass feed stocks as biomass is the most suitable and largest resources of energy in the world. The biomass available in the world is about 220 billion tons per annum. The energy generated from biomass feed stock can substitute conventional energy resources and utilizing the biomass based fuels can reduce emission of green house gases in the world (Aliyu, 2017).

Nigeria and indeed Imo State has abundant, diverse and unexploited renewable energy resources for the production of fuel, which undoubtedly will help in no small measures to solving the energy crisis and poverty (Asikong, Ekpoke, Eja, Mathew & Effiom 2013; Itodo, Agyio & Yusuf, 2007; Mashandete & Parawira, 2009). Importantly, Guruswamy, Kannani, & Nskumar (2003) and Alvarez & Qunnar (2007), identified two challenges in the 21<sup>st</sup> century which are the development and use of renewable energy to decrease over dependence on fossil fuel and the management of waste generated by human activities and this clearly describes the real picture of the developing countries of the globe such as Nigeria.

Holistically, achieving the Millennium Development Goals (MDGs) in Africa requires a significant expansion of access to modern and alternative renewable energy such as biogas, This is of interest for the sustainable management of our waste and is a major breakthrough in the search for a renewable energy to reduce the over-dependence on non-renewable fossil fuel (Nagamaini & Ramasamy, 2003; Adeyanju, 2008). Proper management of wastes is an issue in the developing countries as diseases and sickness are associated with improper waste handling and disposal. It has been identified that one of the ways of preserving the environment which is

being affected by global warming due to heavy consumption of fossil fuels, is by the use of alternative renewable energy fuels (Okoroigwe, Ibeto & Ezema, 2014).

Energy is an essential input for economic growth, social development, human welfare and improving the quality of life. Every sector of the economy be it agriculture, industry, transport, commercial and domestic needs inputs of energy as a result, consumption of energy in all forms has been steadily rising all over the country. This growing consumption of energy has also resulted in many countries becoming increasingly dependent on fossil fuels such as coal, oil and gas. The world is facing the crises of fossil fuel shortage and environmental degradation as a result of growth in population, urbanization and industrialization (Harilal, Sorathia, Pravin, Rathod, Arvind & Sorathiya 2012). Most countries find themselves under considerable energy constraints as the growing demand for domestic energy use decreases fuel wood reserves and increases deforestation rates affecting the environment negatively.

The crisis faced globally in terms of energy generation has kindled interest in the use of agricultural waste in biogas production as a substitute for fossil fuels as they have large potentials as an energy source. The increase in agricultural activities resulting to increased agricultural wastes and the expansion of the renewable energy sector shows that agricultural wastes could play a vital role in future's biofuels sources. The high cost of petroleum and the adverse effects as non-renewable resources are the major reasons for increased interest in renewable sources of energy (Tsunatu, Azuaga, Chia & Agobison, 2014a).

In the struggle for food and fuel, large areas of vegetation, trees, and shrubs are felled and stripped for firewood, cultivation and fodder. The consequence is impairment of ecological process in these countries and the permanent destruction of normally renewable sources. To improve the quality of life, ensure the future and safeguard the resource base, there is an urgent

need for rural development that combines short measures for survival with long term measures. Unfortunately, many people in rural communities are so poor that they lack the economic flexibility that would enable them to differ in the consumption of resources in need of restoration. Conservation measures are needed that will, at least, maintain the standard of living of these communities, or improve it, while taking into account their own knowledge of the ecosystem and finding effective ways to ensure that these resources are used sustainably (UNECA,2012).

Different systems for the recovery and utilization of household and community waste are gaining a more prominent place in the world. Today's issues arise from the spread of deserts, the loss of forest, the erosion of soils and the growth of human population and industrialized animals husbandry, the destruction of ecological balances, and the accumulation of wastes (Marchaim, 1992). One of such system is the use of anaerobic digestion in an integrated resources recovery system in developing countries which is important to solve both ecological and economic problems.

Anaerobic digestion is the most widely studied technology for organic waste treatments for its efficiency in waste reduction while producing renewable energy (Mohammed, Ali, Mohd, Salmiati, Shamila & Norhayati, 2013). Anaerobic treatment is the use of biological process in the absence of oxygen for the breakdown of organic matter and the stabilization of these materials, by conversion to methane and carbondioxide gases and a near- stable residue (Ganiyu & Oloke, 2012; Monnet, 2003; Shefali 2002). It provides some exciting possibilities and solutions to such global concerns as alternative energy production, handling human, animal, municipal solid wastes, biodegradable waste, energy crops or other biodegradable feed stocks (Ilaboya, Asekhome, Ezeugwu, Erameh & Omofuma 2010). Biogas, as its name suggests, is produced by

extracting chemical energy from organic materials. This process takes place in a sealed container known as biogas digester.

Through anaerobic digestion, animal manure can be converted to methane rich biogas and sludge, which is nearly odourless and useful as a fertilizer. Furthermore, biogas is a valuable fuel that can be used in a variety of applications such as cooking and home heating. It can also be converted into compressed natural gas (CNG) after a scrubbing process that removes carbon dioxide and hydrogen sulphide. Biogas has the greatest potentials for mitigating greenhouse gas emissions. It is as a substitute for coal in electricity generation due to coal's role as the primary source of carbon-dioxide emissions from the power sector (Cueller & Webber, 2008).

Research and common experience have shown that the important characteristics of sustainable waste management are mainly in the recovery of materials and energy from the waste generated, and the stabilization of these residues. Thus, communities that explore material recovery and energy generation from wastes would convert the nuisance that waste create into an opportunity for development. Through organized set-ups, these communities would avoid environmental hazards caused by improperly disposed wastes. It is known that potentially all organic waste materials contain adequate quantities of the nutrients essential for the growth and metabolism of the anaerobic bacteria during biogas production (Ojolo, Bamgboye, Ogunsina & Oke 2008).

In Nigeria today, pig farming industries represents a constant pollution risk due to the production of large amount of pig manure and slurries resulting to a magnitude of possible negative impact on the environment. Also, pig dung which should be used as a source in solving the energy problem in the country is solely used as a soil conditioner, to replenish the soil with nutrients that will improve crop yield. It has been estimated that a medium sized pig of 110Ibs (50kg) produces

manure weighing 1600lbs (750kg) in one year (Iortyer et al., 2012). Unfortunately, the increased production of pig waste (pig dung) has created a lot of problem in the society via environmental pollution and disease spread (Iortyer, Ibrahim & Kwaghger, 2012).

Palm Oil Mill Effluent is liquid waste generated from the oil extraction process from Fresh Fruit Bunch (FFB) in palm oil mills. POME as an effluent is a thick brownish liquid with high Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) (Poh, Young, &Chong 2010). Its high solids concentration and acidity makes it to be unsuitable for direct discharge into the environment. For each ton of crude palm oil (CPO) produced, it is estimated that 5 to 7.5 tons of water is used and more than 50% of water ends up as POME (Ahmad, Ismail & Bhatia, 2003). This implies that about 2.5 – 3.75 tons of POME will be generated per ton of CPO produced. Thus huge quantity of POME will pollute the environment of the palm oil mills without proper waste management (Zahrim, 2014). This problem has become more apparent as the number of palm oil mill industries in Nigeria continues to increase rapidly and varieties of waste biomass are generated.

Therefore, the supplementation of palm oil mill effluent POME in anaerobic digestion of pig manure can be beneficial as POME provides the required energy while simultaneously resolving the environmental issues that are associated with POME discharge (Agrahari & Tiwari, 2014).

Biogas generation from anaerobic digestion of POME to generate electricity is becoming the trend for Palm Oil millers (Chow, 2013; Chin, Poh, Tey, Chan& Chin, 2013).

Biomass is the mass or weight of a group of organic materials currently made from plants and animals. It is the fourth largest source of energy in the world, providing about 14% of primary energy. Developing countries, as a whole, derives 35% of their energy from biomass and in many, it offers over 90% of the total energy used in form of traditional fuels, e.g. fire wood and

dung (Chaiprasert, 2011). Since 90% of the world's population may reside in developing countries by 2050, biomass energy is likely to remain a substantial energy feedstock. Biomass (plant and animal materials) produce renewable energy that comes from the sun through the process of photosynthesis. As the plants capture the sun's energy, they release it when they are burnt making the biomass to function as a sort of natural battery for storing solar energy (Chaiprasert, 2011).

There is a significant increase in biomass cultivation for bioenergy purpose, especially for biogas production via anaerobic digestion. Methane-rich biogas is produced from a wide variety of biomass types like organic wastes (Heiermann, Plochi, Linke, Schelleh & Hermann, 2009).

A few decades ago, pollution control has been given full attention, and knowledge of biological processes was successfully developed with the aim of low utilities and process operation cost (Najafpour & Sadeghpour, 2012). The concern for the environment has come to the consciousness of the world, and we now understand better that our environmental heritage must be protected for the sake of the quality of our lives and those of our children. This attitude is now beginning to govern our behaviour in waste management and is encouraged increasingly by governments in both developed and developing countries (UNECA, 2012). The organic compound fraction of Municipal Solid Waste (MSW) in the US represents 70% of the waste composition and consists of paper, garden waste, food waste and other organic waste including plastics. The biodegradable fraction (paper, garden and food waste) accounts for 53% of waste composition and treatment of these wastes is therefore an important component of an integrated solid waste management strategy to reduce both the toxicity and volume of waste requiring final disposal in a landfill (Shefali, 2002).

The energy needs of mankind have led to search for energy from various resources. Wind, water, animals and plants have been used over the ages as energy source in their most basic forms. However, the dawn of the industrial age brought with it a high demand for energy needed to sustain the fast pace of development that resulted into ways of qualitatively exploiting available energy resources. The result of these activities saw the development of such technologies as wind energy technology, hydroelectric technology, solar energy technology, gasifiers and digesters in which producer gas and biogas that can be combusted to provide useful energy are extracted from organic matter (Iortyer et al., 2012). Of all these technologies, anaerobic digestion to generate biogas from organic wastes which is regarded more as a rural technology is simpler and cheaper. Its application in developing countries where a large percentage of the citizenry suffers from inadequate supply of electricity for basic needs such as cooking and household lighting can positively impact on their standard of living and reduce deforestation which results from increased usage of fuel wood.

The by-products of anaerobic generation of biogas from organic wastes like digested waste slurry can be used as manure for improvement of farm yields. Biogas generation from biomass has been researched by various scientists such that a lot has been known about the quantity and quality of biogas generated from several plants and animal wastes (Aragwa, Andargie & Gessesse 2013; Srinidhi, Ramya, Shankar, Jagadish & Geetha, 2012; Babae, Shayegan & Roshani 2013). However, most researchers tend to focus on the generation of biogas from a particular waste type presupposing adequate availability of such waste type for sustainable biogas generation for the satisfaction of energy demand. More often than not, most people in need of this type of technology do not have enough of any particular type of waste for a sustained satisfaction of their energy needs. Thus, for such people, there arises a need for the

combination of whatever waste types available at any particular time for the production of biogas for their daily energy requirement. To this end, some scientists have carried out research into the effect of combining various waste types on biogas generation (Aragwa et al., 2013)

There are various renewable energy resources such as hydro, solar, wind and biomass that have the potential for exploitation in Nigeria. There are also numerous technologies for harnessing these inexhaustible renewable sources. The technology of anaerobic digestion has not yet realized its full potential for energy production (NCAR, 2008). In most industrialized countries, biogas technology is also potentially useful in the recycling of nutrients back to the soil. Burning non commercial fuel sources such as dung and agricultural residues, in countries where they are used as fuel instead of fertilizer leads to a severe ecological imbalance, since the nutrients, nitrogen, phosphorus, potassium and micro-nutrients are essentially lost from the ecosystem. Biogas production from organic materials not only produces energy but preserves the nutrients, which can in some cases be recycled back to the land in form of slurry (Anonymous, 2005).

More than half of the world's population cook and heat with firewood, dung and field residues. For most of these people, cheap oil is little cause for celebration while bioenergy development, on the other hand, is a realistic proposition for a better social and economic life. Nigeria is one of the countries in the world with impending energy crises and there is no standard waste management operation and equipment for proper waste treatment, thus these wastes are disposed indiscriminately in drainages and road sides and the resultant effect is environmental pollution and greenhouse gas emission. Therefore calls for research studies to X-ray the possibility of applying biogas technology, using piggery waste, palm oil mill effluent, cow dung and other readily available agricultural wastes/organic wastes to generate energy (biogas) via anaerobic digestion primarily for domestic use, with a view of alleviating the chronic and lingering

problem of kerosene scarcity, power and energy, create employment and more importantly better the life of Nigerians in particularly, will undoubtedly help in no small measure in solving the energy crisis and poverty.

## **1.2 Problem Statement**

The depleting supply and high cost of fossil fuel, coupled with the indiscriminate disposal of wastes in drainages and road sides with the resultant effect which is environmental pollution and greenhouse gas emission make alternative renewable energy like biogas a better option as it is less expensive and eco-friendly.

## **1.2 Aim of the Study**

The aim of the study was to optimize biogas produced from some agrowastes in a batch system bioreactor.

## **1.3 Specific Objectives**

The objectives of this study included:

- a. To investigate the effect of substrate ratio of cassava peels co-digested with cow dung on biogas production
- b. To determine the effect of pH on biogas production
- c. To determine biogas yield and flammability.
- d. To determine the composition of biogas produced
- e. To optimize conditions/parameters for anaerobic digestion using Box Behnken's design (response surface methodology).

#### **1.4 Justification of the study**

The issue of indiscriminate waste disposal, sanitation and environmental pollution coupled with the high cost of fossil fuel which is non renewable, make biogas production from organic wastes which is renewable, a better option. In addition by the adoption of this technology, these wastes can be converted to wealth, hence maintaining a clean and green environment and also generating soil conditioner.

Finally biomethane production from agrowaste is a panacea to greenhouse gas emission and mitigation of global warming.

## CHAPTER TWO

### LITERATURE REVIEW

#### 2.1 Anaerobic Digestion

Anaerobic digestion is defined as a biological process which can generate biogas through the decomposition of organic matter by microorganisms in the absence of oxygen (Frigon & Guiot, 2010). The technologies of anaerobic digestion technologies have been used effectively in energy recovery from municipal waste, kitchen waste and sewage sludge (Zhang, Xuefei, Zhanguang, Xiaahua & Yu, 2014; Samaras, Stasinakis, Thomaidis, Mamais & Lekkas, 2014). Researchers are developing the models to control, adjust and predict the anaerobic digestion system to improve the methane yield. (Batstone et al 2015; Lauwers et al., 2013 and Chen et al., 2016).

Anaerobic digestion can also be defined as a collection of processes by which microorganism breakdown biodegradable material in the absence of oxygen (NNFCC, 2011). The process is used for industrial or domestic purposes to manage waste or to produce fuels. Much of the fermentation used industrially to produce food and drink products, as well as home fermentation, uses anaerobic digestion.

Anaerobic digestion is a natural biological decomposition process that converts organic matter, in a controlled environment in the absence of oxygen, into a gaseous mixture mainly composed of methane and carbon dioxide through the concerted action of a close-knit community of bacteria (Schanique & Alicia, 2008). In this oxygen-depleted zone, bacteria are employed to decompose the proteinaceous and carbonaceous materials producing biogas and sludge. Anaerobic digestion provides an array of positive environmental benefits such as reducing greenhouse gas emissions, replacing mineral fertilizers, producing renewable energy and treating

waste. However pitfalls in anaerobic digestion such as poor methane yields, process instability, process failure and regional shortages of feedstock have limited the full exploitation of the anaerobic digestion process (Fagbohunge, Hurst, Ibeto, Li, Usman & Semple, 2017).

Anaerobic digestion is the most widely used method of organic waste disposal due to its high performance in volume reduction and stabilization and the production of biogas that makes the process profitable. However, biological hydrolysis, which is the rate-limiting step for the anaerobic degradation has to be improved to enhance the overall process performance and to reduce the associated cost (Tiehm, Nickel, Zellhorn & Neis, 2001). Several mechanical, thermal, chemical or biological pretreatment methods have been considered to improve hydrolysis and anaerobic digestion performance. These pretreatments result in the lyses or disintegration of cells and release of intracellular matter that becomes more accessible to anaerobic microorganisms, thus improving anaerobic digestion (Bougrier, Albasi, Delgenes & Carrere, 2006).

In today's energy demanding life style, there is always a need for exploring and exploiting new sources of energy which are renewable as well as eco-friendly. The renewable energy resource systems such as solar, wind hydro wave, geothermal and biomass, offer attractive prospects because they are unlimited and cheap. Biomass, which is made up of a wide variety of agricultural residues, is found in large quantity and it is the major contributor to renewable energy (occupying approximately 10% of the total energy). It is considered as a worldwide valuable energy alternative to fossil fuel because it can be converted to a variety of usable forms of energy such as biogas and liquid transportation biofuels (Okeh, Onwosi & Odibo, 2014).

Anaerobic digestion is a technology that utilizes various organic wastes (animal manure, energy crops and industrial wastes) to produce biofuel methane, which holds promise for the future

while simultaneously addressing ecological and agricultural issues (Srinidhi, Ramya, Shankar, Jagadish & Geetha,2012; Zerrouki, Rihani & Bentahar,2013).

Production of waste materials is an undeniable part of human society. The wastes are produced by several sectors including industries, forestry, agriculture and municipalities. The accumulation of waste and the “throw-away philosophy” result in several environmental problems, health issues and safety hazards, and prevent sustainable development in terms of resource recovery and recycling of waste materials. A perspective aimed at promoting greater sustainable development and resource recovery has influenced solid waste management practices and is gradually becoming implemented through policy guidelines at national levels in a number of industrialized and even developing countries. Guidelines and directives to reduce waste generation and promote waste recovery are laid down according to the “waste management hierarchy”, in which waste prevention, reuse, recycling and energy recovery are designed to minimize the amount of waste left for final safe disposal (Taherzadeh & Karimi, 2008)

During the past two decades, developing countries and particularly Nigeria has witnessed increased level of waste generation due to population explosion, increased agricultural activities, and the growth of industries. Consequently, there is intense scrutiny of possible alternative of solid waste utilization through biogas production using organic residues which include pig dung, poultry droppings, cattle dung and kitchen wastes. Government and industries are constantly on the lookout for technologies that will allow for more efficient and cost effective waste treatment. One technology that can successfully treat the organic fraction of wastes is anaerobic digestion (Ojolo et al., 2008).

There are currently strong incentives for increased use of renewable fuels in the transport sector worldwide. However, some bioethanol and biodiesel production routes have limitations with

regard to resource efficiency and reduction of greenhouse gases. A complementary strategy to these is to increase the production of biogas from the digestion of organic residues and crops, or from byproducts of ethanol and biodiesel production. Biogas often has several advantages from an environmental and resource-efficient perspective. This provides the motivation for further technological development aiming to reduce costs and thereby increased economic competitiveness of biogas as a vehicle fuel (Borjesson & Mattiason, 2007).

In United States, livestock animals produce over one billion tons of manure annually. Currently, most of this manure is collected in Lagoons or stored outdoors to decompose. Animal waste stored in this fashion can emit unpleasant odour, harmful air pollutants and greenhouse gases. The air pollutants emitted from manure include ammonia, volatile organic compounds (VOC), hydrogen sulphide and particulate matter. Many of these emitted can cause health problems in humans (Borjesson & Mattiason, 2007).

Besides polluting the air, ammonia emission from manure can contaminate ground water and lead to eutrophication of the soil. Manure also emits methane and nitrous oxide, two potent greenhouse gases. Using standards developed by the Intergovernmental Panel on Climate Change (IPCC), methane has 21 times the global warming, potential of carbon dioxide and nitrous oxide has 310 times the warming, potential of carbon dioxide over a 100 year time Span (Cuellar & Webber, 2010). Because of the scale and growth in greenhouse gas emissions from manure, finding other approaches to manure management that decrease these emissions represents a valuable starting point for mitigating concerns about global climate change in the agricultural sector (Mtui, 2009).

In Thailand, the governmental institutions have restructured the national energy economies to strategically focus on the use of renewable energy (Tonrangklang, Therdyothin & Preechawutti,

2017). The use of Indian floating drum system was the biogas technology that was introduced in Thailand in the early 50's for dairy farms, more advanced systems were introduced by the Ministry of Agriculture Thailand (Hoogwijk, Faaij, Eickhout, De Vries and Turkenburg, 2005). Currently, wastewater from cassava starch producing factories and wastes from pig farms are now used for the production of biogas in Thailand (Barz & Delivand, 2011).

The total energy potential of biomass from agricultural residues, new plantation, animal waste, biomass conservation, fuel substitution, municipal solid waste (MSW), industrial wastewater, black liquor and palm oil Mill effluent were calculated by Prasertsan & Sajjakulnuki (2006), in 1997 as 475.4 PJ covering 15% of the main energy consumption of the country.

Also in India and several other developing countries, small towns and villages are using biogas for fulfillment of their daily household burning and electricity production. Cattle dung along with other organic feedstocks are one of the most commonly used and highly accepted substrate for methanogenesis, hence degradation of cellulose containing feedstocks under anaerobic conditions is a very slow process and results into lower production rate of gas. For the reason, an attempt was made to increase the rate of biogas production by providing partially digested cellulose rich feedstock to the fermentor vessel (Gohel, Ghosh & Braganza, 2013).

In Europe, the energy policy is to reduce greenhouse gas (GHG) by using less, cleaner and locally produced energy, such as energy recovery from different kinds of waste, reduce exposure to volatility of fossil fuel prices and encourage new competitive technology innovation and works (i.e. Germany has the targets of increasing renewable energy usage from 9.1% to 20% by 2020). After several years research, scientists have found some acceptable ways to get renewable energy. Among the different choices, anaerobic digestion technology to produce biogas with

waste and wastewater was found to be an ideal cost-effective biological method to meet the European targets (Gohel et al., 2013).

There are various renewable energy sources such as hydro, solar, wind and biomass that have the potential for exploitation in Ghana. There are also numerous technologies for harnessing these inexhaustible renewable energy resources. Amankwah, (2011) studied the possibility of integrating biogas technology into the farming systems in Ghana, using animal and agricultural wastes to generate energy (biogas) for agricultural production. Biogas is one of the most important bio-energy in the world today which can be used in place of natural gas.

Biogas can be produced from simple organic raw materials such as animal dung, food wastes, agricultural residues, municipal waste materials, energy crops and human wastes. The biogas generated from these materials could be used for electricity and heat generation, pressurized for use in public transport and as a fuel for cooking in homes hence is a substitute for natural gas (Santhosh & Revathi, 2014)

## **2.2 Biogas Production from Different Agricultural Waste and Energy Crops**

Reports abound on anaerobic digestion and biogas production from various agricultural wastes and energy crops (Okeh et al., 2014; Tsunatu et al., 2014a; Agrahari & Tiwari, 2014; Chaiprasert, 2011). The production of biogas by anaerobic digestion of organic solid, such as grassland biomass, municipal waste sewage sludge and animal manure have been studied intensively due to an increase in demand for renewable energy (Malee, Snunkheam&Warangkana, 2014).

Opurum., Nweke, Nwanyanwu & Nwachukwu (2019) carried out a research on the kinetic study of anaerobic digestion of goat manure with poultry dropping and plantain peels for biogas production and observed that the individual digester feeds used in the study exhibited very

reasonable compositional characteristics, indicating a high potential for biogas production and that mono-digestion of goat manure can suitably be adopted in biogas production.

Archinas, Li & Achinas, (2019) examined biogas potential from the anaerobic digestion of potato peels and observed that pretreatment of potato peels led to an elevation of the methane amount to 9% and 17% respectively and alleviated the kinetics of biogas production.

Bolaji & Adebajo (2018) carried out a research on biogas production potentials of cassava peels (CP) that was co-digested with yam peels (YP) in a batch reactor at mesophilic temperature at combinations of 100% CP, 100% YP, 25% CP and 75% YP, 75% CP and 25% YP, finally 50% CP and 50% YP for 30days hydraulic retention time. They observed that Cassava peels co-digested with yam peels had very good biogas production potentials especially when co-digested at 75% CP and 25% YP.

Nwankwo (2014) carried out an experiment on production of biogas from cassava and plantain peels blended with cowdung in a 5L capacity batch reactor for 35days and the result revealed that plantain peels blended with cow dung gave the highest yield of biogas and flamed easily when compared with cassava peels alone.

Mayen & Emmanuel (2018) carried out an investigation on biogas production using fresh/ dry cassava peels/ swine dung and observed that fresh samples had higher biogas yield than dried samples.

Eze & Ezeokonkwo (2018) carried out comparative studies on biogas production from cow dung and a blend of three commercial fruit wastes; orange, tomato and garden egg and the result showed that the cow dung was a more efficient substrate for biogas production than the fruit wastes.

Paepatung, Nopharatana & Songkasiri (2009) carried out a research on wastes from four agro-industries (cassava pulp, pineapple peel, decanter cake and empty fruit bunches) and two weeds (Cat-tail (*Typhaangustifolia L.*) and water hyacinth (*Eichlornia crassipes Solms*)) for biogas production. The result revealed that methane potential assays varied from 0.34 to 0.40m<sup>3</sup> CH<sub>4</sub> kg<sup>-1</sup> VS. The maximum specific methane production rates comparing extent and digestibility of each material in a descending order were that of pineapple peel of 36.77ml CH<sub>4</sub>d<sup>-1</sup>, cassava pulp of 36.57ml CH<sub>4</sub>d<sup>-1</sup>, decanter cake of 32.86ml CH<sub>4</sub>d<sup>-1</sup>, empty- fruit bunches of 13.48 ml CH<sub>4</sub>d<sup>-1</sup>, cat-tails of 11.63ml CH<sub>4</sub>d<sup>-1</sup> and water hyacinth of 10.98ml CH<sub>4</sub>d<sup>-1</sup>.

Spyridon, Gerrit & Willem, (2019) carried out an experiment to investigate the impact of ternary mixture on the anaerobic digestion process performance and connoted that ternary digestion with cow manure: food waste: garden waste mixing ratio of 40:50:10 yielded higher biogas amount.

Rubio, Romero, Wikie & Gracia-Morales, (2019) carried out a research to demonstrate the feasibility of co-digestion of agrowastes in a mesophilic temperature. It was observed that the methane production and methane yields were higher in the co-digested substrates compared to individual substrate's yield.

Sambo, Etonihu & Mohammed (2015) carried out a research on co-digestion of plantain peels/rice husk, banana peels/plantain peels and banana peels/rice husk in the ratio of 1:1 in a locally fabricated digester (10L capacity) for 50 days hydraulic retention time and observed that plantain peels/rice husk generated the highest volume of gas (biogas, methane and others).

Zainab & Ali (2014) examined the recycling of date palm waste as a source for biogas production and revealed that the effect of inoculum addition was more significant than alkaline pretreatment of raw waste materials.

Rachadaporn, Hathaikarn, Nirattisai & Chairat, (2016) carried out a research on modeling of anaerobic co-digestion of pig manure and domestic organic waste and found out that the best operating condition was 35°C, 16% Total Solid and the pig manure to domestic waste ratio of 75:25 (3:1) at pH7.

Sapkota, Aryal, Thapa & Karki, (2012) carried out a research to test the feasibility of selected biodegradable materials for biogas production and observed that cow dung had the highest biogas yield of 41,121 m<sup>3</sup> kg<sup>-1</sup> VS per kg of TS.

Umar, Firdausie, Sharifah & Alwi, (2013) evaluated the co-digestion of palmoil mill effluent with cow manure for biogas production and observed that palmoil mill effluent (POME) and cow manure (CM) are excellent substrates for biogas production. Biogas potentials from POME and CM as a single substrate as well as co- substrates were investigated. Batch anaerobic digesters used for the digestion were operated at ambient temperature (28°C to 34°C) for 21 days. The results showed that biogas yield from the digesters were improved by co-digestion as compared to the digestion of POME alone or CM alone.

Omar, Harun, Mohd, Wan, Idris & Yunus, (2008) observed an improvement in biogas up to 0.207 m<sup>3</sup>/kg<sup>-1</sup> VS added with average methane content of 65% in the anaerobic treatment of cattle manure by addition of palm oil mill effluent in a laboratory scale bioreactor.

Dioha, Ikeme, Nafi'u, Soba & Yusuf, (2014) investigated the effect of Carbon to Nitrogen Ratio and observed that carbon to nitrogen ratio affects the volume of the generated biogas. The production of biogas depends to a large extent, on the choice of feedstock and its carbon to nitrogen ratio.

Ray, Mohanty & Mohanty (2014) studied the anaerobic digestion of kitchen wastes, the biogas production, purification and application in internal combustion engines. He then concluded that biogas is required to be upgraded through purification by removing H<sub>2</sub>S and CO<sub>2</sub> before use.

Falah, Banihani, Almad, Mohammad & Abdallah (2015) carried out a research on biogas production enhancement from mixed animal wastes at mesophilic anaerobic digestion. Mixtures of various cattle dung (CD): poultry droppings (PD) ratios (100%:0%; 50%:50%; 60%:40%; 80%:20%; 0%:100%) were prepared, analysed and then anaerobically digested for a period of 40 days. It was observed that 80%:20% had the highest yield, followed by 100%:0%. Addition of CD to PD enhanced the PD production of biogas, while addition of a small portion of PD to CD gave the maximum yield.

Mohammed, Ali, Mohd, Salmiati, Shamila & Norhayati (2013) evaluated the influence of palm oil mill effluent (POME) as inoculums on anaerobic digestion of cattle manure for biogas production and found out that POME as inoculum has better COD percentage reduction and also has an influence on the start-up time and the rate of biogas produced.

Okoroigwe et al. (2014) conducted an experiment on the anaerobic digestion of dog waste and the result shows that five microorganisms were identified at the beginning of the charging period and one was identified at the end of the test and biogas produced. It was concluded that the pathogen reduction through anaerobic digestion justifies its conversion to energy.

Malee et al. (2014) carried out an evaluation of agricultural wastes for biogas production. It was observed that increase in agricultural wastes loading from 25 to 125 g d<sup>-1</sup> resulted in increase in the volumetric flow rate of biogas by almost 400 ml d<sup>-1</sup>.

Ogiehor & Ovueni (2014) evaluated the effect of temperature, pH, and solids concentration on biogas production from poultry wastes. The result showed that biogas yield tend to increase with

solids concentration, however, highest biogas yield was observed at 35°C and slurries with starting pH of 7 gave relatively consistent high gas yields.

Young, Seung, Kook & Chang (2014) evaluated the effects of substrate to inoculum ratio on the biochemical methane potential of piggery slaughter house wastes. It was observed that for biochemical methane potential assay in an anaerobic reactor, the substrate to inoculum ratio of anaerobic reactor should be above 0.1 and the inoculum should be sufficiently stabilized to avoid further degradation during the assay.

Naphon, Kanokorn & Sombat (2014) evaluated the effect of with / without agitation of agricultural waste on biogas production from anaerobic co-digestion. The maximum biogas production and methane concentration of 98.56L/days and 64.07% was obtained at stirring of the digester. This gave an increase of 7.56 over without stirring digester. Thus agitation of digester can be used effectively as an operating strategy to optimize biogas production.

Tsunatu et al (2014a) evaluated the effect of total solids concentration on biogas yields of agricultural wastes. The agricultural wastes are groundnut shell, maize cobs and rice straw. The result showed that biodigester C (rice straw) at percentage total solids of 9% had the highest yield of biogas with cumulative volume of 680ml. Therefore the efficiency of gas production was seen to decrease with increasing total solid concentration yielding approximately 43.2% more gas/g TS (total solid) at 9% TS than 12% TS.

Asikong, Udensi, Ekpoke, Eja & Antai (2014) examined microbial analysis and biogas yield of water hyacinth, cow dung and poultry dropping fed anaerobic digesters. The result showed that heterotrophic bacterial and fungal counts were substrate specific with poultry dropping fed-digester having the highest. Combining all the substrates yielded the highest biogas (423.80mls).

Tsunatu, Yavini, Nuhu & Namo (2014b) carried out a comparative study of mesophilic biogas production potentials of selected agro- wastes. Biogas production from agricultural wastes (groundnut shell, maize cobs, rice straw and bagasse) inoculated with cattle dung /poultry dropping was investigated. It was observed that the inoculation of agricultural wastes with methanogenic bacteria sources have an important role and efficacy in the quantity of biogas generated.

Zahid, Muhammad, Muhammad, Sumair & Nida (2014) carried out an evaluation of mixing cow dung with apple and banana peels on biogas yield. Different compositions of these wastes were used to produce biogas and at end there was a remedial solution to the wastes of food processing industries.

Heiermann et al. (2009) conducted a laboratory scale batch anaerobic digestion tests under mesophilic conditions according to the German standard procedure VDI 4630 to investigate the suitability of different plant species like barley (*Hordeum vulgare*) rye (*Secale cereal*), triticale (*xTriticosecale wittmack*), alfalfa (*Medicago sativa*), hemp (*Cannabis sativa*) Jerusalem artichoke (*Helianthus tuberosus*) and maize (*Zea mays*) for biogas production. Emphasis was placed on growing stage and maturity, respectively as well as on whole crop silage preparation without additives as a preservation method for biogas crops. Results presented indicate that biogas yield is clearly influenced by plant species and harvest stage. Ensiled matter shows a positive effect on biomethanation with higher biogas yields and methane contents than fresh matter investigated. Hence, ensiling can be considered as pre-treatment which has also potential to improve methane production from plant matter.

Ojolo et al. (2008) examined the potential of (putriscible) component of municipal solid wastes in terms of biogas production. The vegetable component of the waste was used as substrate in a

batch-fed 200dm<sup>3</sup> capacity anaerobic digester, which was consecutively loaded with a 10-20kg ranged weight of vegetable. The total solid (TS) of the substrate was 8-10% over a retention period of 40days. The temperature of the substrate during bio-digestion was maintained within 29-30<sup>0</sup>C. The average biogas yield varies from 5-15dm<sup>3</sup>/kg TS to 5.83dm<sup>3</sup>TS. This study focused on Lagos Island municipal solid waste (MSW). The result demonstrated that MSW could be converted into biofuel and can serve as an effective way of reusing the MSW and reducing the high cost of clearing the wastes.

Adelekan & Bamgboye (2009) conducted a research on biogas productivity of cassava peels mixed with poultry, piggery, and cattle waste in ratios of 1:1, 2:1, 3:1 and 4:1, using 220L capacity batch type anaerobic digesters for a retention period of 30days within the mesophilic temperature range. It was observed that all livestock waste types mixed with peels in the ratio of 1:1 produced higher biogas volumes and the highest observed in piggery waste.

Putri, Saputro & Budiyono, (2012) carried out a research for 60days to determine the effect of manure, rumen and water composition of livestock waste in biogas production and the result indicated that manure/ water with a 1:3 ratio and manure/ rumen with 1:2 ratio, produced the higher volumes of biogas compared to other ratios. The highest biogas production occurred on average at day 23.

Kavitha & Kurian (2007) conducted a batch digestion of vegetable waste slurry in the laboratory using 2.5L capacity bottle reactors for 60days at ambient temperature conditions. Gas production was measured at a fixed time each day by water displacement method. The substrate was mixed once a day at the time of gas measurement to maintain intimate contact between the microorganisms and the substrate. The biogas yield from vegetable waste over the length of the

digestion time was observed to be 0.31L/g of VS fed. The methane content of the biogas generated from the reactors was in the range of 67-70%.

A study on biogas production from fruits and vegetables waste materials and their effect on plants when used as fertilizer (using digested and undigested sludge) were carried out by Sagagi, Garba & Usman (2009). It was observed that the highest weekly individual production rate was recorded for the cow dung (control) slurry with average production of 1554cm<sup>3</sup>, followed by pineapples waste which had 965cm<sup>3</sup> of biogas, then by orange waste which had 612cm<sup>3</sup> of biogas and lastly pumpkin and spinach wastes had 373cm<sup>3</sup> and 269cm<sup>3</sup> respectively. The results obtained showed that the difference in the production of biogas to a large extent depended on the nature of the substrate. All the substrates used appeared to be good materials for biogas production and their spent slurries can be used as a source of plant nutrients.

Okoli, Edo, Ogbuewu, Nwajiobi, Enemor & Okoli, (2005) evaluated the rate of biogas generation and biochemical changes in pig dung used in a simple mobile biogas digester design. Biogas production started 4days after charging the digester with pig dung and the daily biogas volume was 0.005m<sup>3</sup> and 0.035m<sup>3</sup> on the 5<sup>th</sup> and 6<sup>th</sup> day. The daily biogas production dropped between 0.001m<sup>3</sup> and 0.002m<sup>3</sup> from the 7<sup>th</sup> to the 22<sup>nd</sup> day, and then increased to 0.003m<sup>3</sup> on the 23<sup>rd</sup> day. A daily biogas yield was then maintained at the volume of 0.009m<sup>3</sup> from the 24<sup>th</sup> to 27<sup>th</sup> day, then a constant volume of 0.008m<sup>3</sup> biogas was produced for the next 5 days from the 33<sup>rd</sup> day.

Abubakar & Ismail (2012) investigated the effectiveness of cow dung for biogas production. The averaged cumulative biogas yield and methane content observed was 0.15 L/kg VS added and 47%, respectively.

Alvarez & Liden (2009) studied biogas production in farm-scale units under low temperature from mixture of llama, cow and sheep for producing domestic fuel and stabilizing animal waste for use as fertilizer. The results show that the mixture at low temperature (18<sup>0</sup>C–25<sup>0</sup>C), had a methane concentration of between 47% and 55%.

Imam, Khan, Sarkar & Ali (2013) investigated biogas production from selected fermentable materials such as cow dung, poultry waste and water hyacinth. It was observed that the percentage of methane content in biogas produced from different fermentable materials was almost the same.

Castrillon, Maranon, Nava, Ormaechea & Quiroga, (2013) studied biogas production from cattle manure by adding food waste and crude glycerin from the biodiesel industry as co-substrates. It was observed that glycerin addition doubled the methane yield in thermophilic anaerobic co-digestion up to 78% and up to 93% COD removal was achieved.

Westerholm, Hansson & Schnürer (2012) evaluated whole stillage as sole substrate and co-digested with cattle manure for biogas production in five mesophilic laboratory-scale biogas reactors, operating semi-continuously for 640 days. The result showed that co-digestion with manure improved biogas productivity and process stability, also indicated increased methane yield.

Xie, Wu, Lawlor, Frost & Zhan (2012) studied the anaerobic co-digestion of the solid fraction of separated pig manure with dried grass silage in three identical continuously stirred tank reactors at 308±1 K. It was observed that the organic loading rate affected the digester performance more than the feedstock proportion.

Quiroga, Castrillon, Nava, Maranon, Negral, Iglesias & Ormaechea (2014) presented a study of the effect of applying ultrasound pre-treatment in the production of methane when co-digesting

mixtures of cattle manure with food waste and sludge. It was found that there was up to 67% increase in methane yield at 55°C and 31% at 37°C.

Borowski, Domanski & Weatherley (2014) carried out a research on the anaerobic digestion of municipal sewage sludge with swine manure and poultry manure. The experiment showed that a 30% addition of swine manure to sewage sludge significantly increased biogas production by nearly 40%, compared to that with sewage sludge alone.

Zhang, Zhang, Zhou, Chen, Liang & Wei (2013) assessed the anaerobic co-digestion of food waste and cattle manure, in order to identify the key parameters that determine the biogas and methane yield. They suggested that the C/N ratio and the higher biodegradation of lipids might be the main reasons for the biogas production improvement.

Ray et al. (2014) analysed the performance of a 1.5 m<sup>3</sup> volume CSTR digester processing the screened liquid fraction of dairy manure. The digestate yielded an attractive amount of gas, 28.4% of that produced in the CSTR, which implies that the digestive tank should be covered to capture its residual methane yield.

Ajay-Kumar, Jianzheng, Oiaoying, Liguu & Bowei (2012) carried out a research on the effect of mixing on dry anaerobic digestion of cow dung for methane production. The result showed that the performance of the mixed digester was quite different and better than the unmixed digester. The mixed digester produced methane that was 7.50% higher than the unmixed digester.

Iortyer et al. (2012) experimented on the effect of mixing ratio of cattle and piggery dung on Biogas generation and found that the generation of biogas from cattle dung is larger than that from piggery and that of piggery is larger than that from the mixture.

Furthermore, a large-scale production of biogas from agro-wastes (plants and animals), kitchen wastes and human faeces is embarked upon in Africa by Songhai Integrated Farms in Porto-

Novo, Republic of Benin and Songhai center Amokpe Delta State, Nigeria. This has shown that biogas has been made an alternative energy which can replace fossil fuel.

Biogas production is basically produced as a result of fermentation of carbohydrate to acetate, propionate and butyrate and this methane (CH<sub>4</sub>) gas produced serves as heat energy which can be used in heating hatcheries and even residential places when the weather is too cold. Biogas production also helps in creating employment opportunities, sanitizing the environment as well as controlling indiscriminate dumping agro- wastes. (Adeyosoye, Adesokan, Afolabi & Ekeocha, 2010).

In China, biogas production of all household is about 2,000 million m<sup>3</sup> per year. In Southern China, biogas yield of family size digesters is about 300m<sup>3</sup> per year while in the Northern China, it is about 200m<sup>3</sup> per year. Biogas is used by about 25 million people in China for cooking and lighting for 8-10 months a year. (Opurum et al., 2019).

Many rural households are equipped with both biogas stoves and improved cooking stoves. Improved and cheap biogas stoves and lamps have been developed and are distributed to every biogas owner. The cost of one biogas lamp varies between 6-12 Yuan. Lamps and burners are adapted to low pressures of about 2N/m<sup>2</sup> at which RPM digesters operate. There are about 400 biogas power stations with a total capacity of 5,800HP, 800 to over 17,000 households in China and this has made the country to have sound experience in running diesel and gasoline engine with biogas (Marchaim, 1992).

## **2.3 Substrates Used in the Study**

### **2.3.1 Palm Oil Mill Effluent (POME)**

Palm tree (*Elaeis guineensis*) is one of the most versatile crops in the tropical world (Bala, Lalung & Ismail, 2014). Palm oil is also one of the two most important vegetable oils in the world's oil and fats market following soya beans (Hartley, 1988). Oil palm (*Elaeis guineensis*) is the most productive oil producing plant in the world, with one hectare of oil palm producing between 10 and 35 tons of fresh fruit bunch (FFB) per year (Ma, Tajima, Asahi & Hannif, 1996). The palm has a life of over 200 years, but the economic life is 20-25 years (nursery 11-15 months, fist harvest is 32-38 months from planting and peak yield is 5-10 years from planting). Usually, the harvest part is the "fruit bunch" whereby oil is obtained from the fleshy mesocarp of the fruit. Oil extraction from flesh amounts to at least 45-46% while kernel accounts for at least 40-50%. The palm has a highly varied nutrient demand which depends mainly on the yield potential determined by the genetic make-up of the planting material and on yield limit set by climatic factors such as water, effective sunshine and temperature (Igwe & Onyegbado, 2007).

Crude palm oil contains fatty acid ester of glycerol commonly referred to as triglycerides, therefore, contributing to the world's need of edible oil and fats. It is composed of approximately 50% saturated fats (primarily palmitic acid) and 40% unsaturated fats (principally linolenic and Oleic acid). The distinctive colour of the oil is due to the fat soluble carotenoids (pigments) which are also responsible for its vitamins E (tocopherols and tocotrienols) content (Igwe & Onyegbado, 2007).

The production of palm oil, however results in the generation of large quantities of polluted wastewater commonly referred to as palm oil mill effluent (POME) (Najafpour, Zinatizadeh, Mohamed, Hasnain & Nasrollahzadeh, 2006). Typically, 1 ton of crude palm oil production

required 5-7.5 tons of water; over 50% of which ends up as POME (Ma, 1999a; Ma 1999b; Ahmad et al., 2003). This wastewater is a viscous, brownish liquid containing about 95-96% water, 0.6-0.7% oil and 4-5% total solids (including 2-4% SS, mainly debris from the fruit). It is acidic (pH 4-5), hot (80-90<sup>0</sup>C), non-toxic (no chemicals are added during oil extraction), has high organic content (COD 50,000mg/L, BOD 25,000mg/L) and contains appreciable amounts of plant nutrients (Borja, Banks & Sanchez, 1996).

Palm oil mill effluent (POME) is an important source of inland water pollution when released into local rivers or lakes without treatment. POME contains lignocellulolic wastes with a mixture of carbohydrate and oil. Chemical oxygen demand (COD) and biochemical oxygen demand (BOD) of POME are very high and COD values greater than 80,000mg/L are frequently reported. Incomplete extraction of palm oil from the palm nut can increase COD values substantially (Oswal, Sarma, Zinjarde & Pant, 2002).

Treatment and disposal of oily wastewater such as palm oil mill effluent is presently one of the serious contributors of environmental problems. Palm oil mill wastes have existed for years but their effects on environment are at present more noticeable. The only waste has to be removed to prevent interfaces in water treatment units, avoid problems in the biological treatment stages, and comply with water-discharge requirements. Oily wastewater containing oil and grease are considered as hazardous pollutants particularly in the aquatic environments, because they are highly toxic to the aquatic organisms (Ahmad, Sumathi & Hameed, 2005).

Characteristics of palm oil mill effluent depend on the quality of the raw material and palm oil production processes in palm oil mills. The extraction of crude palm oil from fresh fruit bunches (FFB) requires huge amount of water (Rupani, Sinnedge, Ibrahim, & Esa, 2010). Yacob, Hassan,

Shrai, Wakisaka & Subash (2005) estimated that about 0.5-0.75 ton of POME will be discharged from mill for every ton of fresh fruit bunch.

Wastewater composition depends mainly on the season, raw matter quality and the particular operation being conducted at any given time. Typically, palm oil mill wastewater is low in pH because of the organic acids produced in the fermentation process, ranging about 4-5. It also contains large amounts of total solids (40, 500mg/L), oil and grease (4000mg/L) (Ma, 2000). Wastewater includes dissolved constituents such as high concentration of protein, carbohydrate, nitrogenous compounds, lipids and minerals, which may be converted into useful materials using microbial processes. The effluents of palm oil mill can cause considerable environmental problems, if discharged untreated (Davis & Reilly, 1993). Therefore, the challenge of converting POME into an environmental friendly waste requires an efficient treatment and effective disposal technique.

The raw or partially treated POME has an extremely high content of degradable organic matter. As no chemicals are added during the oil extraction process, POME is considered as non-toxic, but it is identified as a major source of aquatic pollution by depleting dissolved oxygen when discharged untreated into the water bodies (Khalid & Wan Mustafa, 1992). However it also contains appreciable amounts of N,P,K, Mg and Ca, which are the vital nutrient elements for plant growth. Due to the non toxic nature and fertilizing properties, POME can be used as fertilizer or animal feed substitute, in terms of providing sufficient mineral requirements (Habib, Yusuf, Phang & Mohamed, 1997). Agamuthu & Shaifal (1995) has also reported the increase of organic nitrogen leading to the production of a better fertilizer in POME. Muhrizal, Shamshuddin, Fauziah & Husni (2006) reported that POME contains high content of Aluminium as compared to chicken manure and composted sawdust.

POME has generally been treated by anaerobic digestion resulting in methane as a value added product (Sinnappa, 1978; Borja & Banks, 1995; Zinatizadeh, Mohamed, Najafpour, Isa&Nasrollahsadeh, 2006; Busu, Sulaiman, Hassan, Shiran, Abdul-Aziz, Yacob & Wakisaka, 2010; Baharuddin, Hock, Yusof, Rahman, Shah, Hassan, Wakisaka, Sakai & Shirai, 2010; Chotwatanasak & Puetpaiboon, 2011).

Anaerobic treatment is the most suitable method for the treatment of effluents containing high concentration of organic carbon (Perez, Diez & Fdz-Polanco, 2001). Considering the high organic character of POME, anaerobic process is the most suitable approach for its treatment.

Anaerobic treatment of wastewater has been considered to have a number of advantages over the conventional aerobic process. It saves the energy needed for aeration, converts organic pollutants into methane gas, a readily useable fuel, needs low nutrient requirement and produces low biomass as the technology is applied in recent years (Faisal & Unno, 2001).

Anaerobic digestion has been employed by most palm oil mills as their primary treatment of POME and for biogas production as it has become a worldwide focus of research, because it produces energy that is renewable and environmentally friendly(Tay, 1991).

### **2.3.2 Pig Dung**

The pig farming industry is growing rapidly along with the world human population. The trend is towards more concentrated piggeries in thousands and the numbers herds produce large quantities of wastes, including organic matter, inorganic nutrients and gaseous emission. The trend in piggery waste management is towards treatment of these wastes to minimize negative impact on the health and comfort of workers and animals (Nabila & Nawel, 2015).

The rapid growth of piggery industry is more in the developing countries as they can afford and acquire a taste for more pork meat in their diet. In the past 16 years, the annual global production

of pig has increased from 790 million to 926 million with most of that increase occurring in China, India, Nigeria and other emerging countries (FAO, 2011). The increased demand for pork has resulted in establishment of larger centralized piggeries with herds exceeding 1,000 and sometimes more than 10,000 herds. Wastes from these facilities exceed the capacity for direct land disposal without severe environmental impacts. These include odour, attraction of rodents, insects /other pests and release of animal pathogens. Atmospheric methane and ammonia, nitrogen, phosphorus and other nutrients are also released into ground and surface water (Hatfield, Brumm & Melvin, 1998).

The characteristics of swine wastes vary with a number of factors, including the age and diet of the pigs, type of housing, cleaning and waste removal (Day & Funk, 1998). Effluent or manure from pigs in piggeries can be converted into biogas, liquid fuel and nutrient rich solids. Piggery wastewater contains urine and dung of pigs and may also contain washwater and chemicals such as disinfectants used in washing the pens. The dung/urine of pig that dries before being collected is called manure (Song, Zhu, Yuan, Hong & Ding, 2010).

Piggery waste is one of the major problems in Nigeria and other developing countries and effective ways of disposal and management is required to prevent environmental pollution. Piggery waste also contains valuable nitrogen and phosphorus and can equally be anaerobically digested to produce energy in the form of biogas (Young et al., 2014)

Finally with the increasing market demand for pork, the growth of swine herds leads to a large increase in swine manure worldwide. The pollution impact of swine waste on water, soil and air is a growing concern in many countries like Nigeria. The sustainability of an efficient disposal mechanism for manure is becoming a key factor in the expansion of pig industry in China (Yin, Liu, Zhai, Yang, Wang & Feng, 2014). Biogas production with Pig manure is a suitable method

for the treatment of this organic waste, yielding biogas as a useful by-product. This process could also produce renewable energy (cheap and clean methane), soil conditioner, and liquid fertilizer that are valuable for crop production ((Zhang, Zhang, Zhang, Fan & Zhang, 2012, Wang, Yang, Feng, Ren & Han2012; Angelidaki&Ellegaard 2002and Mottet, Steyer, Deleris, Vedrenne, Chauzy & Carrere, 2009).

### **2.3.3 Cow Dung**

In Nigeria, cows are reared by Fulani herdsmen and they produce wastes called cow dung. Cow dung or faeces is indigestible plant material which has passed through the animal's gut. It serves as manure to soil but when they are dumped indiscriminately, they will be digested anaerobically by microorganisms producing toxic greenhouse gases such as methane and carbondioxide into the atmosphere. The abundant availability of animal manure in Nigeria (mostly cow dung) which can cause serious health hazard and environmental pollutions can be used as biodigester feeds to produce biogas.This can be commercialized for sale to people in rural and urban areas.Thus animal waste has turned to a source of wealth generation (El-Mashad & Zhang, 2010)

### **2.3.4 Poultry Manure**

Poultry manure is the feaces of chickens, which can be used as organic fertilizer to improve nitrogen content of the soil. This is because it contains the highest amount of nitrogen, phosphorus and potassium. Chicken manure can be stored to retain its liquid content as large quantity of nitrogen is found in the urine. Fresh chicken manure has 0.8% potassium, 0.4% - 0.5% phosphorus and 0.9% - 1.5% nitrogen in it. Also it is assumed that one chick produces approximately 8 to 11 pounds of manure monthly which can be used as fertilizer in the farm. (Itodo & Awulu, 1999).

Poultry manure can also be used in the production of biogas as about 32% dry matter of chicken manure gas efficiency is estimated at 70m<sup>3</sup> to 90m<sup>3</sup> of biogas per ton of farm manure with 60% of methane (Hansen, Schmidt, Angelidaki, Marca, Jansen, Mosbaek & Christensen, 2004).

### **2.3.5 Cassava Peels**

Cassava (*Manihot esculenta*) is a short-lived perennial shrub which is grown in almost all the poor countries of the world (Ekop, Simonyan & Ewwierhoma, 2019). It grows well in areas that experience long dry season than rainy season, thus it can be planted as a crop of choice in drought-prone areas. Cassava is a major root crop in the tropical countries and serves as one of the cheapest source of calories for human consumption (Ekop et al., 2019). Cassava is one of the most important crops in Nigeria and many tropical countries. Cassava species, *Manihot esculenta* Crantz or *Manihot utilisima* pohl is believed to have originated from Brazil and was introduced into West Africa by the Portuguese (Oparaku, Ofomatah & Okoroigwe, 2013).

Cassava roots play an important role in the African diet as it is one of their staple food and they are processed using simple traditional methods into fermented products such as "garri" and "fufu" or flour. It is estimated that about 10 million tons of cassava is processed for garri annually in Nigeria alone. During the processing of cassava products, the peels which constitute about 20-35% of the total weight of the tuber are usually peeled off and these peels are regarded as wastes and are usually disposed of indiscriminately (Obadina, Oyewole, Sanni & Abiola, 2006). The wastes generated at present pose a disposal problem and would even be more problematic in the future with increased industrial production of cassava products such as in garri production, cassava flour and fufu (Obadina et al., 2006).

Cassava peels continue to constitute waste in the cassava processing industry despite the fact that small portion of it is used as animal feed. This is because of the harmful effect of the hydrocyanic acid content of the peels to the growth and development of non-ruminant animals. (Adeyosoye et al., 2010). Due to the high demand of cassava fermented products in Nigeria, the rate of cassava production has increased drastically in recent years and this increase has resulted to a lot of problem in the society via environmental pollution as about 46% of wastewater are produced in Africa, 33% in Asia and 21% in Latin America (Obinna & Okoli, 2010).

### **2.3.6 Cabbage waste**

Cabbage (*Brassica oleracea*) is a greenish leafy biennial plant that has very high nutritional value. As of 2014, the Food and Agricultural Organization of the United Nations (FAO) reported that about 71.8 million metric tons of cabbage and other brassica were produced in the world, with China accounting for total production (FAOSTAT, 2017).

Brazil produced 377,108 tons of cabbage in 2006 and had 1.3% lost in the field as waste, although approximately 45% of the 60 million tons of cabbage being produced in the world is usually wasted during harvest and 16% lost during commercial processing (FAO, 2020). Therefore ensiling process can be adopted as an alternative to reduce waste as it can be used in feeding animals (Makkar & Ankers, 2014). It can also be used as substrates for renewable energy production through anaerobic digestion (Kafle, Bhattarai, Kim, and Chen, 2014). Proper precautionary measures must be taken during the ensiling process of cabbage to avoid contamination by some undesirable microorganisms. (Cao, Cai, Takahashi, Yoshida, Tohno, M., Uegaki, Nonaka & Terada, 2011).

## **2.4 Renewable energy production**

The issue of waste disposal, sanitation and environmental problems coupled with the high cost of fossil fuel make biogas production a better choice. Many of the farming communities in Ghana are far away from the national grid and cannot even dream of getting connected thus the use of biogas technology in these communities could address their energy challenges (Amankwah, 2011). Already agriculture is known to contribute significantly to the global climate change, nevertheless, the transformation of animal wastes and other agricultural wastes will reduce the impact agriculture has on the environment. The issue of climate change with its concomitant challenges due to increasing CO<sub>2</sub> in the atmosphere could be addressed by the use of renewable energy resources such as biogas.

The world today is experiencing global warming due to the use of fossil fuel and wood fuel but the use of biogas does not contribute to global warming neither does the methane produced has any effect on the atmosphere. The integration of biogas technology into agriculture popularly called biological or ecological farming will improve the income levels of farmers and facilitate the achievement of effective and low-cost productivity in our agricultural system. Biogas technology can provide the link between animal husbandry and crop farming thus playing an important role in self-sustaining eco-farming.

The idea of renewable energy resources in Ghana started some years ago, however its exploitation has far been left behind. Renewable energy is now seen as the way forward for energy supply especially in rural communities. Agricultural communities in rural areas produce a chunk of the food for the nation yet lack the energy needed to process and add value to the food they produce. The main source of energy for the rural dwellers is wood fuel and charcoal which increases deforestation leading to the degradation of the environment. It was estimated that

Ghana used about 18 million tones of wood fuel in 2000 and the total energy consumption of Ghana during 1999 was 7, 108 metric tons of which 5,196 metric tons were from traditional sources (Trossero, 2002).

The bulk of wood fuels amounting to 90% are obtained directly from the natural forest and approximately 60% of the world's total wood removals from forest and outside forests are used for energy purposes. The developed countries use only 30% of wood produced for energy while the developing countries use about 80% for the same purpose. It was estimated that 6.5million hectares of forest in Ghana have been deforested at an alarming rate of 22,000 hectares per annum and the rest of being encroached upon. As at 1985, 12,000,000m<sup>3</sup> of fuel wood and charcoal were consumed and this forms about 70% of the country's energy consumption. Developed countries use 155,000,000m<sup>3</sup> of wood as firewood of which 0.4% of all energy used is equivalent to 10% of total wood used while developing countries use 1.2 billion cubic metre of wood for firewood of which 25% of all energy use of firewood is equivalent to 90% of total wood used (Amankwah, 2011).

Considering the above trend of wood consumption, it is important to consider a cheap but reliable alternative source of energy such as biogas. It is worth noting that most of the wood fuel is used in rural communities as energy for cooking. Farmers in the rural communities also engage in charcoal production especially during the lean season which is transported to the cities for consumption (Amankwah, 2011).

Integration of biogas technology into the farming system will reduce the use of firewood for cooking and reduce the involvement of farmers in charcoal production as they are likely to be busy on their farms. It is estimated that about 70% of Ghana's population are rural dwellers that use wood fuel as their main source of energy for their domestic and commercial activities.

Farming communities in the three northern regions namely; Northern, Upper East and Upper West Regions cannot boast of basic energy on their farms to process their crops. Lack of basic energy coupled with the use of wood fuel and charcoal call for the need to integrate biogas technology into the farming systems to meet those challenges (Amankwah, 2011).

## **2.5 Co-digestion of Substrates**

Anaerobic digestion of organic matter or biomass as a single substrate has been extensively researched and evaluated for biogas production. This is because of the low biodegradability which leads to the low yield of biogas production from single digestion of the substrates. Amongst the approaches of enhancing the economics of anaerobic digestion for organic matter or biomass is to improve their biogas yield rate by co-digesting more than one organic matter to augment the missing nutrients in the digesters (Mata-Alvarez, Macc & Llabres, 2000).

Co-digestion is the immediate breakdown of the homogenous mixture of several substrates. It has a lot of benefits which includes better digestibility, enhanced biogas production and methane yield due to availability of additional nutrients (Mshandete & Parawira 2009). Co-digestion has taken much attention since it is one of the interesting ways of improving the yield of anaerobic digestion. Most of the investigations on co-digestion were carried out in batch operation mode and many researchers have pointed out the influence of synergy due to a balanced mixture composition on methane yield (Oparaku et al., 2013).

Studies have shown that co-digestion of several substrates, for example, banana and plantain peels, spent grains and rice husk, pig waste and cassava peels, sewage and brewery sludge, among many others have resulted in improved methane yields by 24 to 47% compared to that obtained from single substrates (Aragwa et al., 2013, Sawyerr, Trois, Workneh & Okudoh, 2017).

## **2.6 Microbial Metabolism in Anaerobic Digestion**

The degradation of organic matter to produce methane relies on the complex interaction of several different groups of bacteria. Stable digester operation requires that these bacterial groups be in dynamic and harmonious. Changes in environmental conditions can affect this equilibrium and result in the buildup of intermediates which may inhibit the overall process. It is of utmost importance to understand the basic microbiological and biochemical pathways in order to master the biogas digestion system (Babae et al., 2013).

Effective anaerobic degradation of organic matter proceeds in the absence of oxygen and requires the combined and coordinated metabolism of different kinds of carbon catabolizing anaerobic bacteria. It is the consequence of a series of metabolic interactions among various groups of microorganisms. There are four key biological and chemical stages (Dioha, Ikeme, Nafi'u, Soba & Yusuf, 2013).

The first is hydrolysis, where complex organic molecules are broken down into simple sugars, amino acids and fatty acids. The second stage is the biological process of acidogenesis where a further breakdown by acidogens into simpler molecules, Volatile fatty acids (VFAs) occurs, producing ammonia, carbon-dioxide and hydrogen sulphide as byproducts. The third stage is the biological process of acetogenesis where the simple molecules from acidogenesis are further digested by acetogens to produce carbon dioxide, hydrogen and mainly acetic acid. The fourth stage is the biological process of methanogenesis where methane, carbon dioxide and water are produced by methanogens (Shefali, 2002).

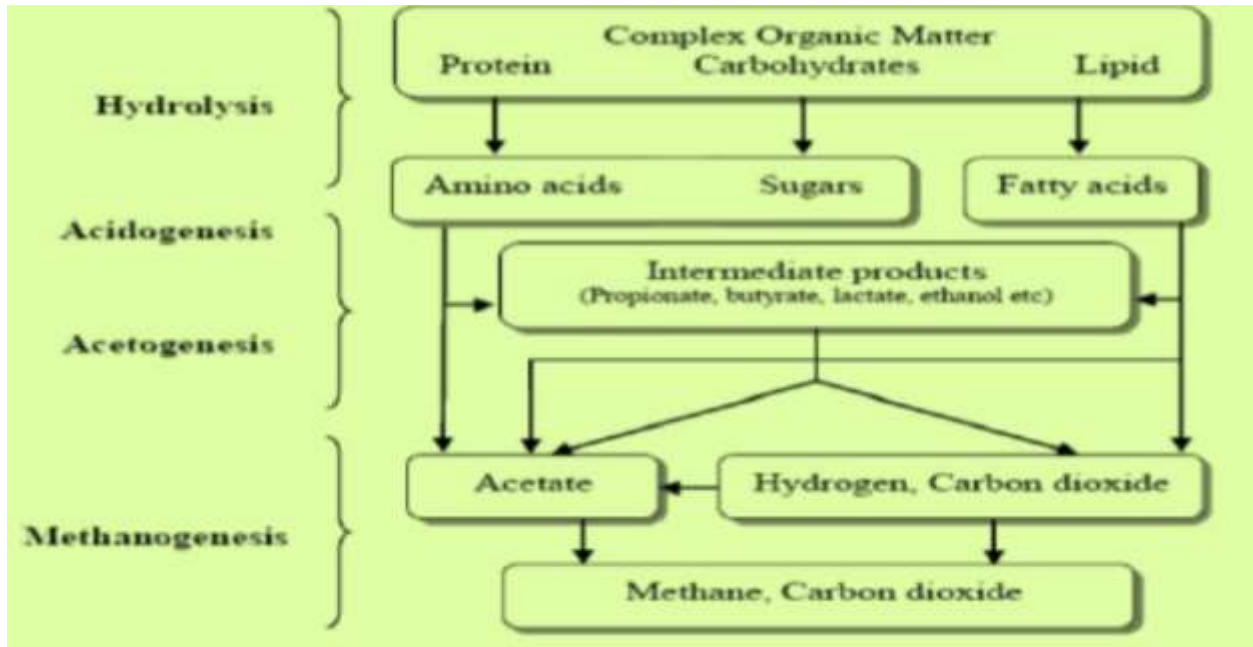


Figure 2.1: Anaerobic digestion pathways of organic degradable substrate (Shefali, 2002).

### 2.6.1 Hydrolysis

In the first stage, fermentative bacteria convert the insoluble complex organic matter, such as cellulose and starch into soluble molecules such as sugar, amino acids and fatty acids. The complex polymeric matter is hydrolyzed to monomer and proteins to peptides or amino acids by hydrolytic enzymes (lipase, protease, cellulases, amylases etc.) secreted by microbes. The hydrolytic activity is of significant importance in high organic waste and may become rate limiting. Some industrial operations overcome this limitation by the use of chemical reagents to enhance hydrolysis. The application of chemicals to enhance the first step has been found to result in a shorter digestion time and provide a higher methane yield (Shefali, 2002).

Hydrolysis/Liquefaction reactions

Lipids → Fatty acids

Protein → Amino acids

Polysaccharides → Monosaccharides

Nucleic acids → Purines and pyrimidines

### 2.6.2 Acidogenesis

In the second stage, acidogenic bacteria, also known as acid formers, convert the products of the first phase to volatile organic acids, carbon-dioxide and hydrogen. The principal acids produced are acetic acid, propionic acid ( $\text{CH}_3\text{CH}_2\text{COOH}$ ), butyric acid ( $\text{CH}_3\text{CH}_2\text{CH}_2\text{COOH}$ ) and ethanol ( $\text{C}_2\text{H}_5\text{OH}$ ). The products formed during acidogenesis are due to a number of different microbes eg *Syntrophobacter woliniia*, propionate decomposer and *Syntrophomonas wolfei*, a butyrate

decomposer. Other acid formers are *Clostridium* spp, *Peptococcus anerobus*, *Lactobacillus* and *Actinomyces*.

It has been reported that seventeen fermentative bacterial species have played important role in the production of biogas. However, the nature of the feedstock determines the type and extent of fermentative bacteria present in the digester (Nagamany & Ramasamy, 1999). Bori, Adebuseye, Lawal & Awotiwon, (2007) reported the population distributions of the microflora in anaerobic digestion of banana and plantain peels as consisting of mainly *Micrococcus luteus*, *Bacillus subtilis*, *Escherichia coli* and *Clostridium perfringes* while the methanogens identified belong to the genera, *Methanobacterium*, *Methanococcus* and *Desulfovibrio*. Some researchers also observed higher amylolytic microorganisms in cow dung digester system and found higher proteolytic population in poultry dung fed digester systems (Bori et al., 2007). Further observation indicated a clear distinction that existed in the type of cellulolytic bacterial distribution in rumen and biogas systems. The observation indicated that while in rumen, *Ruminococcus* spp. alone accounted for 60% of the total population, the predominant species belonging to genera *Lacteriodes* and *Clostridium* were found in biogas system (Nagamany & Ramasamy, 1999).

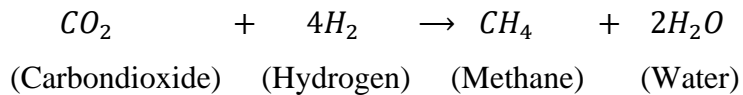
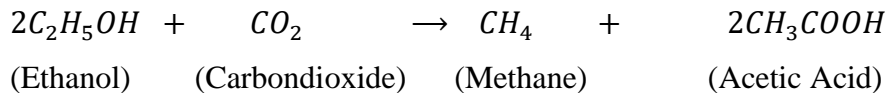
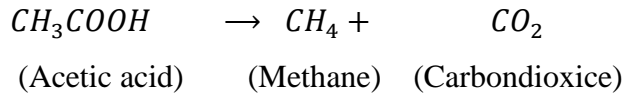
### **2.6.3 Acetogenesis**

Acetogenic bacteria convert fatty acids like propionic acid, butyric acid and alcohol into acetate, hydrogen and carbondioxide which are used by methanogens. This group of bacteria requires low hydrogen tension for acid conversion and therefore a close monitoring of hydrogen concentration is necessary. Under relatively high hydrogen partial pressure, acetate formation is reduced and substrate is converted to propionic acid, butyric acid and ethanol rather than methane. (Shefali, 2002)

#### 2.6.4 Methanogenesis

Methanogenesis is a reaction facilitated by the methanogenic microorganisms. In this final stage, methane is produced by bacteria called methane formers (also known as methanogens) in two ways: either by means of cleavage of acetic acid molecules to generate carbondioxide and methane or by reduction of carbondioxide with hydrogen (Ray et al., 2014).

Methane production is higher from reduction of carbondioxide but limited hydrogen concentration in digesters results, in that the acetate reaction is the primary producer of methane (Shefali, 2002). The methanogenic bacteria include *Methanobacterium*, *Methanobacillus*, *Methanococcus* and *Methanosarcina*. Methanogens can also be divided into two groups: acetate and H<sub>2</sub>/CO<sub>2</sub> consumers. *Methanosarcina* spp and *Methanotherix* spp. (also *Methanosaeta*) are considered to be important in anaerobic digestion both as acetate and H<sub>2</sub>/CO<sub>2</sub> consumers. The methanogenesis reaction can be expressed as follows:



(Tsunatu et al., 2014b; Dioha et al., 2013)

## **2.7 FACTORS AFFECTING ANAEROBIC DIGESTION AND BIOGAS PRODUCTION**

Several factors affect the performance of the anaerobic digestion either by process enhancement or inhibition. These factors include operational factors, pH, temperature, hydraulic retention time (HRT), free ammonia concentration, Carbon:Nitrogen ratio, substrate characteristics / biodegradability, biodigester design, the nature of the substrate, loading rate, toxicity, stirring, nutrients, slurry concentration, digester construction and size, acidity and alkalinity, initial feeding, total volatile acids, chemical oxygen demand (COD), total solid (Ts), volatile liquid etc.(Roubik, Mazancova, Bonout & Verner, 2016 ). However, Xie et al. (2012) reported that temperature, biodegradability, Organic Loading Rate and Hydraulic Retention Time have great impact on the anaerobic digestion of animal manure and other factors should not be overlooked.

While anaerobic digestion is a natural process, the efficient management of anaerobic digestion plant, yielding maximum biogas and proper breakdown of organic feed material is a complex process. For instance, water content of raw material must be monitored because digestion of material with total solid content lower than 5% is usually not economically viable. Temperature must be maintained relatively constant to sustain gas production. The acid-alkaline chemical balance must be controlled for efficient digestion. Also, the carbon to nitrogen ratio must also be closely managed. The implication is that the efficient management of a large-scale anaerobic digestion plant requires trained full-time staff (Anonymous, 2005).

Stable digester operation requires that bacterial groups be in dynamic and harmonious equilibrium as changes in environmental conditions can affect it and result in the buildup of intermediates which may inhibit the overall process. (Sagagi et al. 2009)

The concentration of water-soluble substances such as sugar, amino acid, protein and minerals decrease with age of plants because non-water soluble substances such as lignin, cellulose, hemicellulose and polyamides increase in content with the age of the plant. This means that vegetable matter from younger plants produce more biogas compared to those from the older plants. For waste products from animals, its feeding and living conditions, the age and storage of the waste product are factors affecting the quality and quantity of the gas produced. In general finely ground waste products produce more biogas due to large surface area of contact with bacteria (Sagagi et al., 2009).

### **2.7.1 Temperature**

In anaerobic digestion process, temperature is not only important for microbial metabolic activities but also for the overall digestion rate, specifically the rates of hydrolysis and methane formation. In general, anaerobic digestion process can occur within a wide range of temperatures (Seadi, Rutz, Prassl, Köttner, Finsterwalder, Volk & Janssen, 2008). Based on temperature also, anaerobic microorganisms can be categorized into psychrophiles (<20 °C), mesophiles (25–37 °C) and thermophiles (55–65 °C) (Wan,Wan, Karim & Azni,2009). Some methanogenic species exhibit a preference of extreme heat (90–100 °C) thus, are classified as hyperthermophilic methanogens (Chen,Cheng & Creamer, 2008). Examples are *Methanocaldococcusjannaschii* and *Methanococcusvulcanius*. Experimentally, most of the anaerobic digestion system are designed to operate at mesophilic range, between 30 to 38<sup>0</sup> C, and some of them are designed for thermophilic temperature range of 50 to 57<sup>0</sup>C. Generally, thermophilic digestion processes potentially allow higher loadings with reduced hydraulic retention times, higher conversion efficiencies and pathogen disinfection while mesophilic

digestion is more stable, less at risk from ammonia nitrogen toxicity and requires less process heat (Metcalf & Eddy, 2003).

Many modern anaerobic digestion plants have chosen thermophilic process temperatures as the thermophilic process provides some advantages, compared to mesophilic and psychrophilic processes, although they have some disadvantages as well (Seadi et al, 2008):

#### **2.7.1.1 Advantages of thermophilic process**

- Destroy pathogens effectively
- With reduced retention time, the digestion process become faster and more efficient
- Provide better degradation and utilization of substrates
- better potential for solid liquid separation

#### **2.7.1.2 Disadvantages of thermophilic process**

- Higher degree of imbalance
- Higher energy demand due to high temperature
- Higher risk of ammonia inhibition

Temperature can be considered as the most important environmental factor influencing the growth of microbes. Although each microorganism has a certain temperature range within which it can grow and multiply. When temperature is increased within a certain range, the chemical and enzymatic reactions increase at a faster rate and growth increases (Vindis, Mursec, Janzekovic & Cus, 2009). However, above optimum temperature, key chemical reactions in the different metabolic pathways being catalyzed by enzymes cannot occur because enzymes are irreversibly destroyed since they are protein - like in nature. Enzymes are crucial to metabolism because they allow organisms to drive desirable

reactions that require energy, by coupling them to spontaneous reactions that release energy.

However, different microbial species respond differently to abrupt changes in temperature. Moreover, temperature does not only influence the rate of metabolism of the microorganisms but also affect other process parameters such as organic loading rate (OLR) and ammonia concentration (Marchaim, 1992). However, the ratio of free ammonia to total ammonium ion is higher at thermophilic temperature ranges. Consequently, animal wastes (containing nitrogen and ammonia compounds) are digested at mesophilic temperature range (25–37 °C) in a bid to avoid ammonia mediated inhibition of methanogenesis (Marchaim, 1992). Moreover, thermophilic treatment requires high energy thereby may reduce the net energy obtained from the overall digestion. In spite of the above mentioned drawbacks of thermophilic fermentation; it undoubtedly causes significant destruction of pathogens and weed seeds and also causes higher metabolic rate resulting in higher methane yield (Vindis et al., 2009).

### **2.7.2 pH and Alkalinity**

In anaerobic digestion, the pH level has an enzymatic activity in the microorganism since each has its activity in one specific range of pH and its maximum activity with its optimal pH (Ahring, Ibrahim & Mladenovska, 2001). Hence a stable pH indicates system equilibrium digester stability. Moreover, a falling pH decrease can point towards acid accumulation digester instability.

When large amount of organic acids are produced in the beginning of the fermentation, pH inside the digester might decrease below 5; since the digester has a high concentration of volatile acids, the methane fermentation process in the digester will be inhibited and even stopped. The medium

with low pH (below 6.5) will have a toxic effect on the methanogenic bacteria (Ahring et al., 2001).

In regards to anaerobic digestion, it is also more appropriate to discuss pH alongside alkalinity since the later can be used to control pH thus buffering the acidity of the system derived from acidogenesis phase. Therefore, the amount of alkalinity present in an anaerobic digester represents the buffering capacity. The pH range of anaerobic digestion normally occurs near neutral pH range and it is dependent on the OLR (which depends on reactor type) and the buffering capacity of the substrate. Livestock wastes (rich in ammonia and nitrogen compounds) such as cow, pig and poultry manure have high buffering capacity as they produce alkalinity when degraded upon by microorganisms. Furthermore the range of acceptable pH for the bacteria participating in digestion is from 5.5-8.5, though closer to neutral (Yadvika, Kohli & Rana, 2004). However, anaerobic digestion of these wastes is often maintained at higher pH values of 7.6 . Most methanogens prefer a narrow pH range and the optimal is reported to be 7 to 8. Acidogens usually have a lower value of optimum pH. The optimum pH interval for mesophilic digestion is between 6.5 and 8 and the process is severely inhibited if the pH value falls out of this range (Seadi et al, 2008). Most anaerobic bacteria including methane forming bacteria function in a pH range of 6.5 to 7.5, but optimally at a pH of 6.8 to 8.0 and the rate of methane production may decrease if the pH is lower than 6.3 or higher than 8.0 (Seadi et al., (2008).

An increase in OLR with a corresponding decrease in HRT can result to accumulation of volatile fatty acids which causes a drop in pH due to increased acidity of the digesting medium. However, in instances where the pH has to be adjusted, several chemicals such as sodium hydroxide, potassium bicarbonate, sodium bicarbonate, calcium carbonate (lime),

calcium hydroxide etc. can be added for alkalinity supplementation (Pang, Liu, Li, Wang & Yuan, 2008).

### **2.7.3 Ammonia Concentration**

Anaerobic digestion of urea and protein-rich wastes such as animal wastes is often faced with the challenge of high levels of free ammonia. This is due to their high organic nitrogen concentration which upon biological degradation results in high concentration of total ammonium ion plus free ammonia. The quantity of ammonia produced during the digestion process is attributed to substrate concentration of nitrogen, reactor loading, C/N ratio, buffering capacity and temperature. Ammonia toxicity is influenced by the operating pH and temperature (Ganiyu & Oloke, 2012). An increase in pH will cause increase ammonia toxicity of the system since a greater part of the total ammonia nitrogen will be free ammonia, the form that has been recognized as a toxic agent. On the other hand, reduction in pH to a level within the optimum pH necessary for growth of the microorganisms will help to counteract free ammonia concentration. However, process instability provoked by ammonia toxicity often results in increased level of volatile fatty acids with a corresponding decrease in methane yield (Ganiyu & Oloke, 2012). Therefore, in the presence of elevated levels of ammonia in a fermentor, a shift occurs in the biomethanation process from acetoclastic methanogenesis (performed by acetate-utilizing methanogens) to syntrophic acetate oxidation conducted by syntrophic acetogens in collaboration with hydrogenotrophs (Wan et al., 2009).

Furthermore, chemical equilibriums especially of free ammonia concentration at a fixed total ammonium concentration can be affected by the operating temperature (i.e., mesophilic and thermophilic temperature ranges) of the digester system

(Richards,Cummings, Jewell & Herndon, 1994). Therefore, anaerobic digestion of animal manure at mesophilic temperature offers better process stability and performance of the digester system than at thermophilic temperatures. (Vindis et al., 2009).

#### **2.7.4 Hydraulic Retention Time (HRT) and Organic Loading Rate (OLR)**

HRT is the average period of time that the substrate resides in the anaerobic digester and OLR describes the amount of organic matter expressed in g COD/L or g TS/L or g VS/L added to the digester per reactor volume and unit time. HRT is inversely proportional to OLR and both are very useful parameters that contribute knowledge on design and performance of the reactor (Anonymous,2005). Biological decomposition of animal manure is affected greatly by its retention time in the reactor (Harasimowicz, Orluk, Zakrzewska-Trznadel & Chmielewska, 2007). Retention time is determined by solid content of manure, temperature as well as the type of reactor used for treatment. More elaborately, a higher OLR beyond the optimum capacity elevates the rate of production of intermediary products (fatty acids) by hydrolytic and acidogenic bacteria. Subsequently, these fatty acids would accumulate due to the slow rate of their consumption by methanogens thus; pH will drop thereby inhibiting methanogenic activity.

#### **2.7.5 Volatile Solids**

The weight of organic solids burned off when heated to about 538°C is defined as volatile solids. The biogas production potential of different organic materials can also be calculated on the basis of their volatile solid content. The higher the volatile solid content in a unit volume of fresh dung, the higher the gas produced. For example, a kilogram of volatile solids in cow dung would yield about 0.25m<sup>3</sup> biogas (Moller, Sommer & Ahring, 2004).

### **2.7.6 Particle Size**

The size of the feedstock should not be too large otherwise it would be difficult for microbes to carry out digestion and would result in the clogging of the digester. Thus smaller particles on the hand would provide large surface area for absorption of the substrates that would result in increased microbial activity and hence increased gas production (Izumi, Okishio, Nagao, Niwa, Yamamoto & Toda, 2010).

### **2.7.7 Substrate Characteristics and Heavy Metals**

The constituents of manure directly determine the biogas yield and the level of biochemical reactions that would take place within the digester system. The composition of the manure will depend on the livestock operations which includes the diet and the handling/storage procedure of the wastes. Evidently, for the proper functioning and continuous reproduction of microbes implicated in the anaerobic digestion process, there is a need for available sources of energy; carbon for the synthesis of new cellular materials, inorganic elements such as nitrogen, phosphorus, potassium, sulfur, calcium and magnesium as well as organic nutrients (Yadvika et al., 2004). As a consequence, the physical and chemical characteristics including the moisture content, total solids content, volatile solids content, phosphorus, nitrogen and carbon content of the feedstock must be evaluated before commencement of the digestion process (Okeh et al., 2014). The term biodegradability of manure is indicated by biogas or methane yield and percentage of solids (total or volatile solids) that are destroyed in the anaerobic digestion process (Yue, Teater, Liu, Maclellan & James, 2010).

Microorganisms require a trace amount of some metals (nickel, cobalt, copper, iron, zinc, molybdenum etc) for optimum growth and performance. However, process failure

caused by trace element deficiency has been demonstrated during anaerobic digestion of single substrates such as maize silage as some of these mono substrates cannot provide both the micro and macro nutrients essential for the growth of anaerobic microbes that are present in the anaerobic digestion process (Dosta, Galí, Macé & Mata-Álvarez, 2007). Therefore, they have to be supplemented with other substrates for more nutrients before commencement of the digestion process. Better still, they can be co-digested with animal manure that provides good buffering capacity and required nutrients as the energy crop provides increase in the energy yield of the process (Dosta et al., 2007). However, too high concentration of these heavy metals would lead to toxicity of the system thereby inhibiting the biological process via the enzymes involved by interfering with their function and structure.

### **2.7.8 Toxic Substances**

Presence of toxic inhibitory compound can adversely affect the anaerobic process of microorganism. A wide variety of inorganic and organic toxic and inhibitory substances can cause anaerobic digester failure. The commonly present toxic substance in anaerobic digesters include ammonia, sulfide, light metal ions, heavy metals etc. (Chen et al., 2008).

Some inhibitory substance as explained in 'Digestion Inhibitors' documents by The Sustainable Energy Authority of Ireland are listed as follows:

- a. Oxygen and light; high amount of them could inhibit the activity of methane producing bacteria.
- b. Disinfectants such as herbicides, heavy metals or antibiotics found in poultry/chicken manure can also disturb the process if present in high concentration.

- c. Hydrogen sulphide ( $H_2S$ ) is a product of the digestion process but can be found in the organic material as well. Hydrogen sulphide and sulphuric acid are highly corrosive and could seriously affect the components of digester.
- d. A high ammonia concentration, which is a cellular poison, could be caused by high nitrogen and ammonium ( $NH_4$ ) concentrations under certain circumstances. In such case, substrates with a high nitrogen concentration like chicken manure/pig slurry should be diluted or mixed with another nitrogen-poor substrates.

### **2.7.9 Carbon and Nutrients Availability**

Nutrients like carbon, nitrogen, phosphorus and sulphur are very important for the survival and growth of anaerobic microorganism. Insufficient amount of these nutrients and trace elements can cause inhibition and instability in anaerobic digestion process. The ideal carbon to nitrogen (C: N) ratio for anaerobic digestion ranges from approximately 20:1 to 30:1 (Sapkota et al.,2012).The optimal nutrient ratio for the carbon, nitrogen, phosphorous, and sulphur (C: N: P: S) is considered to be 600:15:5:1 (Seadi et al, 2008). It is also reported that to maintain optimum methanogenic activity, desirable liquid phase concentration of nitrogen, phosphorus and sulphur should be in the order of 50, 10 and 5 mg/l (Metcalf and Eddy, 2003). In addition, it is suggested that level for iron, cobalt, nickel and zinc should be 0.02, 0.004, 0.003 and 0.02 mg/g acetate produced respectively (Metcalf & Eddy, 2003).

### **2.7.10 Mixing**

Mixing in anaerobic digestion helps the animal/ plant manure to be in contact with a viable bacterial population.It improves the contact between the microorganisms and substrate which gives the bacterial population the ability to obtain nutrients. The benefits of mixing digester contents during anaerobic digestion process are to prevent scum formation inside the

digester, ensure uniform distribution of microorganisms and substrates throughout the mixture and intensifies contact between them, prevents stratification within the digester. This enables uniform distribution of heat throughout the mixture and release of gas. However, precautionary measures should be taken during this process as excessive mixing can disrupt the microbial activity (Chukwuma, Umeghalu & Orakwe, 2013)).

#### **2.7.11 Product Concentrations**

The stability of the anaerobic digestion process also depends on concentration of some products produced during the organic break-down process, like Volatile Fatty Acid (VFA). During the acidogenesis process, different fatty acids like acetate, propionate, butyrate, lactate etc. are produced. Excessive accumulation of these acids can drop the pH value inside the reactor when the buffering capacity of the digester is exhausted. The buffering capacity of the digesters and how they will react to certain amount of VFA concentration vary from digester to digester based on its microbial population (Seadi et al, 2008).

### **2.8 Anaerobic Configuration**

Anaerobic digesters can be designed and engineered to operate using a number of different configurations and can be categorized into batch vs. continuous process mode, mesophilic vs. thermophilic temperature conditions, high vs. low portion of solids, and single stage vs multistage processes. Higher heat energy is demanded in a thermophilic system compared to a mesophilic system and has a larger gas output capacity and higher methane gas content. For solids content, low will handle up to 15% solid content. Above this level is considered high solids content and can also be known as dry digestion. In a single stage process, one reactor houses the four anaerobic digestion steps. A multistage process utilizes two or more reactors for digestion to separate the methanogenesis and hydrolysis phases (Monnet, 2003).

### **2.8.1 Batch process**

Anaerobic digestion can be performed as a batch process or a continuous process. In a batch system, biomass is added to the reactor at the start of the process. The reactor is then sealed for the duration of the process. The feedstock digests and subsequently the whole system is emptied. In its simplest form batch processing needs inoculation with already processed material to start the anaerobic digestion. There can be severe odour issues if a batch reactor is opened and emptied before the process is well completed. As the batch digestion is simple and requires less equipment and lower levels of design work, it is typically a cheaper form of digestion. Using more than one batch reactor at a plant can ensure constant production of biogas (Shefali, 2002).

### **2.8.2 Continuous process**

In continuous digestion processes, organic matter is constantly added or added in stages to the reactor. The end products are constantly or periodically removed, resulting in constant production of biogas (Shefali, 2002).

### **2.8.3 Temperature**

The two conventional operational temperature levels for anaerobic digesters determine the species of methanogens in the digesters (Song, Kwon & Woo, 2004)

- a. Mesophilic digestion takes place optimally around 30 to 38 °C, or at ambient temperatures between 20 and 45 °C, where mesophiles are the primary microorganism present.
- b. Thermophilic digestion takes place optimally around 49 to 57 °C, or at elevated temperatures up to 70 °C, where thermophiles are the primary microorganisms present.

Mesophilic species outnumber thermophiles, and they are also more tolerant to changes in environmental conditions than thermophiles. Mesophilic systems are, therefore, considered to be

more stable than thermophilic digestion systems. In contrast, while thermophilic digestion systems are considered less stable, their energy input is higher, with more biogas being removed from the organic matter in an equal amount of time. The increased temperatures facilitate faster reaction rates, and thus faster gas yields. Operation at higher temperatures facilitates greater pathogen reduction of the digestate (Gohel, Ghosh & Braganza, 2013)

#### **2.8.4 Solids content**

Typical three different operational parameters are associated with the solids content of the feedstock to the digesters:

- a. High solids (dry—stackable substrate)
- b. High solids (wet—pumpable substrate)
- c. Low solids (wet—pumpable substrate)

High solids (dry) digesters are designed to process materials with a solid content between 25 and 40%. The primary styles of dry digesters are continuous vertical plug flow and batch tunnel horizontal digesters. Continuous vertical plug flow digesters are upright, cylindrical tanks where feedstock is continuously fed into the top of the digester, and flows downward by gravity during digestion. In batch tunnel digesters, the feedstock is deposited in tunnel-like chambers with a gas-tight door.

High solids (wet) digesters process thick slurry that requires more energy input to move and process the feedstock. High solids digesters will typically have a lower land requirement due to the lower volumes associated with the moisture. (Shefali, 2002)

Low solids (wet) digesters can transport material through the system using standard pumps that require significantly lower energy input. Low solids digesters require a larger amount of land than high solids due to the increased volumes associated with the increased liquid-to-feedstock

ratio of the digesters. There are benefits associated with operation in a liquid environment, as it enables more thorough circulation of materials and contact between the bacteria and their food. This enables the bacteria to more readily access the substances on which they are feeding, and increases the rate of gas production (Shefali, 2002).

### **2.8.5 Complexity**

Digestion systems can be configured with different levels of complexity. In a **single-stage digestion system** (one-stage), all of the biological reactions occur within a single, sealed reactor or holding tank. Using a single stage reduces construction costs, but results in less control of the reactions occurring within the system.

In a **two-stage digestion system** (multistage), different digestion vessels are optimised to bring maximum control over the bacterial communities living within the digesters.

Under typical circumstances, hydrolysis, acidogenesis and acetogenesis occur within the first reaction vessel. The organic material is then heated to the required operational temperature (either mesophilic or thermophilic) prior to being pumped into a methanogenic reactor. The initial hydrolysis or acidogenesis tanks prior to the methanogenic reactor can provide a buffer to the rate at which feedstock is added.

## **2.9 Biogas Utilization**

Biogas is mainly composed of methane and carbon dioxide, but also contains small amounts of hydrogen sulfide, ammonia, and traces of hydrogen, nitrogen, carbon monoxide, saturated or halogenated carbohydrates, and oxygen. Biogas is usually saturated with water vapor and may also contain particles and *siloxanes*. The energy content is determined by the methane content (1 kWh per m<sup>3</sup> of biogas with 10% of methane). The biogas can be used in as many applications as

the natural gas (heating, combined heat and power systems, fuel cells). There may be different specifications for biogas to be used in different applications, especially when biogas is to be used in stationary appliances or to be fed to a pipeline grid. The biogas needs purification to improve its quality in most cases (Amankwah, 2011). Hydrogen sulfide and its oxidation products are the major “contaminants” of the biogas (corrosive) and corrosion starts when the concentration of H<sub>2</sub>S is above 50 ppm and it reacts with most metals. Sulfur dioxide also lowers the dew point of the gas. Humidity should also be removed, because the presence of water favors the formation of sulfur oxidation products. Carbon dioxide must also be removed if the biogas has to meet natural gas specifications especially if biogas is to be used as a vehicle fuel, its methane content should be enriched until it becomes higher than 90% (Harasimowicz et al., 2007). Halogenated compounds (present in landfill biogas) and oxygen (due to air entrance when landfill biogas is collected) must be removed too (Jerger & Tsao, 2006).

**Table 2.1: Composition of Biogas**

<b>Components (Vol.)</b>	<b>%</b>	<b>Household Waste</b>	<b>Wastewater treatment plant sludge</b>	<b>Agricultural wastes</b>	<b>Waste of agro food industry</b>
<b>Methane (CH<sub>4</sub>)</b>	50-60		60-75	60-75	68
<b>Carbondioxide (CO<sub>2</sub>)</b>	38-34		33-19	33-19	26
<b>Nitrogen (N<sub>2</sub>)</b>	5-0		1-0	1-0	-
<b>Oxygen (O<sub>2</sub>)</b>	1-0		<0.5	<0.5	-
<b>Water vapour</b>	6(@40 <sup>0</sup> C)		6(@40 <sup>0</sup> C)	6(@40 <sup>0</sup> C)	6(@40 <sup>0</sup> C)
<b>Total</b>	100		100	100	100
<b>H<sub>2</sub>S (mg/m<sup>3</sup>)</b>	100-900		1000-4000	3000-10,000	400
<b>NH<sub>3</sub>mg/m<sup>3</sup></b>	-		-	50-100	-

(Amankwah, 2011)

The decomposition of organic matter in the absence of air could be elicited by the use of physical or chemical processes at high temperature and pressure, or the use of microorganisms at low temperature and atmospheric pressure. The preferred method to be used depends on the relative polluting impacts on the environment. However, despite the method used, gas is produced, but it can only be referred to as biogas if generated as a result of the action of microorganisms on the organic wastes (Amankwah, 2011).

## **2.10 Types of Digester**

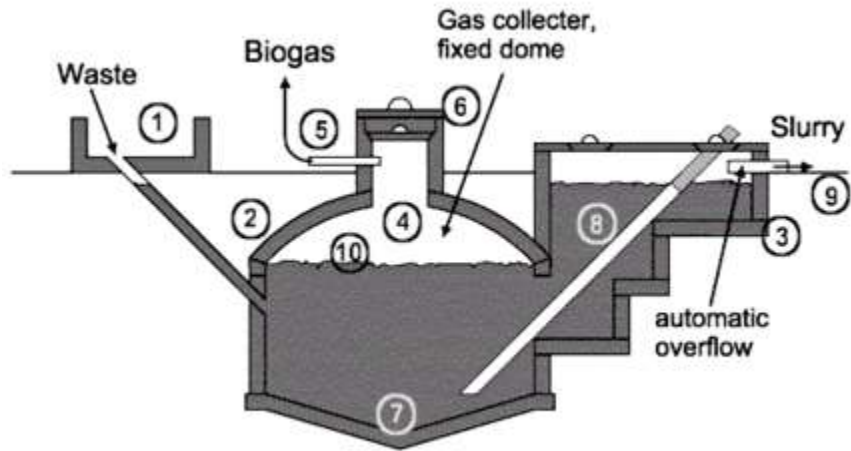
### **2.10.1 Balloon Plants**

The balloon plant consists of a bioreactor bag (e.g PVC) in the upper part of the plant. The in-let and out-let are attached directly to the plastic skin of the balloon. The gas pressure is achieved through the elasticity of the balloon and by added weights placed on the balloon (Anonymous, 2005).

### **2.10.2 Fixed-dome Plants**

A fixed-dome plant consists of a digester with a fixed, non-movable gas holder, which sits on top of the digester. When gas production starts, the slurry is displaced into the compensation tank. Gas pressure increases with the volume of gas stored and the height difference between the slurry level in the digester and the slurry level in the compensation tank. The costs of a fixed-dome biogas plant are relatively low. It is simple as no moving parts exist. There are also no rusting steel parts and hence a long life of the plant (20 years or more) can be expected. The plant is constructed underground, protecting it from physical damage and saving space. While the underground digester is protected from low temperatures at night and during cold seasons, sunshine and warm seasons take longer to heat up the digester. No day/night fluctuations of temperature in the digester positively influence the bacteriological processes. The construction of

fixed dome plants is labor-intensive, thus creating local employment (Chaudhary, Pathak & Fulekar, 2012).

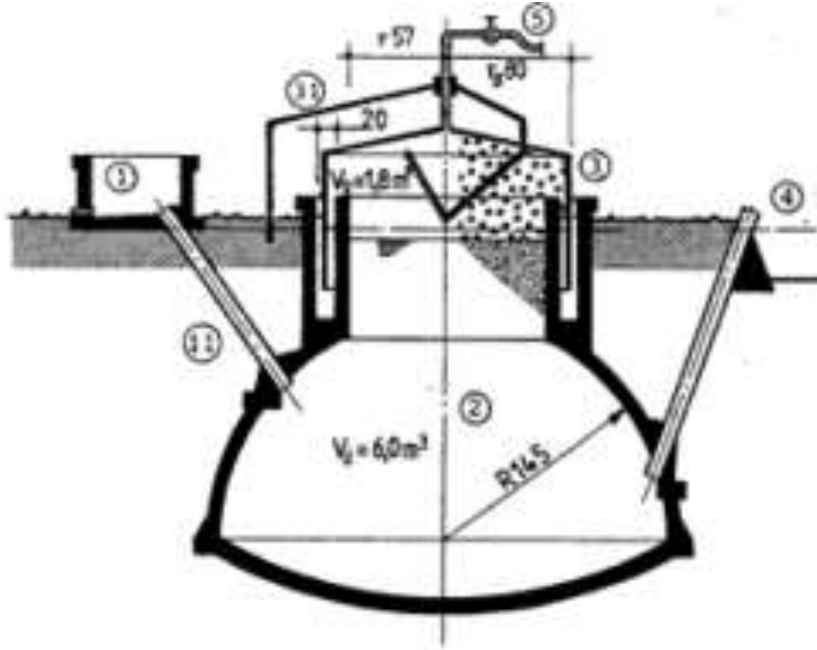


**Figure 2 2: Fixed dome plant Nicarao design (Chaudhary et al., 2012)**

1. Mixing tank with inlet pipe and sand trap.
2. Digesters.
3. Compensation and removal tank.
4. Gas holder.
5. Gas pipe.
6. Entry hatch, with gastight seal.
7. Accumulation of thick sludge.
8. Outlet pipe.
9. Reference level.
10. Supernatant scum, broken up by varying level.

### **2.10.3 Floating-drum Plants**

Floating-drum plants consist of an underground digester (cylindrical or dome-shaped) and a moving gas-holder. The gas-holder floats either directly on the fermentation slurry or in a water jacket of its own. The gas is collected in the gas drum, which rises or moves down, according to the amount of gas stored. The gas drum is prevented from tilting by a guiding frame. When biogas is produced, the drum moves up and when it is consumed, the drum goes down. If the drum floats in a water jacket, it cannot get stuck, even in substrate with high solid content (Nagamani & Ramasamy, 1999).



**Figure 2.3: Floating- drum plant (Chaudhary et al., 2012)**

1 Mixing pit, 11 Fill pipe, 2 Digester, 3 Gasholder, 31 Guide frame, 4 Slurry store

## **2.11 Purification/Upgrading Of Biogas**

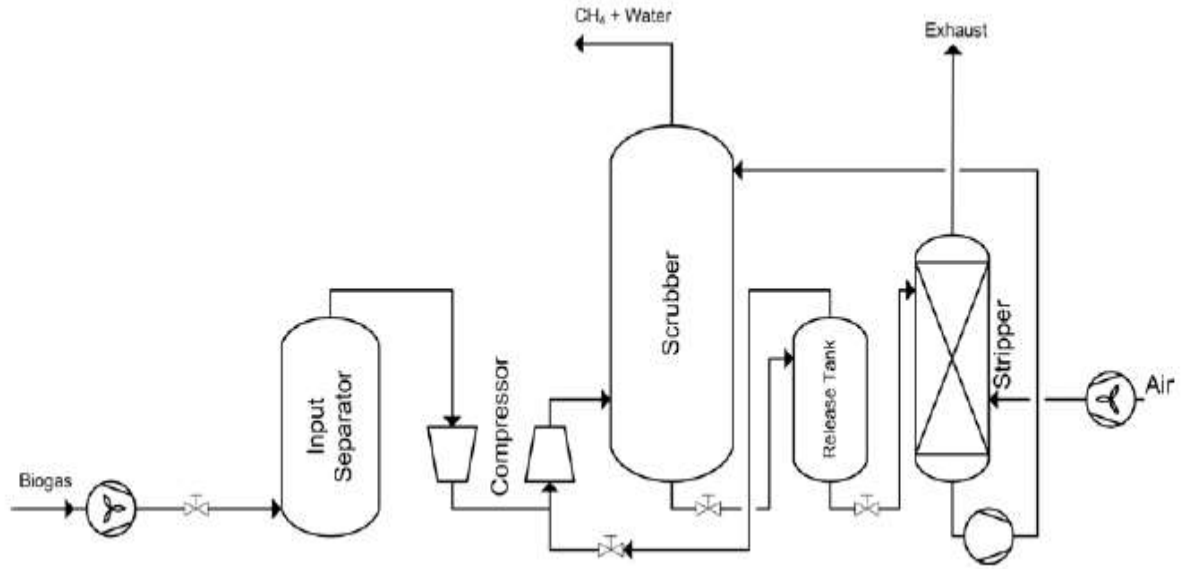
Raw biogas produced during anaerobic digestion is about 60% methane and 29% CO<sub>2</sub> with trace elements of H<sub>2</sub>S, hence there is need for purification of the gas before it can be used as fuel gas for machinery. This is because the corrosive nature of H<sub>2</sub>S destroys the plant.

The biogas produced should be upgraded or purified before use to remove the contaminants in the raw biogas stream and these can be absorbed or scrubbed, leaving more methane per unit volume of gas (Ray et al., 2014).

### **2.11.1 Water and Polyethylene Glycol Scrubbing**

Water scrubbing is used to remove CO<sub>2</sub> and H<sub>2</sub>S from biogas. This is because these gases are more soluble in water than methane. In this process, the biogas will be pressurized and fed to the bottom of a packed column. Water is then fed on the top to aid the absorption process. Then the water which goes out of the column with absorbed CO<sub>2</sub> and H<sub>2</sub>S or CO<sub>2</sub> or H<sub>2</sub>S can be regenerated and re-circulated back to the absorption column. Regeneration is accomplished by de-pressuring or by stripping with air in a similar column. (De Hullu, Maassen, Van Meel, Shazad & Vaessen, 2008).

Polyethylene glycol scrubbing is a physical absorption process that relies on the same mechanism as water scrubbing, because both CO<sub>2</sub> and H<sub>2</sub>S are more soluble than methane in the solvent e.g. Selexol. CO<sub>2</sub> and H<sub>2</sub>S are more soluble in Selexol than in water and water and halogenated hydrocarbons (contaminants in biogas from landfills) are removed when scrubbing biogas with Selexol (De Hullu et al. 2008).



**Figure 2.4: Water and Polyethylene Glycol Scrubbing Process (De Hullu et al. 2008)**

### **2.11.2 Chemical Absorption**

Chemical absorption involves formation of reversible chemical bonds between the solute and the solvent. Regeneration of the solvent involves breaking of these bonds by employing relatively high energy input. Chemical solvents generally employ either aqueous solutions of amines or alkaline salts.

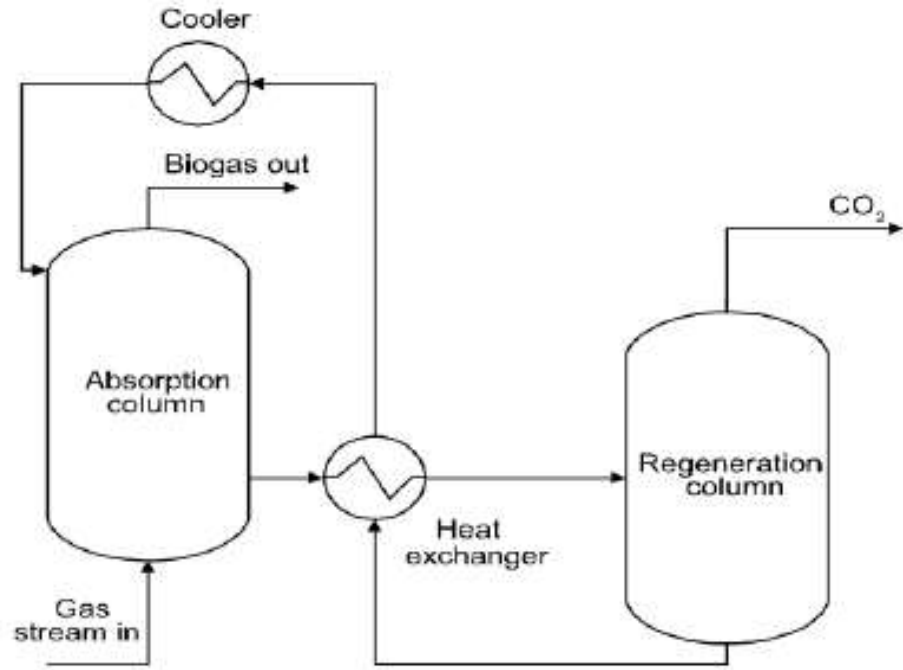


Figure 2.5: Chemical Absorption Process (De Hullu et al., 2008)

Bubbling biogas through a 10% aqueous solution of mono-ethanolamine reduces the CO<sub>2</sub> content of biogas by 0.5–1.0% by volume. This process is commonly used in industrial applications, including natural gas purification (Kim, Park, Kim, Lee, Kim, Kim & Lee 2004; Palmeri, Cavallaro & Bart, 2008).

### **2.11.3 Pressure Swing Adsorption**

Pressure Swing Adsorption (PSA) is a technology used to the separation of some gas from a mixture of gases. It operates at near-ambient temperatures. Special adsorptive materials (e.g., zeolites and active carbon) are used as a molecular sieve, preferentially adsorbing the target gas species at high pressure (Cavenati, Grande & Rodrigues, 2005).

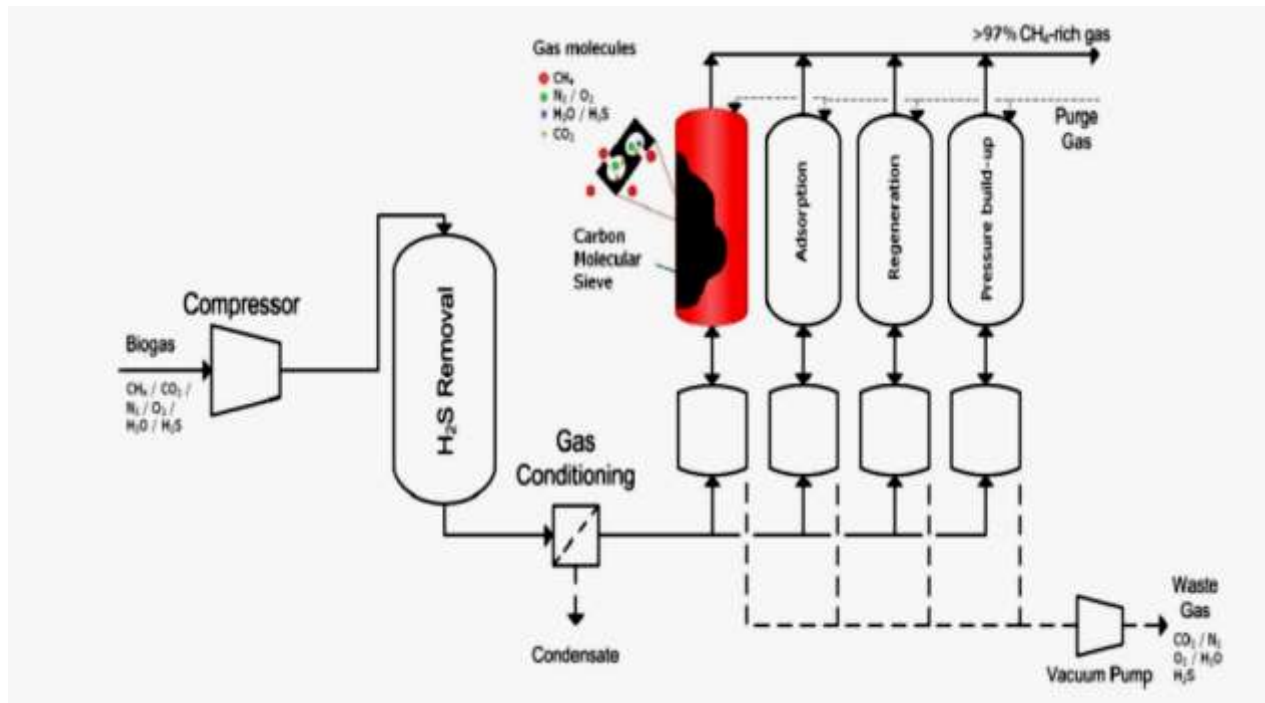


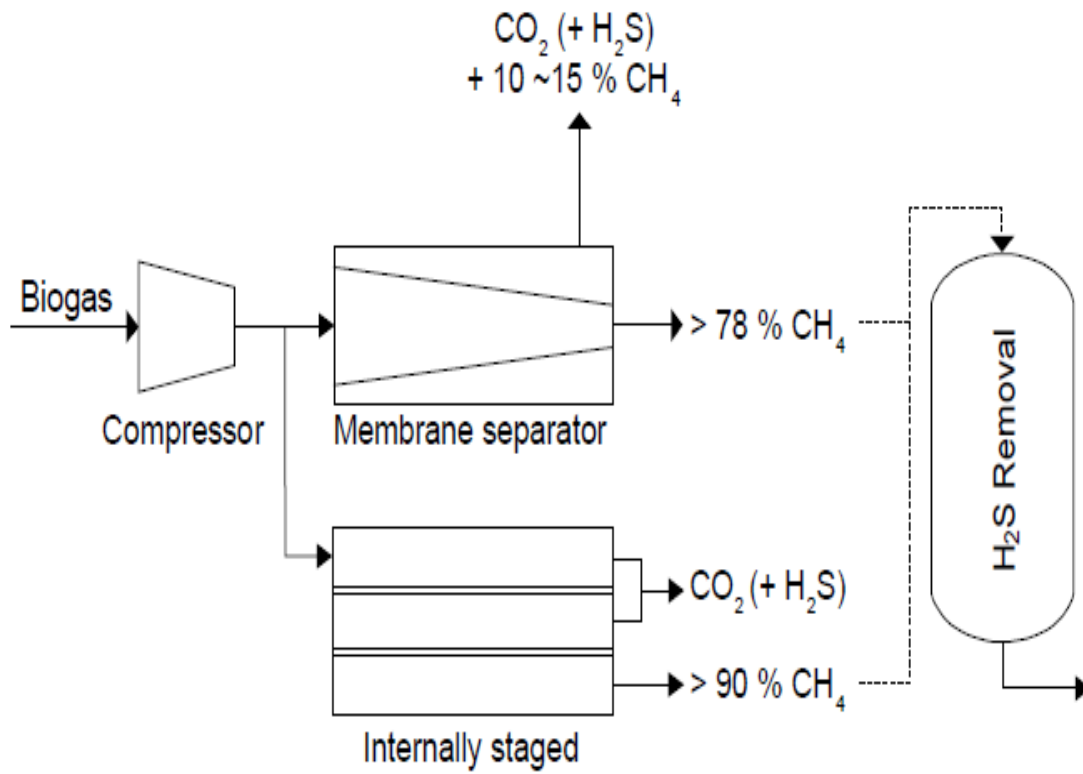
Figure 2.6: Pressure Swing Adsorption Process (De Hullu et al.,2008)

The PSA process relies on the fact that under pressure, gases tend to be attracted to solid surfaces or adsorbed. The higher the pressure, the more gas is adsorbed and when the pressure is reduced, the gas is released or desorbed. (Pipatmanomai, Kaewluan & Vitidsant, 2009)

PSA using zeolites or activated carbon at different pressure levels is an effective method for the separation of CO<sub>2</sub> from methane (Grande & Rodrigues, 2007; Pinto, Pires & Rocha, 2008). The reaction is best achieved at 7 to 8 bar (unit of pressure) and 50 to 70°C (De Hullu et al., 2008).

#### **2.11.4 Membrane Separation**

The principle of membrane separation is that some components of the raw gas are transported through a thin membrane while others are retained (De Hullu et al., 2008). The permeability is a direct function of the chemical solubility of the target component in the membrane. The purity of the upgraded gas can be improved by increasing the size or number of the membrane modules (De Hullu et al., 2008).



**Figure 2.7: Membrane Separation Process (De Hullu et al.,2008)**

There are two membrane separation techniques: high pressure gas separation and gas-liquid adsorption. The high pressure separation process selectively separates H<sub>2</sub>S and CO<sub>2</sub> from CH<sub>4</sub>. Usually, this separation is performed in three stages and produces 96% pure CH<sub>4</sub>. Gas liquid adsorption is a newly developed process that uses micro-porous hydrophobic membranes as an interface between gas and liquids. The CO<sub>2</sub> and H<sub>2</sub>S dissolve into the liquid while the methane is collected for use (Chatterjee, Houde & Stern, 1997). The advantages of membrane separation are that the process is compact, light in weight, has low energy and maintenance requirements and easy processing. The disadvantages of membrane separation are relatively low CH<sub>4</sub> yield and high membrane cost (De Hullu et al. (2008).

#### **2.11.5 Bio-filter**

Biological processes are used for H<sub>2</sub>S removal, mostly in biogas applications, because they are often economical and eco- friendly (Duan,Koe,Yan &Chen,2006; Van der Zee,Villaverde, Garcia&Fdz-Polanco, 2007). Chemotropic bacterial species (*Thiobacillus* genus) are used in this process.

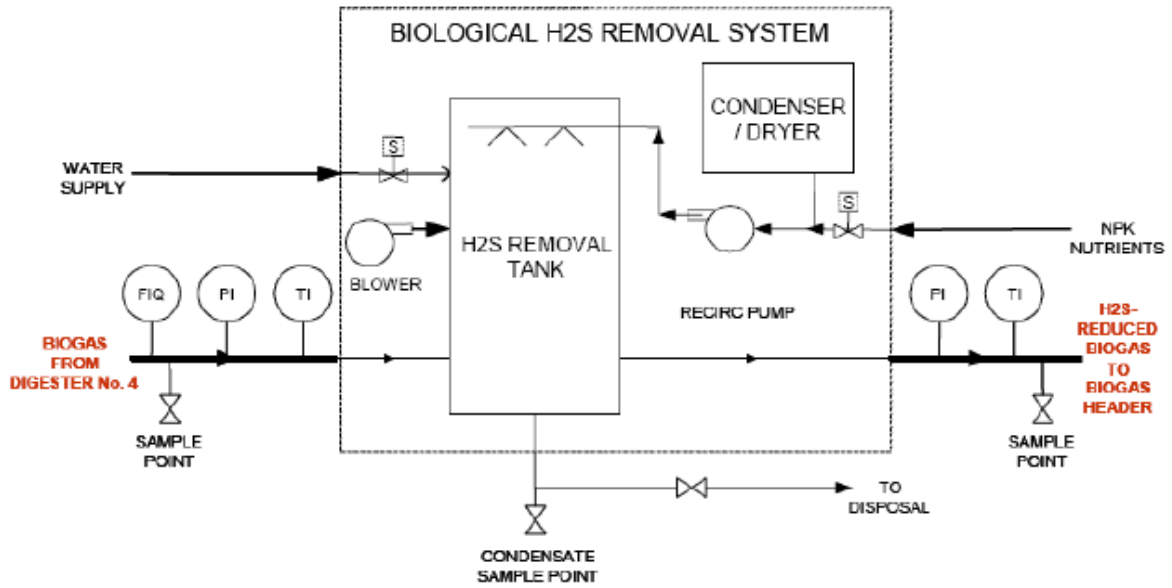
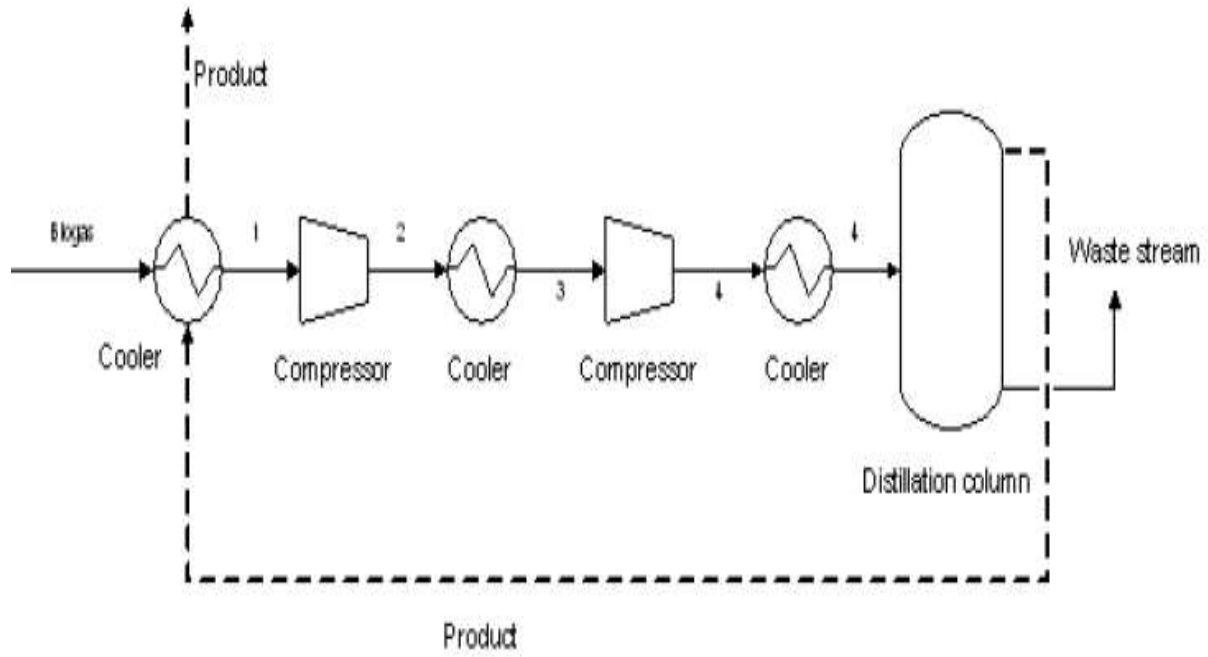


Figure 2.8: Bio-filter Process (De Hullu et al., 2008)

The biological methods require low energy and production of elemental sulfur as byproduct. This sulfur can be reused to produce sulfuric acid, hydrogen sulfide or agricultural applications (Kim, Kim, Chung & Xie, 2002; Vannini, Munz, Mori, Lubello, Verni & Petroni, 2008). Biological methods also have some disadvantages such as; additional nutrients are required for growing bacteria. Secondly a small amount of O<sub>2</sub> and N<sub>2</sub> are left in the treated biogas. The H<sub>2</sub>S removal efficiency depends on the activity of bacteria.

#### **2.11.6 Cryogenic Separation**

Cryogenic separation of biogas separates CO<sub>2</sub>, H<sub>2</sub>S and all other biogas contaminants from CH<sub>4</sub> based on the fact that each contaminant liquefies at a different temperature-pressure domain. This separation process operates at low temperatures, near -100°C, and at high pressures of about 40 bars using a linear series of compressors and heat changers.



**Figure 2.9: Cryogenic Separation Process (De Hullu et al., 2008)**

This heat exchanger makes use of the product stream as cooling medium, which is energy efficient and has the advantage of preheating the upgraded biogas before leaving the plant. The first cooling step is followed by a cascade of compressors and heat exchangers which cool the inlet gas down to  $-100^{\circ}\text{C}$  and compress it to 40 bars before it enters the distillation column. Finally, the distillation column separates  $\text{CH}_4$  from the other contaminants, mainly  $\text{H}_2\text{S}$  and  $\text{CO}_2$ . The main advantage of cryogenic separation is the high purity of the upgraded biogas (99%  $\text{CH}_4$ ), as well as the large quantities that can be efficiently processed. The main disadvantage of cryogenic separation is that cryogenic processes require the use of considerable process equipment, mainly compressors, turbines and heat exchangers (De Hullu et al., 2008).

#### **2.11.7 Selexol**

In the Selexol process, the Selexol solvent dissolves the acid gases from the feed gas at relatively high pressure, usually 300 to 2000 psi (2.07 to 13.8 MPa). The rich solvent containing the acid gases is then let down in pressure and steam stripped to release and recover the acid gases.

Selexol is a physical solvent, unlike amine based acid gas removal solvents that rely on a chemical reaction with the acid gases. Since no chemical reactions are involved, Selexol usually requires less energy than the amine based processes (De Hullu et al., 2008).

#### **2.11.8 Amine gas treater**

$\text{H}_2\text{S}$  and  $\text{CO}_2$  can be removed with this technology. The chemistry involved in the amine treating of such gases varies with the particular amine being used. For one of the more common amines, monoethanolamine (MEA) denoted as  $\text{RNH}_2$ , the chemistry may be expressed as:



A typical amine gas treating process includes an absorber unit and a regenerator unit. In the absorber, the downflowing amine solution absorbs H<sub>2</sub>S and CO<sub>2</sub> from the upflowing gas to produce a gas stream free of hydrogen sulfide and carbon dioxide as a product and an amine solution rich in the absorbed acid gases (De Hullu et al., 2008).

## 2.12 Benefits of Anaerobic Digestion

The benefits of anaerobic digestion technology can be classified broadly under the following: Social and Economic, Ecological, Health and Sanitary, and Environmental benefits.

The main objective of anaerobic treatment systems is usually waste stabilization or disposal. Environmental benefits are achieved by reducing bacterial contamination (ie. Destruction of pathogens) under correct operating conditions (hydraulic retention time, temperature, pH etc.) .Using anaerobic digestion technologies can help to reduce the emission of greenhouse gases in a number of key ways:

- Replacement of fossil fuels
- Reducing or eliminating the energy footprint of waste treatment plants.
- Reducing methane emission from landfills.
- Displacing industrially produced chemical fertilizers.

### 2.12.1 Power Generation

In developing countries, simple home and farm-based anaerobic digestion systems are used as the potential for low-cost energy for cooking and lighting. Methane and power produced in anaerobic digestion facility can be used to replace energy derived from fossil fuels, and hence

reduce emission of greenhouse gases, because the carbon in biodegradable material is part of a carbon cycle. Anaerobic digestion facilities have been recognized by the United National Development programme as one of the most useful decentralized sources of energy supply from 1975. China and India have government backed up schemes for adaptation of small biogas plants for use in the household for cooking and lighting. Biogas from sewage works is sometimes used to run a gas engine to produce electrical power. The biogas is useful as a fuel substitute for firewood, dung, agricultural residues, petrol, diesel and electricity depending on the nature of the task and local supply conditions and constraints. Biogas used as fuel gas can be burnt directly for cooking, heating and lighting or can be used for generating electrical and thermal energy used in the farm or supplied to the whole nation (Ray et al., 2014).

### **2.12.2 Waste Treatment**

Anaerobic digestion is particularly used in organic material, effluent and sewage treatment. Anaerobic digestion can greatly reduce the amount of organic matter which might otherwise be dumped at sea, landfills or burnt in incinerators. Pressure from environmentally related legislation on solid waste disposal methods in developed countries has increased the application of anaerobic digestion as a process for reducing waste volumes and generating useful by-products. It may either be used to process source-separated fraction of municipal waste or alternatively combined with mechanical sorting systems to process residual mixed municipal waste. (Ojolo et al., 2008).

### **2.12.3 Fertilizer and Soil Conditioner**

The solid, fibrous component of the digested material can be used as a soil conditioner to increase the organic content of soils. Digester liquor can be vital nutrients to soils instead of chemical fertilizer that require large amounts of energy to produce and transport. Anaerobic digestion increases the proportion of nutrients immediately available for uptake by plants. During the digestion process nutrients are mineralized, which allows improved plant uptake (Amankwah, 2011). For instance, digestate has 25% more accessible inorganic nitrogen ( $\text{NH}_4\text{-N}$ ) and a high pH value than untreated liquid manure, though some research suggests that in excess of 80% of the nitrogen in digestate can be available to plants depending on the mixture of slurries (eg. Cattle, pig, poultry etc.), the nutrient balance of digestate may be more balanced for agricultural application. Anaerobic digestion transforms organic bound nutrients to a mineral form which is readily available for crops. With a better nutrient balance and more accessible nutrients the requirement for artificial fertilizers may be lessened, which results in a cost saving to farmers (Diaz, Reyes, Ludi & Horvath, 2011).

### **2.12.4 Domestic Uses**

The primary domestic uses of biogas are cooking, heating and lighting, based on the fact biogas has different properties from other commonly used gases, such as propane and butane and is only available at low pressures (Anonymous, 2005).

### **2.12.5 Agricultural and Industrial Uses**

Biogas can be used as a fuel in stationary and mobile engines, to supply motive power, pump water, drive machinery (e.g. threshers, grinders) or generate electricity. It can be used in both spark and compression (diesel) engines. The spark ignition engine is easily modified to run on biogas by using a gas carburetor. (Ojolo et al., 2008).

### **2.12.6 Use of Biogas for Vehicle Fuel**

Biogas is suitable as a fuel for most purposes, without processing. If it is to be used to power vehicles, however, the presence of CO<sub>2</sub> is unsatisfactory, for a number of reasons it lowers the power output from the engine, takes up space in the storage cylinders (thereby reducing the range of the vehicle) and it can cause problems of freezing at valves and metering points, where the compressed gas expands during running, refueling, as well as in the compression and storage procedure. The biogas produced must be purified before use. (Mata-Alvarez et al., 2000)

### **2.12.7 Other Benefits of Anaerobic Digestion**

In addition to the benefits of energy recovery and displacement of greenhouse gas emissions from fossil fuels, anaerobic digestion produces several additional beneficial outcomes.

Anaerobic digestion destroys a wide range of pathogenic microorganisms. Biogas plants must be fitted with pasteurization/hygienisation unit of minimum treatment of 70<sup>0</sup>C for one hour. Such treatment will kill all pathogens and seeds, thereby eliminating cross-farm contamination of pathogens or weeds (Noyola & Tinajero, 2005).

Anaerobic digestion substantially reduces odours associated with animal slurries by as much as 80%, compounds associated with offensive odours including volatile fatty acids and molecules of mercaptans, are degraded into methane and carbondioxide by the anaerobic bacteria (Asikong et al., 2013)

Anaerobic digestion reduces the organic pollution potential of animal slurries. Tests of animal slurries from pilot and farm scale digesters show a reduction of 55% of BOD for cattle slurry, 75% for pigs and 80% for poultry slurries.

## CHAPTER THREE

### MATERIALS AND METHODS

#### 3.1 Sample Collection and Preparation:

##### 3.1.1 Palm Oil Mill Effluent (POME)

POME was collected with clean plastic containers from a palm oil mill industry at Umuagwo in Ohaji-Egbema LGA, Imo State. The sample was allowed to sediment and then filtered to remove the debris.

##### 3.1.2 Pig Dung

The pig dung was collected with clean bags from Agric. Farm of Federal University of Technology Owerri in Owerri West L.G.A, Imo State. The sample was sun dried until it was sufficiently dry. After drying, the sample was milled to finely reduced particle size at Feed Mill Company in Owerri, sieved and subsequently stored in air-tight polythene bags for preservation.

##### 3.1.3 Cow Dung

Cow dung was collected with clean bags from an abattoir near 34 Artillery brigade Obinze Owerri West L.G.A, Imo State. It was sun dried until it was sufficiently dried. After drying the sample was milled to a finely reduced particle size at a Feed Mill Company in Owerri, sieved and subsequently stored in airtight polythene bags for preservation.

##### 3.1.4 Poultry Manure

The poultry manure were collected with clean bags from the Agric. Farm of Federal University of Technology Owerri in Owerri West L.G.A, Imo State. It was sun dried until it was sufficiently dry. After drying the samples, were milled to a finely reduced particle size at a Feed Mill Company in Owerri, sieved and subsequently stored in airtight polythene bags for preservation.

### **3.1.5 Cabbage waste**

The cabbage waste was collected with clean bags from Onitsha road fruit market in Owerri, Imo State. The sample was sun dried until it was sufficiently dry. After drying the sample was milled to finely reduced particle size at a feed mill company in Owerri, sieved and subsequently stored in air tight polythene bags for preservation.

### **3.1.6 Cassava Peels**

The cassava peels were collected with clean bags from local farmers in Umuagwo, Ohaji L.G.A, Imo State, who are into cassava processing to produce garri. The samples were sun dried until they were sufficiently dry. After drying the samples were milled to finely reduced size at a Feed Mill Company in Owerri, sieved and subsequently stored in air tight polythene bags for preservation.

### **3.2 Cow Rumen Liquor (Inoculum)**

Cow rumen waste was used as the inoculum to stabilize the wastes. The fresh cow rumen waste was collected with clean air tight container to preserve the anaerobic microbes immediately after slaughtering the cow. The cow rumen waste was collected from an abattoir near 34 Artillery Brigade, Obinze Owerri West L.G.A and it was filtered with cheesecloth, then the liquor was stored in the air-tight container to maintain the anaerobic condition required by the methanogens needed for methane production.

### **3.3 Proximate Analysis of the Bioreactor Feeds**

The proximate analysis of the substrates; Pig dung, Palm oil mill effluent (POME), Cow dung, Poultry droppings, Cassava peels and Cabbage were evaluated. The moisture content, ash, total solid, volatile solid, crude fat, crude fibre, crude protein, carbon: nitrogen ratio etc. were determined using AOAC method of 2012.

Other physico-chemical analysis of POME carried out were pH, percentage carbohydrate, protein, total N<sub>2</sub>, BOD, COD, Metals like Phosphorus, Potassium, Magnesium, Calcium, Iron and Sodium (AOAC, 2012).

**3.3.1 Determination of Moisture Content of the Substrates:**

Three porcelain crucibles were pre-heated at 105<sup>0</sup>C and cooled in desiccators to ensure that they are moisture free, and it was weighed, (W<sub>1</sub> each). Five gram of the prepared (unfermented) sample was thinly spread in each of the porcelain crucibles and weighed rapidly, W<sub>2</sub> each. The porcelain crucibles and their contents were heated in an oven at 105<sup>0</sup>C for 3 hours and allowed to cool in a desiccator to a constant weight, W<sub>3</sub> each. The heating and cooling of the loaded porcelain crucibles were repeated 3 times, until a constant weight, W<sub>3</sub> was obtained. Average weight was calculated at the end of each step. The percentage by weight of the moisture content of the samples was evaluated as follows:-

$$\text{Moisture content (\%)} = \frac{W_2 - W_3}{5.0g} \times 100 \dots\dots\dots (\text{equa. 1})$$

Where:

W<sub>2</sub> = Average weight of the porcelain crucibles and their contents before heating

W<sub>3</sub> = Average weight of the porcelain crucibles and their contents after heating.

**3.3.2 Determination of Ash content of the substrates:**

Three (3) porcelain crucibles were pre-heated in a muffle furnace to 550<sup>0</sup>C. The pre-heated porcelain crucibles were allowed to cool and weighed, W<sub>1</sub> each. Five gram 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  $550^{\circ}\text{C}$ . The porcelain crucibles and their contents were maintained at this temperature (i.e  $550^{\circ}\text{C}$ ) for 8 hours. The heating was stopped and the loaded porcelain crucibles were allowed to cool there in the muffle furnace, after which they were transferred to desiccators and weighed,  $W_3$  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:

$$\text{Ash (\%)} = \frac{W_3 - W_1}{5.0g} \times 100 \dots\dots\dots (\text{equa. 2})$$

Where:

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

$W_1$  = Average weight of the porcelain crucibles alone.

**3.3.3 Determination of Organic Matter Content of the Substrates: (Volatile solid)**

This was estimated by subtracting the average weights of the moisture and ash earlier obtained 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:

$$\text{Volatile solid (\%)} = 100\% - [\text{moisture (\%)} + \text{ash (\%)}] \dots\dots\dots (\text{equat. 3})$$

**3.4 Bioreactor Design and Construction**

The bioreactors were constructed with plastic containers (10L capacity) and connected to 13L transparent buckets and 3L transparent buckets inverted in it which served as the gas collectors.

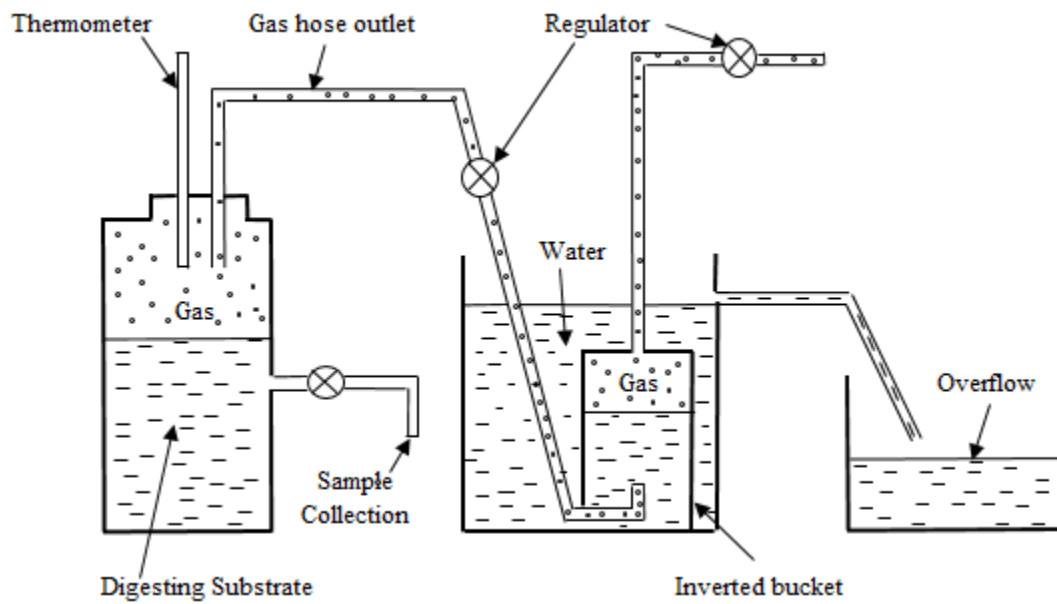
Downward water displacement method was used for the gas collection. This was achieved by measuring and recording the quantity of water displaced due to the pressure exerted by production of gas on daily basis from the buckets.

The bioreactors were made up of 153 transparent plastic containers of 10L capacity. The cover/cap of the 10L containers were drilled using a hot soldering iron to make holes, for the passage of  $\frac{1}{4}$  inch gas hose which serves as a channel for gas outlet from the digester. This was held firmly using the 3-ton epoxy glue, which was applied at both top and under the cover/cap, sealing up every possible aperture that might allow the entrance of air into the anaerobic digester. Proper care was taken to ensure that the glue does not cover the mesh which allows the cover fit in tightly to the mouth of the digester when covered. An opening, wide enough to allow the end of the  $\frac{1}{4}$  inch hose from the digester to pass through, was made by the side of the 3L bucket. Also, small holes were bored by the side of the 3L bucket to allow excess gases pass out into the surrounding environment when the 3L bucket gets filled up with water to reduce pressure. After these holes have been drilled, a small portion of the  $\frac{1}{4}$  inch hose was fitted through the drilled holes at the centre of the 3L bucket held firmly by 3-ton epoxy glue. Then the 3L was inverted in the 13L bucket with its three (3) holes on its flap in perfect alignment with those at the base of the 13L bucket. Nuts and bolts were then used to screw the two buckets together. The other end of the  $\frac{1}{4}$  inch hose attached to the 3L bucket was fixed to the gas regulator. Holes were also made on the cover of the 13L bucket to allow both the passage of  $\frac{1}{4}$  inch hose from the digester and the  $\frac{1}{4}$  inch hose from the 3L gas collector bucket. A hole was drilled by the side of the 13L bucket and a short hose was attached and held firmly by epoxy glue for collection of displaced water into a small bowl. The above set up was applied in the construction of all the digesters used in the study. This was done in triplicate as shown in figure 3.1.

Bioreactor design, experimental set-up and operation were carried out as described by Opurum, Nweke, Nwanyanwu & Nwachukwu, (2015).



**Plate 3.1, Bioreactor Design (Opurum et al., 2015)**



**Figure 3.1: Schematic Diagram of the bioreactor set-up**

Source: Opurum et al. (2015)

The gas collection was by downward water displacement method (Opurum et al., 2015; Aragaw et al 2013). The quantity of water displaced from the 13L buckets were proportional to the volume of biogas produced after 24hrs. The 13L buckets were refilled after biogas reading every 24hrs. The pH values during the hydraulic retention time were monitored with digital pH meter. The pH meter with electrodes were calibrated using buffer solutions (pH4 and pH7) before pH reading was taken. (Opurum et al., 2015).

### **3.5 Microbiological Analysis**

The samples for microbiological analysis were collected in sterile bottles immediately after the digesters were set up for charging. The analysis was carried out to determine and estimate the microbial load present in the slurry.

#### **3.5.1 Preparation of media and diluents**

Nutrient agar (NA), Eosin Methylene Blue Agar (EMBA), MacConkey Agar (MCA), Potato Dextrose Agar (PDA), *Salmonella-Shigella* Agar (SSA), Mannitol salt agar, Centrimide agar (CA) and Sabouraud dextrose agar (SDA) were prepared as specified by the manufacturers. Nutrient agar (NA), was used for the isolation of heterotrophic bacteria, Potato Dextrose Agar and Sabouraud dextrose agar (SDA), for isolation of total fungi, *Salmonella Shigella* Agar (SSA) was used for the isolation of *Salmonella* and *Shigella*, Centrimide agar (CA) was used for *Pseudomonas aeruginosa*, Mannitol salt agar (MSA) was used for *Staphylococcus* sp., while Eosine methylene blue agar (EMBA), and MacConkey (MCA) were used for selecting *Escherichia coli* and other related coliforms respectively.

Physiological saline used as diluents was prepared by dissolving 9.8g of sodium chloride in 1000ml of distilled and dispensed into 9ml portions. Both diluents and media were sterilized in an autoclave at 121<sup>0</sup>C for 15mins (Cheesbrough, 2003).

### **3.5.2 Preparation of samples and inoculation**

One milliliter of the sample transferred into 9 ml of the diluents and serially diluted until  $10^6$  dilution was obtained. Aliquot portion (0.1ml) of appropriate diluted sample was inoculated into the sterile and surface dried medium. The inocula were spread evenly to ensure that the colonies are uniform and countable. Inoculated plates were incubated at ambient temperature for 48 hours to isolate heterotrophic bacteria (Bergey's Manual of Determinative Bacteriology, 1994).

### **3.5.3 Determination of microbial population**

Colony forming units per gram (CFU/g) were used to express the colony counts on the media to obtain total population (Cappucino & Sherman, 1981).

### **3.5.4 Characterization and identification of microbial isolates**

Cultural (colonial), microscopic and biochemical methods were used in the characterization and identification of all the microbial isolates with reference to standard manuals as described by Cheesbrough (2003). The isolates were cross-matched based on their identities with reference to standard manuals for the identification of bacteria (Bergey's Manual of Determinative Bacteriology, 1994).

### **3.5.5. Microscopic characterisation**

#### **3.5.5.1 Gram staining**

The Gram staining technique was used for the bacterial isolates as described by Cheesbrough (2003). A smear of the isolate was made on grease free glass slide with a drop of water and allowed to dry. The smear was fixed by mild heating, flooded with crystal violet and allowed to stand for 60 seconds. The crystal violet was rinsed off with water for 5 seconds; Lugol's iodine was flooded and allowed to stand for 60 seconds. This was washed off with water and

decolourized with alcohol for 10-30 seconds. It was counter stained with Safranin for 30 seconds and rinsed with water. The wet slide was allowed to air dry. A drop of oil immersion was added on the slide and viewed using X100 objective lens of the microscope.

### **3.5.5.2 Sporestaining**

The spore stain was used to confirm the presence of spores when indicated in the Gram stain. Isolates were heat fixed on a slide and flooded with 5% malachite green. It was steamed for 3 minutes (without allowing it to boil), dried and cooled. It was then rinsed off and stained with Safranin for 30 seconds. This was rinsed, dried with filter paper and viewed under the microscope using oil immersion lens. The positive spores showed green while the vegetative cells were stained pink.

### **3.5.5.3 Motility test**

It is used to determine the motility of bacteria isolated. A semi-solid agar medium was used to carry out the test and it was observed that motile bacteria swam and gave a diffuse spreading growth. The medium was dispensed into test tubes, sterilized and allowed for some time in an upright position. An inoculation needle was used to inoculate it by stabbing it into the test tube with the medium. Then it was incubated at 37°C for 24 hours. Positive result was recorded as a diffuse growth from the straight line of inoculation.

### **3.5.6 Biochemical characterization of bacterial isolates**

Although the presumptive identification of colonies was made using their morphological character and colour standard, other tests were performed in order to characterize and identify the bacterial isolates. In these test, only fresh bacteria between 18hrs-24hrs were used.

Microorganisms that were not identified by the colonial and microscopic characteristics were further subjected to few biochemical tests described by Cheesbrough (2003).

#### **3.5.6.1 Catalase test**

Catalase activity was detected by adding the substrate  $H_2O_2$  to an appropriately incubated (18-24 hours) tryptic soy agar slant culture. Organisms which produce the enzyme breakdown the hydrogen and the resulting  $O_2$  production produces bubbles in the reagent drop indicating a positive test. Organisms lacking the cytochrome system also lack the catalase enzyme and are unable to breakdown peroxide into  $O_2$  and water and are catalase negative.

#### **3.5.6.2 Coagulase test**

In the test, the sample was added to rabbit plasma and held at  $37^\circ C$  for a specified period of time. Formation of clot within four hours is indicated as positive result and indicative of a virulent *Staphylococcus aureus* strain. The absence of coagulation after 24 hours of incubation is a negative result indicative of an avirulent strain.

#### **3.5.6.3 Oxidase test**

The test was carried out by impregnating strips of filter paper with 1% Kovac's oxidase reagent. The paper was smeared with the bacterial colonies to be tested by a glass rod. In a positive result, the smeared area on the filter paper turns deep purple within 10 seconds and no colour change in negative result.

#### **3.5.6.4 Sugar fermentation/oxidation**

This test is used to differentiate between bacterial groups that oxidize carbohydrate such as members of Enterobacteriaceae. One milliliter (1ml) of 10% glucose, maltose, lactose, fructose, mannitol, and sucrose were separately under aseptic conditions transferred into duplicate tubes

containing 9ml of sterile Hugh and Leifson's medium to obtain a final concentration of 1% of each of sugar. The tubes were stab-inoculated in duplicates while two uninoculated tubes serve as control. Vaseline was used to cover one set of the duplicate tubes and one control tube to discourage oxidative utilization of sugar. All tubes were incubated at 37°C for 48h. After the incubation, they were observed for acid production in the culture. Yellow colouration indicates acid production in the open tubes only, suggesting oxidative utilization of the sugar, while acid production in the sealed tubes suggests a fermentative reaction.

#### **3.5.6.5 Hydrogen sulphide production (H<sub>2</sub>S) test**

The test isolates were aseptically inoculated into a tube containing triple sugar iron agar started by stabbing the agar to the bottom and streaking the surface of the slant. The inoculated tube was incubated at 37°C for 72h and was examined daily. Black precipitation and yellow colouration was checked for. Black precipitate indicates H<sub>2</sub>S production and yellow colouration indicates sucrose, lactose and glucose fermentation.

#### **3.5.6.6 Urease test**

This test is used to determine whether an organism has the enzyme urease which breaks down urea. The bacterial isolate was inoculated into urease agar slant in McCartney bottle at 30°C for 4 hours and then incubated overnight. A change in colour of the medium to a pink colour indicates a positive result.

#### **3.5.6.7 IMViC TEST**

This test consists of four different test; they are Indole production, Methyl-Red test, Voges Proskauer test and Citrate utilization test. They are specifically useful in the differentiation of Gram-negative intestinal bacilli, particularly *Escherichia coli* and the *Enterobacter-Klebsiella* group.

### **3.5.6.8 Indole test**

This test demonstrates the ability of certain bacteria to decompose the amino acid-Tryptophan to Indole. The bacteria isolates were inoculated into the medium and incubated at 37°C for 48 hours. At the end of incubation period, 3 drops of kovac's reagents (see appendix) was added and then shaken. A red colour ring at the interface of the medium denotes a positive result.

### **3.5.6.9 Methyl red and Voges-Proskauer test**

Methyl red and Voges-Proskauer test must be considered together since they are physiologically related. Opposite test is usually obtained from the MR and VP test, that is, MR+, VP-, or MR-, VP+.

Methyl red test was performed to demonstrate the capacity of different organisms to produce acid from the fermentation of sugar (dextrose). Methyl-red positive organisms produce a red colouration when five drops of methyl-red indicator is added into 48h old MR-VP broth culture.

The Voges-Proskauer test demonstrates the ability of organisms to produce acetoin from glucose metabolism. Some organisms metabolise glucose to produce pyruvic acid which is further broken down to yield Butane-diol and acetyl-methyl carbinol as an intermediate product.

One milliliter of six percent alcoholic solution of alpha-naphthol and one milliliter of 16% KOH were added into one milliliter of the culture and stood for 15-20 minutes. Development of red to pink colour showed a positive test.

### **3.5.6.10 Citrate utilization test**

It is one of the techniques used to identify Enterobacteria. Simmon's citrate agar is used to carry out the test and the principle is based on the ability of an organism to utilize citrate as its only source of carbon.

Bijou bottles were used to prepare the slopes as recommended by the manufacturers. Then sterile straight wire was used to inoculate on the slope with a saline suspension of the test organisms. The bottles were incubated at 35°C for 48 hrs. A bright blue colouration of the medium indicates a positive result while no change indicates negative citrate test (Cheesbrough, 2003).

### **3.5.7 Identification of Fungi**

Yeasts were isolated, characterized and identified based on cultural and colonial morphologies, microscopic characteristics and their ability to ferment different sugars. Moulds were identified on the basis of spores and hyphal arrangement and pigmentation. Microscopically moulds were identified by spore and conidia arrangement. The identities of organisms isolated were cross matched with identification manual of Barnnet and Hunter (1990).

### **3.6 Experimental Set-Up/ Anaerobic Digestion of the Substrates.**

A total of one hundred and fifty three digesters were used in the anaerobic digestion of the substrates at three different ratios of the substrates (1:1, 2:1 and 3:1), but the same pH range (6.8-8.0) and Hydraulic Retention Time (45 days). Sodium Hydroxide (NaOH) and Hydrogen Chloride (HCL) were used in the adjustment of the pH on daily basis.

#### **3.6.1 Preliminary Study**

From literature so many researchers used 45g, 55g, 65g and 75g bioreactor dilutions in biogas production and it was observed that 65g/L dilution gave the optimum biogas yield and it was adopted. Hence three different substrate: water ratio (S: W) bioreactor dilutions were used in this study to ascertain the best to be used. These were 65g/L, 75g/L and 85g/L and 20% (20ml in 100ml) cow rumen liquor was equally used as an inoculum. Then 10L capacity bioreactors were used in the main work (8L bioreactor content and 2L space), therefore the 8L was used to multiply 65, 75 and 85 to obtained 520g, 600g and 680g respectively of each solid substrate used

in the study. Furthermore, to obtain the quantity of cow rumen to be used as the inoculum, 8L was been divided by 20ml in 100ml cow rumen to obtain 1.6L cow rumen used in the study.

### **3.6.2 Anaerobic digestion of POME as a single substrate and co-digestion of POME with pig dung, cow dung, poultry manure, cassava peels and cabbage waste.**

In this study, 520g, 600g and 680g of each of pig dung, cow dung, poultry manure, cassava peels and cabbage waste were separately weighed with an electronic balance and mixed thoroughly with 3litres of POME and 3.4litres of water for optimum gas production. Also 3L of POME was equally measured out separately as control. Up to 1.6litres of fresh cow rumen liquor was used as the inoculum to pitch the loaded substrates in the digesters. Each of the experiment was designed in triplicate to ensure that the biogas production was not by chance occurrence.

The digesters were covered and tightened to exclude oxygen while the hose from the digester's outlet was connected to the inverted water-filled transparent bucket and they were labelled appropriately. The digesters were shaken on a daily bases to avoid stratification of the substrates and ensure thorough mixing of the contents of the digesters while maintaining intimate contact between the microorganisms and substrates to enhance complete anaerobic digestion of the substrates. The volumes of biogas produced were measured and recorded on daily basis at 24hrs interval. The pH of the slurry was taken and adjusted on daily basis and the gas production/flammability test was also carried out on daily basis. Biogas analysis using Gas Chromatography was carried out immediately the gas started flammimg. The experiment was monitored for 45days (hydraulic retention time).

### **3.6.3 Anaerobic digestion of pig dung as a single substrate and co-digestion of pig dung with cow dung, poultry manure and cabbage waste.**

Three different ratios (1:1, 2:1 and 3:1) each of pig dung with cow dung, pig dung with poultry manure and pig dung with cabbage waste were separately weighed out with an electronic balance, then 520g of pig dung (Control) was equally weighed out separately. Each digester was properly mixed with 6.4litres of water and 1.6litres of fresh cow rumen liquor for optimum gas production. Each of the experiment was designed in triplicate to ensure that the biogas production was not by chance occurrence. The experiment was carried out as stated above.

### **3.6.4 Anaerobic digestion of cassava peels as a single substrate and co-digestion of cassava peels with pig dung, cow dung and poultry manure.**

Three different ratios (1:1, 2:1 and 3:1) each of cassava peels with pig dung, cassava peels with cow dung and cassava peels with poultry manure were separately weighed out as well as 520g of cassava peels (Control) was equally weighed out. Each digester was properly mixed with 6.4litres of water and 1.6litres of fresh cow rumen liquor for optimum gas production. Each of the experiment was designed in triplicate to ensure that the biogas production was not by chance occurrence. The experiment was equally carried out as stated above.

### 3.6.5 Design of optimization of biogas:

Table 3.1: Design table in uncoded units

StdOrder	RunOrder	PtType	Blocks	pH	Substrate	HRT
11	1	2	1	7	1	45
6	2	2	1	9	2	15
2	3	2	1	9	1	30
3	4	2	1	5	3	30
13	5	0	1	7	2	30
1	6	2	1	5	1	30
5	7	2	1	5	2	15
12	8	2	1	7	3	45
8	9	2	1	9	2	45
4	10	2	1	9	3	30
9	11	2	1	7	1	15
10	12	2	1	7	3	15
14	13	0	1	7	2	30
15	14	0	1	7	2	30
7	15	2	1	5	2	45

Box Behnken's design of response surface methodology of three factors at three levels was used for the optimization of the substrate combination from the co-digested substrates above, with the highest biogas production. The three factors were Substrate Ratio, pH and Hydraulic Retention Time. The three levels were substrate ratio (1:1, 2:1 and 3:1), pH (5, 7 and 9), hydraulic retention time (15days, 30day and 45days). The digesters were charged with values obtained from the design. From the design above, 15 digesters were used at three different ratios, three different pH values and three different hydraulic retention times. The bioreactors were also set up and charged as earlier described and the necessary parameters were monitored. Compositional gas analysis of biogas produced was carried out using Gas Chromatography (GC).

### 3.7 Gas Chromatography Analysis

Biogas composition was determined using Gas Chromatography-flame ionization detector (AOAC, 2000). The GC used in this study was produced from Buck Scientific (M910USA) and equipped with FID detector and capillary and capillary column (Elite -5, 30 m X 0.25 $\mu$ m). The workstation was Total Chrom Navigator used for data processing. The temperature of column chamber, inlet chamber and detector were 150°C, 200°C and 250°C, respectively. High purity nitrogen was used as carrier gas in this study, and the flow rate for nitrogen was 2.0ml/min. The split ratio of gas sample in inlet chamber was 20:1 which is used to control the amount of biogas that enters into the column, and to prevent the unconventional peak, such as flat peak, trailing peak. The flow rate was 450ml/min for air produced by automatic air source (BCHPSPB - 300China) and 45ml/min for hydrogen produced by hydrogen gas.

The percentage of each gas produced (%) =

$$\frac{\text{Concentration of each gas produced (ppm)}}{\text{Total concentration of the gas produced (ppm)}} \times \frac{100}{1}$$

### **3.8 Optimization of biogas with the predicted values.**

Co-digestion of Cassava peels with Cow dung had the highest biogas yield and was used for the optimization of biogas. Cassava peels was co-digested with cow dung using the predicted optimal values from the surface plot of responses to verify the experimented result and these values were; pH - 6.3737, substrate ratio – 2.7962 and hydraulic retention time – 15days.

### **3.9 Analysis of Data**

Analysis of variance (ANOVA) was used in the analysis of the cumulative biogas yield in all the treatments and control data generated. Then the means of maximum cumulative biogas production in the treatments were compared using the Post Hoc Duncan test implemented in IBM SPSS version 20.0 Statistics software.

## CHAPTER FOUR

### RESULTS AND DISCUSSION

#### 4.1 Characteristics / physicochemical properties of the bioreactor feeds.

The proximate analysis of palm oil mill effluent (POME), pig dung (PD), cow dung (CD), poultry manure (PM), cassava peels (CP) and cabbage waste (CB) were evaluated to determine the availability of nutrients in the feedstock that can be accessed and digested by the microorganisms in the course of the anaerobic digestion and biogas production. The crude fat, crude fibre, crude protein, carbon: nitrogen ratio, ash content, moisture content, organic carbon, total solid, total volatile solid, crude nitrogen, percentage carbohydrate. Also pH, COD, BOD and POME metallic ions were determined. The results are shown on Table 4.1. This was carried out prior to the anaerobic digestion process because adequate physico-chemical properties of wastes are very necessary for effective biogas production. POME has a Carbon to Nitrogen (C/N) ratio of 10:1, PD 6:1, CD 18:1, PM 14:1, CP 46:1, CB 17:1. It was observed that cassava peels had a higher C/N ratio than all other substrates. It was also observed that none of the substrate has an optimum C/N ratio, hence it is imperative to co-digest the substrates to balance the nutrients and enhance biogas yield.

**Table 4.1: Proximate/ Physico-chemical Characteristics of all the Substrates used for biogas production**

<b>Parameters</b>	<b>Substrates</b>					
	POME	Pig Dung	Cow Dung	Poultry Manure	Cassava Peels	Cabbage waste
<b>Fat content (%)</b>	8.23	11.39	5.43	6.98	3.94	3.83
<b>Fibre content (%)</b>	-	7.20	7.58	10.40	11.07	13.26
<b>Ash content (%)</b>	0.54	34.73	31.31	12.50	6.77	23.81
<b>Crude Protein (%)</b>	3.25	22.09	6.34	9.68	6.778	14.759
<b>Organic carbon content (%)</b>	48.90	20.61	17.96	22.05	49.87	39.54
<b>C: N (%)</b>	10.13	5.84	17.50	14.24	46	16.90
<b>Crude Nitrogen (%)</b>	5.12	3.53	1.02	1.55	1.084	2.361
<b>Moisture content (%)</b>	86.08	15.45	5.62	10.74	11.48	11.15
<b>Carbohydrate (%)</b>	1.90	9.14	43.72	49.70	-	-
<b>Total Volatile Solids (%)</b>	13.39	49.82	63.07	76.76	81.75	65.04
<b>Total Solids (%)</b>	13.93	84.55	94.38	89.26	88.52	88.85
<b>pH</b>	4.06	7.8	6.5	6.9	5.91	6.84

**Table 4.2: Further Physico-chemical analysis of POME metallicions in mg/100g:**

<b>POME metallic ions</b>	<b>Values (mg/100g)</b>
<b>Potassium</b>	23.91
<b>Calcium</b>	249.0
<b>Sodium</b>	28.19
<b>Magnesium</b>	19.0
<b>Iron</b>	0.82
<b>Phosphorus</b>	15.30
<b>BOD (mg/L)</b>	1664.40
<b>COD (mg/L)</b>	4580

## **4.2 Microbiological Analysis of all the Substrates.**

In this study, eleven (11) bacterial species were isolated from the digesting slurry. These were *Bacillus*, *Pseudomonas*, *Enterococcus*, *Staphylococcus*, *Micrococcus*, *Erwinia*, *Vibrio*, *Escherichia coli*, *Enterobacter*, *Salmonella* and *Shigella* species (Table 4.3). Also isolated were five fungal species; *Saccharomyces*, *Aspergillus*, *Rhizopus*, *Penicillium*, *Geotrichum* species (Table 4.4).

**Table 4.3: Characteristics and identification of bacterial isolates from all the substrates**

<b>Colony code</b>	<b>Colonial characteristics</b>	<b>Microscopic morphology</b>	<b>CAT</b>	<b>OXI</b>	<b>COG</b>	<b>IN</b>	<b>MR</b>	<b>VP</b>	<b>CIT</b>	<b>URS</b>	<b>NO<sub>3</sub></b>	<b>GIT</b>	<b>SUC</b>	<b>LAC</b>	<b>MAL</b>	<b>TRE</b>	<b>Identity of isolates</b>
<b>POME/CP</b>	Circular pink colonies with black eye fish in SSA	Short rods in cluster	+	-	-	-	+	-	+	+	+	+	-	-	+	+	<i>Salmonella</i> sp
<b>POME/CP, POME/CB PD/ PM, CP/ PD, CP /PM, CP/ CD</b>	Moist bluish green colonies in NA	Gram negative rods slender, a few in pairs, others in singles	+	+	-	-	-	+	+	+	+	+	+	-	+	+	<i>Pseudomonas</i> sp
<b>POME/PM, POME/CP, POME/CB, PD/ PM</b>	Moist and shiny smooth light pink colonies in EMBA	Gram negative rod	+	-	-	-	+	+	+	-	-	+	-	+	-	-	<i>Enterobacter</i> sp

<b>POME/PM,</b>	Smooth moist and	Gram positive	+	-	-	-	-	-	-	+	+	+	+	+	+	-	<i>Staphylococcus sp</i>
<b>POME/CP,</b>	shiny golden	cocci in clusters															
<b>POME/CB,</b>	yellow colonies in																
<b>POME/CD, PD</b>	NA																
<b>/PM, CP/ CD, CP/</b>																	
<b>PD, PD/ CD, CP/</b>																	
<b>PM, PD/ CB</b>																	
<b>POME/PD,</b>	Dull and dry	Rods in chains and	+	-	-	-	-	+	+	-	+	+	-	-	-	-	<i>Bacillus sp</i>
<b>POME/CD,</b>	serrated flat cream	few in singles.															
<b>POME/ PM,</b>	colony in NA																
<b>POME/CP,</b>																	
<b>POME/CB, PD/</b>																	
<b>PM, PD/ CB,</b>																	
<b>CP/PD, CP/ CD,</b>																	
<b>CP/ PM</b>																	
<b>POME/PD,</b>	Small circular and	Gram positive	-	-	-	-	+	-	+	-	-	+	+	+	-	-	<i>Enterococcus sp</i>



<b>PD/ CD, PD/ PM,</b>	Moist and dull	Gram negative rod	+	+	+	+	+	+	+	-	-	-	-	+	-	+	<b><i>Vibrio sp</i></b>	
<b>CP/ PD, CP/ CD</b>	creamy colony in NA																	
<b>POME/CP,</b>	Purple greenish	Gram negative rod	+	-	-	+	-	+	-	-	+	+	+	+	+	+	<b><i>Escherichia coli</i></b>	
<b>POME/CB, CP/</b>	metallic sheen in																	
<b>PM, CP/ CD</b>	EMBA																	
<b>POME/PD,</b>	Large circular	Gram positive	+	-	-	-	-	+	-	+	+	+	+	+	+	-	<b><i>Staphylococcus sp.</i></b>	
<b>POME/CD,</b>	moist and shiny	cocci,																
<b>POME/PM, CP/</b>	yellow colonies in	predominantly in																
<b>PD, PD/ CB, CP/</b>	MSA	clusters, few in																
<b>CD</b>		pairs and tetrads																
<b>POME/PD,</b>	Large moist and	Small Gram	+	+	-	-	-	+	+	+	+	+	+	+	-	+	+	<b><i>Pseudomonas sp.</i></b>
<b>POME/PM CP/</b>	shiny cream	negative short																
<b>CD, CP/ PD, PD/</b>	colonies in	rods in singles and																
<b>CB, CP/ PM</b>	Centrimide Agar	short chains																

**Key:**

POME= palm oil mill effluent, PD= pig dung, CD= cow dung, PM= poultry manure, CP= cassava peels, CB= cabbage waste.

+ = Positive

- = Negative

CAT= catalase; OXI= oxidase; COAG= coagulase; IN= indole; MR=Methyl Red; VP= Voges Proskauer, CIT= citrate, URS= urease production; NO<sub>3</sub>= nitrate reduction; GLU= glucose; SUC= sucrose; LAC= lactose; MAL= maltose; XYL= xylose

**Table 4.4: Colonial and microscopic characteristics of fungal isolates**

<b>Colony code</b>	<b>Colonial characteristics</b>	<b>Microscopic morphology</b>	<b>Identity of isolates</b>
<b>POME/PM, POME/CP, POME/CB,PD/CB, CP/PM, CP/CD</b>	Circular moist and shiny cream colonies in PDA. Small smooth regular moist and shiny cream colonies with a characteristic fermented smell in SDA	+Spherical Budding cells	<b><i>Saccharomyces sp.</i></b>
<b>POME/CP, POME/CB, CP/PM, CP/CD</b>	Black spores on short mycelium on PDA	Septate hyphae conidia globosed	<b><i>Aspergillus sp.</i></b>
<b>POME/CP,</b>	Tall filamentous hyphae with orange spores at the tip in PDA	Non septate hyphae. Sporangiospore enclosed in a sporangium	<b><i>Rhizopus sp.</i></b>
<b>POME/CP</b>	Thick dirty green spores enclosed in white mycelium in PDA	Septate hyphae, conidia mop head	<b><i>Penicillium sp.</i></b>
<b>POME/CB</b>	White cotton wool-like hyphae in PDA	Septate hyphae and conidia rectangular fork-like	<b><i>Geotrichum sp.</i></b>

**Key:** POME= palm oil mill effluent, PD= pig dung, CD= cow dung, PM= poultry manure, CP= cassava peels, CB= cabbage waste.

### **4.3 Anaerobic digestion and biogas production from POME as a single substrate and co-digestion of POME with pig dung, cow dung, poultry manure, cassava peels and cabbage waste.**

POME was anaerobically digested as a single substrate at ambient temperature for 45 days and this served as the control for other experimental studies. Then it was co-digested in triplicate with pig dung (POME/PD), cow dung (POME/CD), poultry manure (POME/PM), cassava peels (POME/CP) and cabbage waste (POME/CB) at the ratios of 3L: 520g, 3L: 600g and 3L: 680g. The co-digestion of POME/CD, POME/PD, POME/PM, POME/CP and POME/CB in the ratio of 3L: 520g, 3L: 600g, 3L: 680g respectively had higher cumulative biogas yield of 8.035dm<sup>3</sup>, 10.344dm<sup>3</sup>, 7.570dm<sup>3</sup>; 6.529dm<sup>3</sup>, 6.171dm<sup>3</sup>, 9.306dm<sup>3</sup>; 4.601dm<sup>3</sup>, 6.462dm<sup>3</sup>, 7.995dm<sup>3</sup> ; 7.082dm<sup>3</sup>, 5.182dm<sup>3</sup>, 9.055dm<sup>3</sup> and 9.127dm<sup>3</sup>, 9.284dm<sup>3</sup>, 8.332dm<sup>3</sup> respectively than POME alone that had cumulative biogas yield of 4.635dm<sup>3</sup> (Table 4.5). The result has proved that co-digestion of these substrates were able to enhance biogas production.

The bioreactor co-digested with POME/PD, 3L: 520g, 3L: 600g and 3L: 680g ratios started biogas production on the 1<sup>st</sup> day. Flammability test indicated that 3L:680g ratio started on the 3<sup>rd</sup> day while 3L: 520g ratio started on the 4<sup>th</sup> day then 3L: 600g ratio started on the 5<sup>th</sup> day. POME/PD (3L: 680g) which started biogas production on the 1<sup>st</sup> day, started producing flammable gas on the 3<sup>rd</sup> day and the peak recorded on the 4<sup>th</sup> day with biogas yield of 1.679dm<sup>3</sup> and cumulative biogas yield of 9.306dm<sup>3</sup> as shown in Table 4.5.

The bioreactor co-digested with POME/CD, 3L: 520g and 3L: 600g ratios started biogas production on the 1<sup>st</sup> day while 3L: 680g ratio started on the 2<sup>nd</sup> day. Flammability test indicated that 3L: 600g ratio started on the 2<sup>nd</sup> day while 3L: 520g and 3L: 680g ratios flammable biogas production started on the 3<sup>rd</sup> day. POME/CD (3L: 600g) started biogas production on the 1<sup>st</sup> day,

flammable gas production on the 2<sup>nd</sup> day and the peak recorded on the 6<sup>th</sup> day with biogas yield of 2.216dm<sup>3</sup> and cumulative biogas yield of 10.344dm<sup>3</sup> as shown in Table 4.5.

The bioreactor co-digested with POME/PM, 3L: 520g biogas production started on the 2<sup>nd</sup> day, while 3L: 600g and 3L: 680g ratios biogas productions started on the 1<sup>st</sup> day. Flammability test indicated that for 3L: 680g, flammable biogas production started on the 33<sup>rd</sup> day, while 3L: 520g and 3L: 600g ratios did not produce flammable biogas. POME/PM (3L: 680g) which started biogas production on the 1<sup>st</sup> day, recorded the peak on the 3<sup>rd</sup> day with biogas yield of 1.196dm<sup>3</sup> and cumulative biogas yield of 7.995dm<sup>3</sup> as shown in Table 4.5.

The bioreactor co-digested with POME/CP 3L: 520g, 3L: 600g and 3L: 680g ratios biogas productions started on the 2<sup>nd</sup> day. Flammability test indicated that 3L: 680g started on the 11<sup>th</sup> day, 3L: 520g ratio on the 13<sup>th</sup> day and 3L: 600g on the 14<sup>th</sup> day. POME/CP 3L: 680g started biogas production on the 2<sup>nd</sup> day and flammable gas on the 11<sup>th</sup> day, recorded the peak of gas production on the 13<sup>th</sup> day with biogas yield of 1.89dm<sup>3</sup> and cumulative biogas yield of 9.055dm<sup>3</sup> as shown in Table 4.5.

The bioreactor co-digested with POME/CB, 3L: 520g, 3L: 600g and 3L: 680g biogas productions started on the 2<sup>nd</sup> day. Flammability test indicated that 3L: 600g ratio flammable biogas production started on the 5<sup>th</sup> day while 3L: 520g and 3L: 680g flammable biogas started on the 15<sup>th</sup> day. POME/CB 3L: 600g had the highest biogas yield as well as the percentage biomethane content. The biogas production started on the 2<sup>nd</sup> day, recorded the peak of gas production on the 11<sup>th</sup> day with biogas yield of 1.55dm<sup>3</sup> and cumulative biogas yield of 9.28dm<sup>3</sup> as shown in Table 4.5.

The bioreactor with POME alone (control), started its biogas production on the 2<sup>nd</sup> day, flammable biogas production on the 4<sup>th</sup> day and the peak was observed on the 5<sup>th</sup> day with biogas

yield of 1.21dm<sup>3</sup> and cumulative biogas yield of 4.64dm<sup>3</sup> which was recorded as one of the lowest biogas yield obtained in the research work as shown in Table 4.5.

#### **4.3.1 Gas Chromatograms of produced biogas**

The percentage biomethane contents obtained in the study were 77.89% for POME/CD (3L: 600g), 76.66% for POME/PD (3L: 680g), 44.14% for POME/PM (3L: 680g), 68.80% for POME/CB (3L: 600g), 65.28% for POME/CP (3L: 680g) and 56.53% for POME (control). From this study, the mixing ratio of POME/CD proved to be the most suitable mixture to enhance biogas production as well as biomethane content (Table 4.6).

#### **4.3.2 Statistical Analysis**

Post-Hoc Duncan test was used to determine the means of maximum cumulative biogas yield and it was observed that POME/CD 3L: 600g is significantly different from every other treatment, POME/PM 520g and POME alone are significantly lower while other treatments are not statistically different from each other. Also there is significant difference between POME alone and all other treatments except POME/PM 520g, POME/CP 520g and POME/CP 600g.

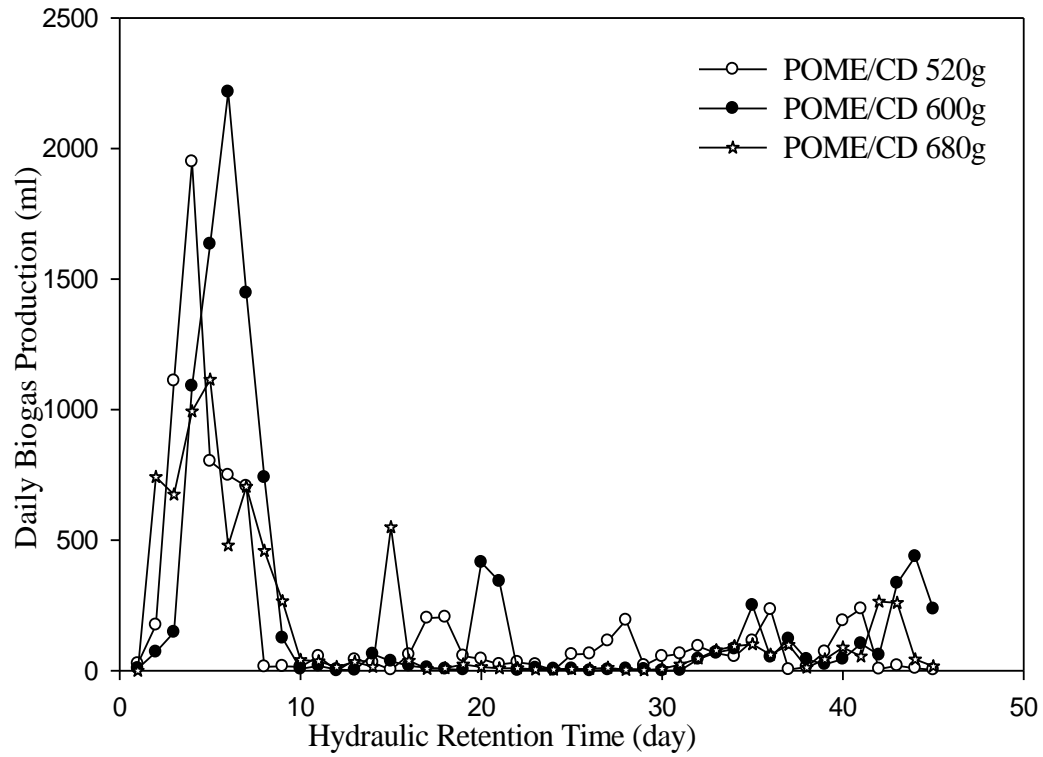
**Table 4.5: Cumulative biogas yield from the different substrate ratios (dm<sup>3</sup>) from POME co-digested with other substrates.**

Treatment	3L:520g	3L:600g	3L:680g
<b>POME/CD</b>	8.035	10.344	7.570
<b>POME/PD</b>	6.529	6.171	9.306
<b>POME/PM</b>	4.601	6.462	7.995
<b>POME/CP</b>	7.082	5.182	9.055
<b>POME/CB</b>	9.127	9.284	8.332
<b>POME control</b>	4.635		

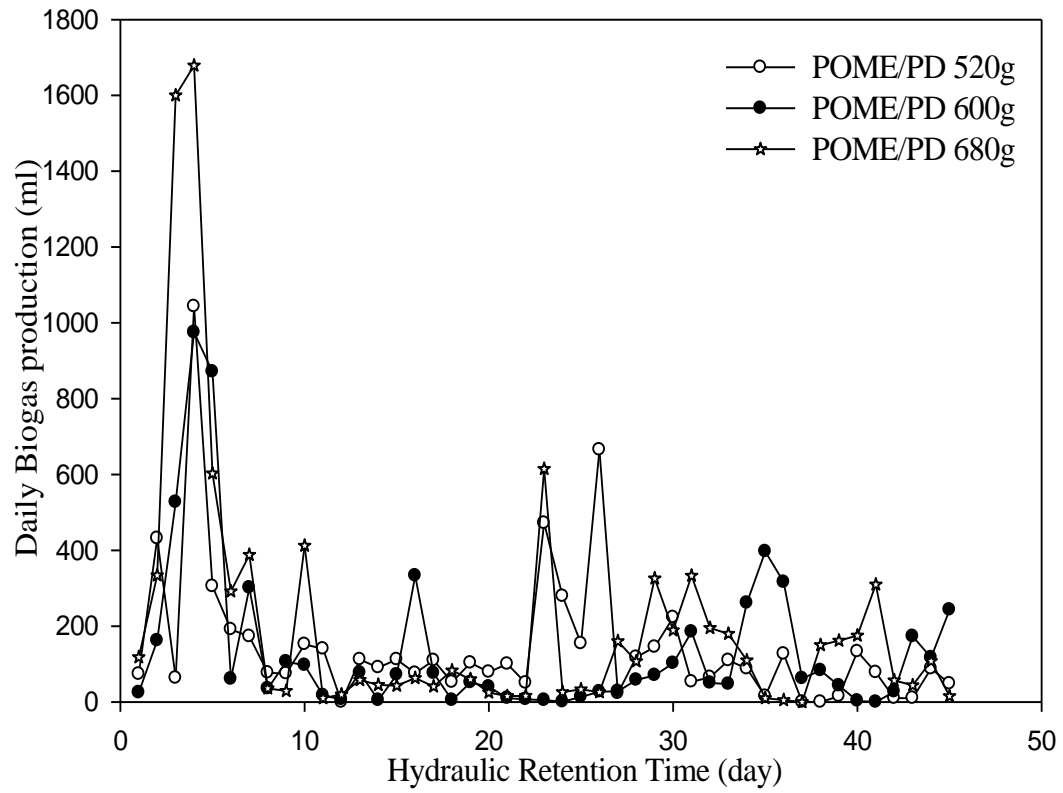
**Table 4.6: Percentage biogas composition of bioreactors with the highest biomethane yield.**

Component	POME/ CD3L: 600g		POME/ PD 3L:680g		POME/ PM 3L: 680g		POME/ CP 3L: 680g		POME/ CB 3L: 600g		POME control	
	Ppm	% composition	Ppm	% composition	Ppm	% compositio n	Ppm	% compositi on	Ppm	% composition	ppm	% composition
<b>Ethylene</b>	-	-	-	-	0.0164	0.224286	-	-	-	-	-	-
<b>Carbondioxide</b>	3.4740	13.34128	3.4762	15.73646	2.8374	38.80417	2.0896	8.759075	2.0896	9.500039	5.4645	30.9169
<b>Methanol</b>	0.6004	2.340221	0.6938	3.140773	-	-	3.4865	14.61453	1.8263	8.302986	1.3374	7.5667
<b>Acetic acid</b>	-	-	-	-	0.9949	13.60621	0.9120	3.822874	0.7737	3.517506	-	-
<b>Methane</b>	19.9844	77.89488	16.9337	76.65742	3.2273	44.13643	15.5738	65.28143	15.5738	68.7997	9.9928	56.5370
<b>Phenol</b>	1.0450	4.073169	0.9489	4.295589	-	-	0.3456	1.448668	0.978	4.446324	0.8000	4.5262
<b>Carbonmonoxide</b>	0.5518	2.150789	0.0375	0.169759	0.2361	3.228895	1.4489	6.073423	1.4213	6.461718	0.0801	0.45319
<b>Ethanol</b>	-	-	-	-	-	-	-	-	-	-	-	-
<b>Total</b>	25.6556		22.0901		7.3121		23.8564		22.6627		17.6748	

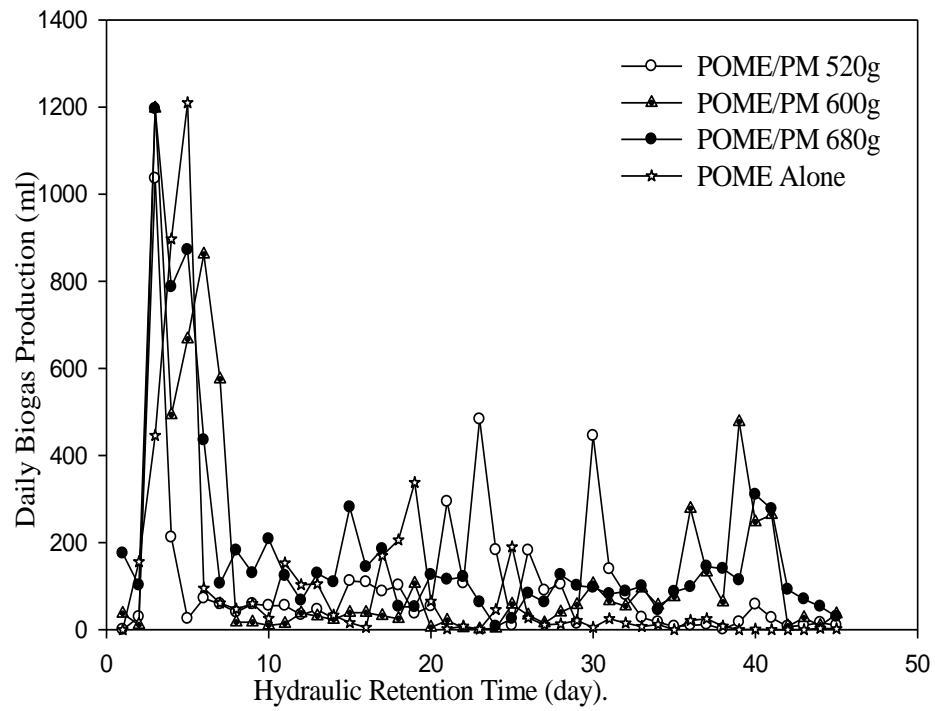
The mean values of biogas produced were plotted against the hydraulic retention time (HRT) as shown in Figures 4.1-4.5.



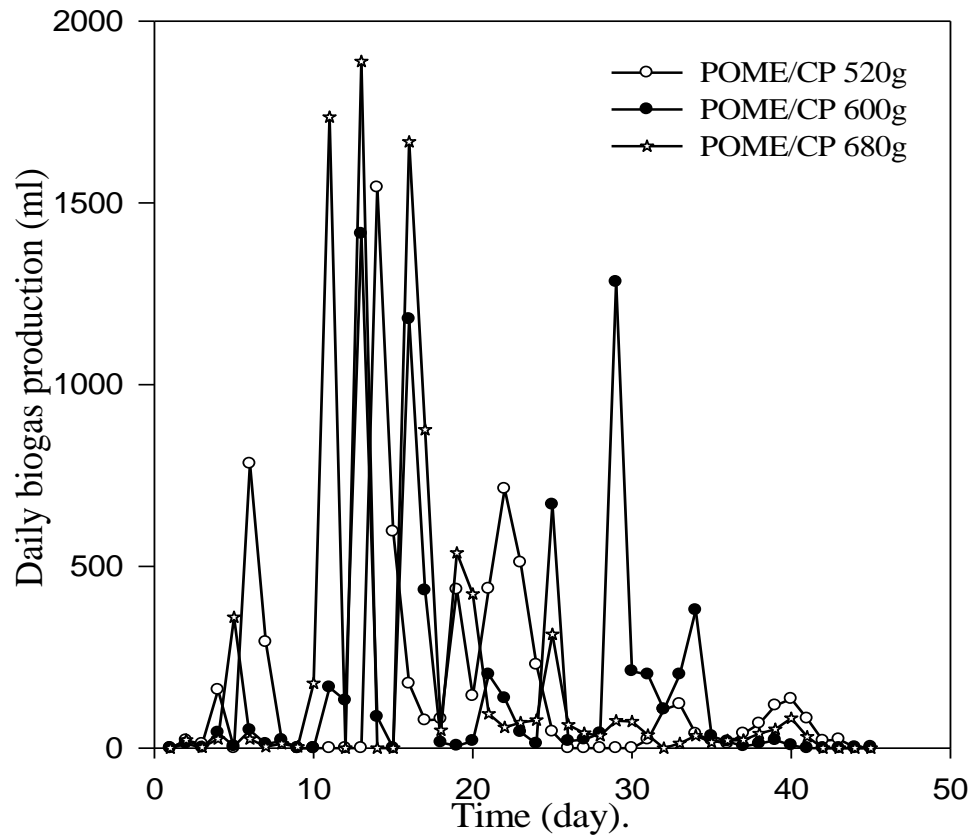
**Figure 4.1:** Profile of daily biogas production from mixtures of POME/CD.



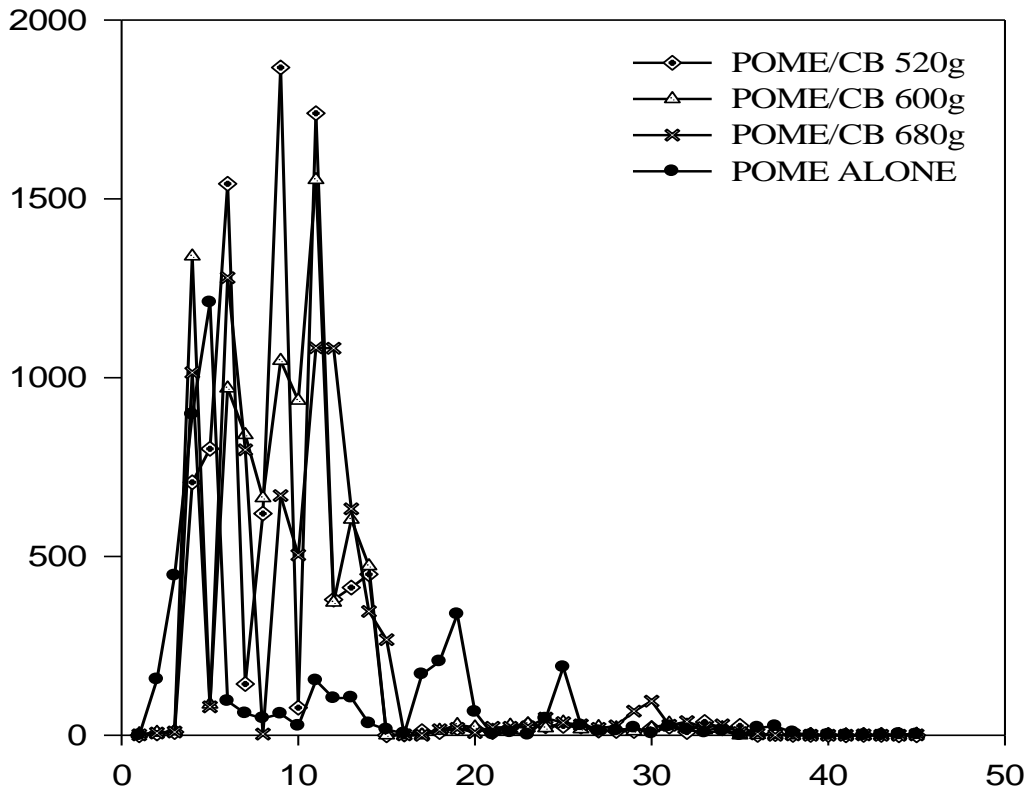
**Figure 4.2:** Profile of daily biogas production from mixtures of POME/PD.



**Figure 4.3:** Profile of daily biogas production from mixtures of POME/PM and POME alone.



**Figure 4.4:** Profile of daily biogas production from mixtures of POME/CP.



**Figure 4.5:** Profile of daily biogas production from mixtures of POME/CB and POME alone.

#### **4.4 Anaerobic digestion and biogas production from pig dung as a single substrate and co-digestion of pig dung with cow dung, poultry manure and cabbage waste.**

Pig dung was anaerobically digested as a single substrate at ambient temperature for 45 days and this served as the control for other experimental studies. Then it was co-digested in triplicate with cow dung (PD/CD), poultry manure (PD/PM) and cabbage waste (PD/CB) at the ratios of 1:1, 2:1 and 3:1. The co-digestion of PD/CD, PD/PM and PD/CB in the ratio of 1:1, 2:1, 3:1 respectively had cumulative biogas yield of 5.777dm<sup>3</sup>, 1.591dm<sup>3</sup>, 1.792dm<sup>3</sup>; 1.189dm<sup>3</sup>, 0.862dm<sup>3</sup>, 4.417dm<sup>3</sup> and 4.794dm<sup>3</sup>, 0.838dm<sup>3</sup>, 4.316dm<sup>3</sup> respectively, then pig dung alone had cumulative biogas yield of 3.37dm<sup>3</sup> (Table 4.7).

The bioreactor co-digested with PD/CD, 1:1 ratio started biogas production on the 2<sup>nd</sup> day. Flammability test indicated that 1:1 ratio started on the 18<sup>th</sup> day while 2:1 and 3:1 ratios did not produce flammable biogas. PD/CD 1:1 which started biogas production on the 2<sup>nd</sup> day, flammable biogas production on the 18<sup>th</sup> day, recorded the peak on the 20<sup>th</sup> day with biogas yield of 7.85dm<sup>3</sup> and cumulative biogas yield of 5.777dm<sup>3</sup> as shown in Table 4.7.

The bioreactor co-digested with PD/PM, 3:1 ratio started biogas production on day the 2<sup>nd</sup> day, 1:1 ratio started on the 3<sup>rd</sup> day and 2:1 ratio started on the 4<sup>th</sup> day. Flammability test indicated that 3:1 ratio started on the 26<sup>th</sup> day, 2:1 ratio flammable biogas production started on the 43<sup>rd</sup> day while 1:1 ratio did not produce flammable biogas. PD/PM 3:1 ratio started biogas production on the 2<sup>nd</sup> day, flammable gas production on the 26<sup>th</sup> day and the peak recorded on the 26<sup>th</sup> day with biogas yield of 1.475dm<sup>3</sup> and cumulative biogas yield of 4.417dm<sup>3</sup> as shown in Table 4.7.

The bioreactor co-digested with PD/CB 1:1 and 2:1 ratios biogas productions started on the 2<sup>nd</sup> day while 3:1 ratio biogas production started on the 3<sup>rd</sup> day. Flammability test indicated that 1:1 ratio flammable biogas production started on the 4<sup>th</sup> day, 2:1 ratio flammable biogas production

started on the 41<sup>st</sup> day while 3:1 ratio did not produce flammable biogas. PD/CB 1:1 ratio biogas production started on the 2<sup>nd</sup> day, recorded the peak of gas production on the 18<sup>th</sup> day with biogas yield of 7.81dm<sup>3</sup> and cumulative biogas yield of 4.794dm<sup>3</sup> as shown in Table 4.7.

The bioreactor with pig dung alone (520g) control, started its biogas production on the 2<sup>nd</sup> day, flammable biogas production on the 25<sup>th</sup> day. The peak was observed on the 15<sup>th</sup> day with biogas yield of 4.92dm<sup>3</sup> and cumulative biogas yield of 3.37dm<sup>3</sup> as shown in Table 4.7.

#### **4.4.1 Gas Chromatograms of produced biogas**

The percentage biomethane content obtained in the study were 65.24% for PD/CD 1:1, 61.39% for PD/PM 3:1, 63.19% for PD/CB 1:1, and 44.14% for pig dung (control). From this study, the mixing ratio of PD/CD proved to be the most suitable mixture to enhance biogas production as well as biomethane content (Table 4.8).

#### **4.4.2 Statistical Analysis**

Post-Hoc Duncan test was used to determine the means of maximum cumulative biogas yield and it was observed that there is no statistical difference between PD/CD 2:1 and PD/CD 3:1 but they are statistically different from all other treatments, PD/PM 1:1, 2:1 and 3:1 is significantly lower than all other treatments. All other treatments are statistically different from each other. All PD/CB treatments are statistically different from each other.

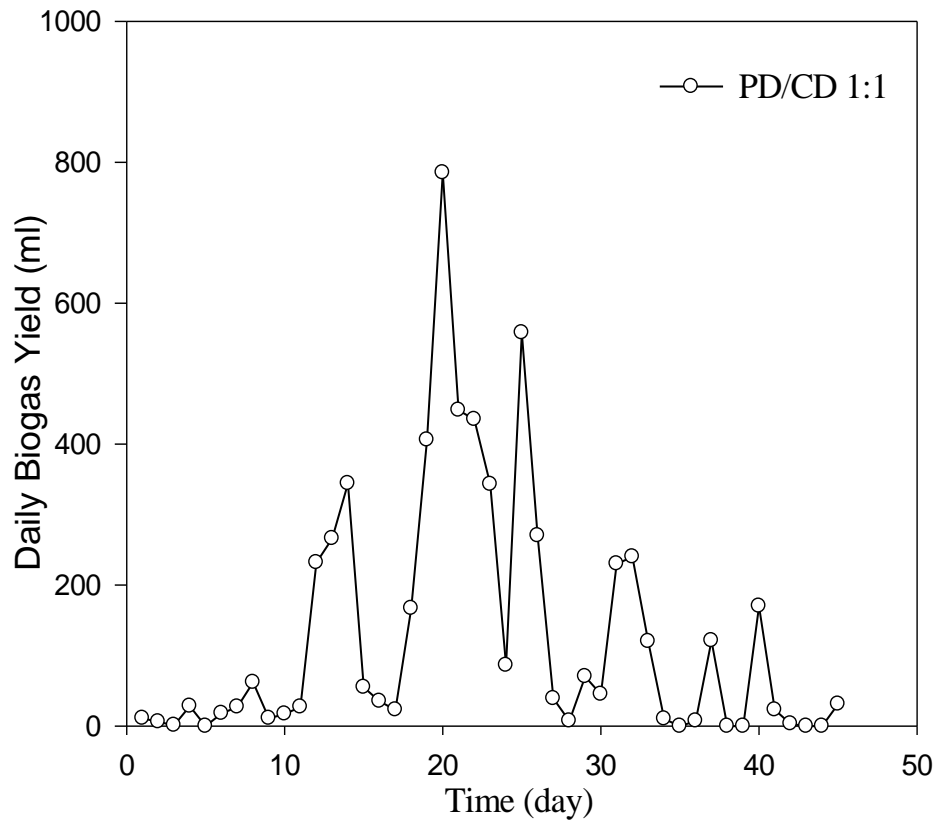
**Table 4.7: Cumulative biogas yield from the different substrate ratios (dm<sup>3</sup>) from pig dung co-digested with other substrates.**

Treatment	1:1	2:1	3:1
<b>PD/CD</b>	5.777	1.591	1.792
<b>PD/PM</b>	1.189	0.862	4.417
<b>PD/CB</b>	4.794	0.838	4.316
<b>PD alone</b>	3.37		

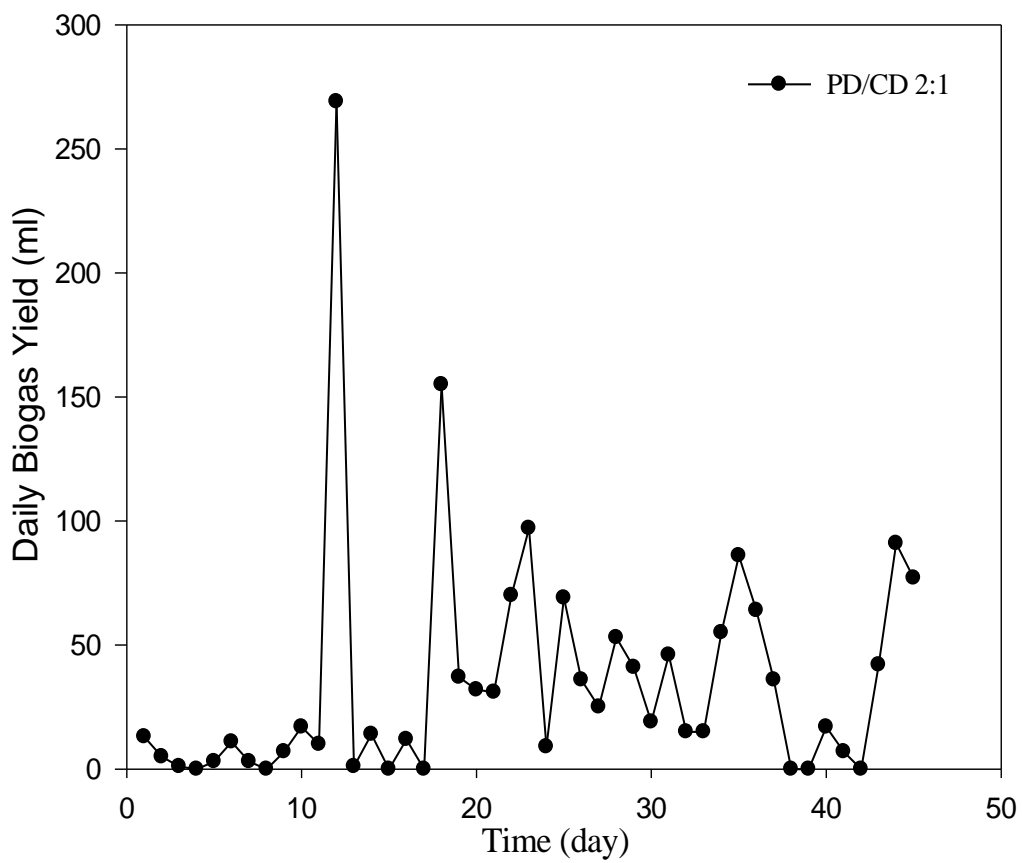
**Table4.8: Percentage biogas composition of bioreactors with the highest methane yield.**

Component	PD control		PD/CD, 1:1		PD/PM, 3:1		PD/CB, 1:1	
	Ppm	%Comp.	Ppm	%Comp.	Ppm	%Comp.	Ppm	%Comp.
<b>CO<sub>2</sub></b>	2.8374	38.80417	11.4654	23.874	49.2338	31.6466	5.3600	16.762
<b>CO</b>	0.2361	3.228895	1.0033	1.778	3.7789	2.429	1.4213	4.444743
<b>Acetic acid</b>	0.9949	13.60621	3.0455	5.3998	1.7453	1.121	-	-
<b>Methanol</b>	0.0164	0.224286	0.7151	1.267	2.8724	1.846	1.5772	4.93228
<b>Ethylene</b>	-	-	-	-	0.2836	0.1822	0.8727	2.729141
<b>Methane</b>	3.2273	44.13643	35.4921	65.2418	95.4998	61.386	20.2080	63.19107
<b>Ethanol</b>	-	-	2.4290	4.4198	2.1374	1.374	-	-
<b>Phenol</b>	-	-	0.2507	0.445	0.0222	0.01427	2.5400	7.943184
<b>Total</b>	7.3121		54.4008		155.5734		31.9771	

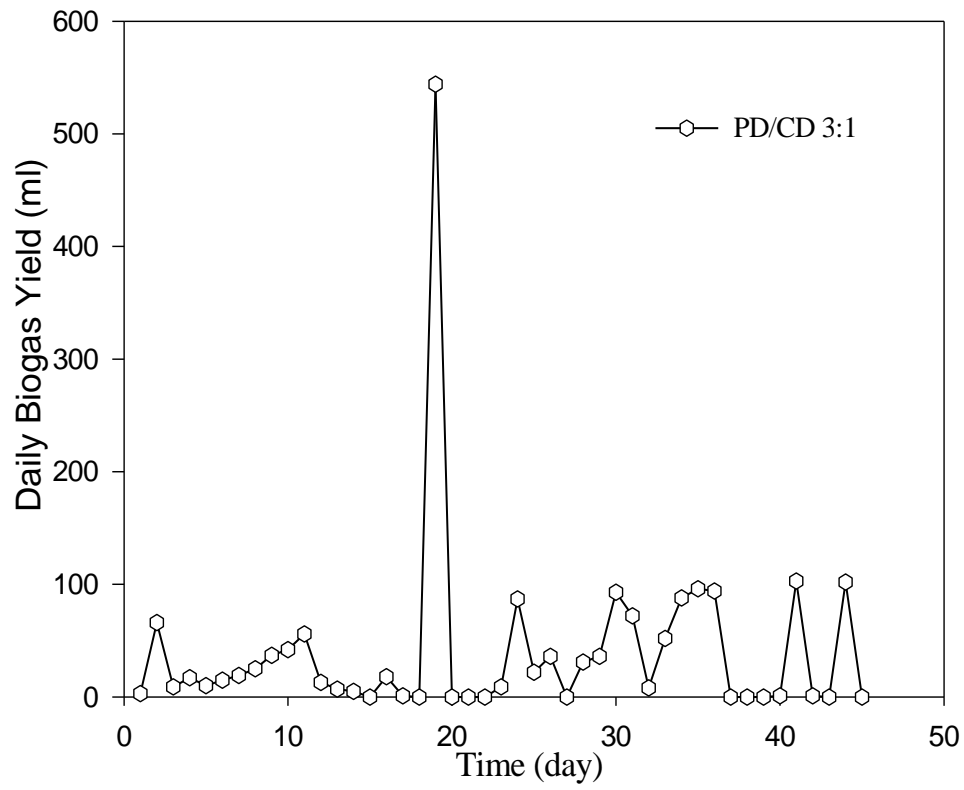
The mean values of biogas produced were plotted against the hydraulic retention time (HRT) as shown in Figures 4.6-4.15.



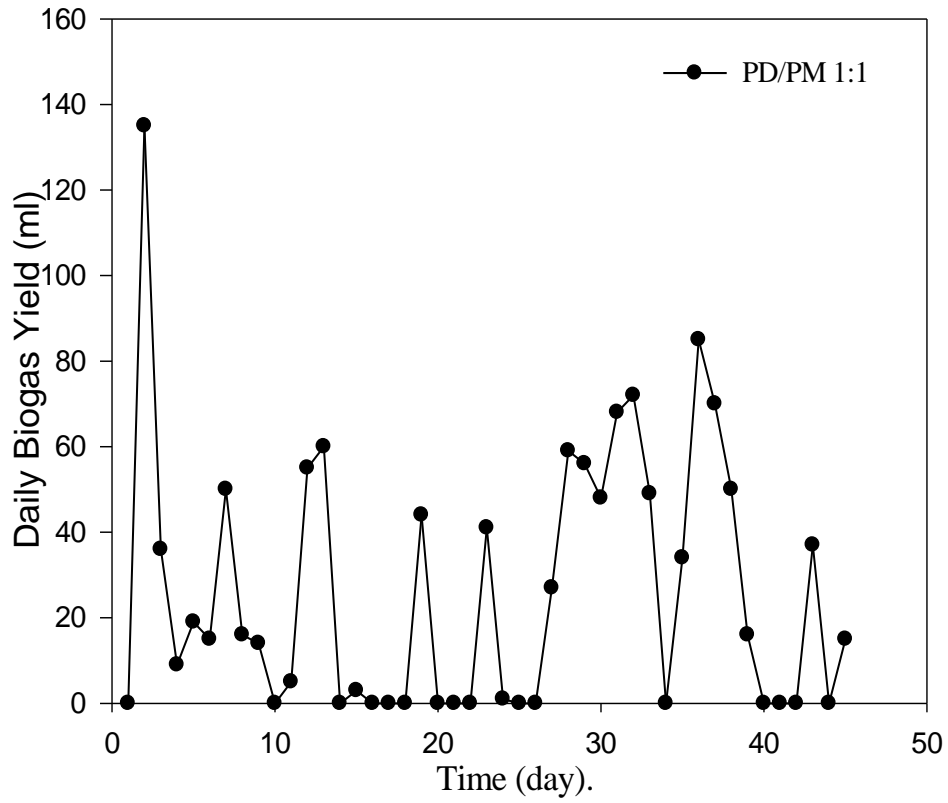
**Figure 4.6:** Profile of daily biogas production from mixtures of PD/CD 1:1.



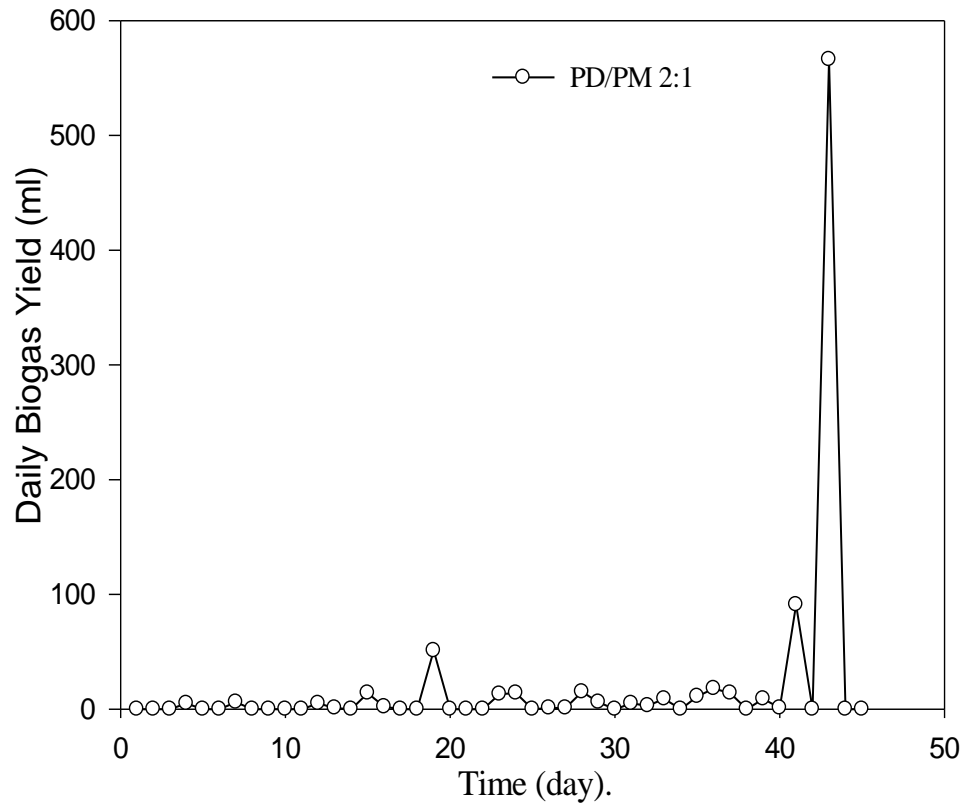
**Figure 4.7:** Profile of daily biogas production from mixtures of PD/CD 2:1.



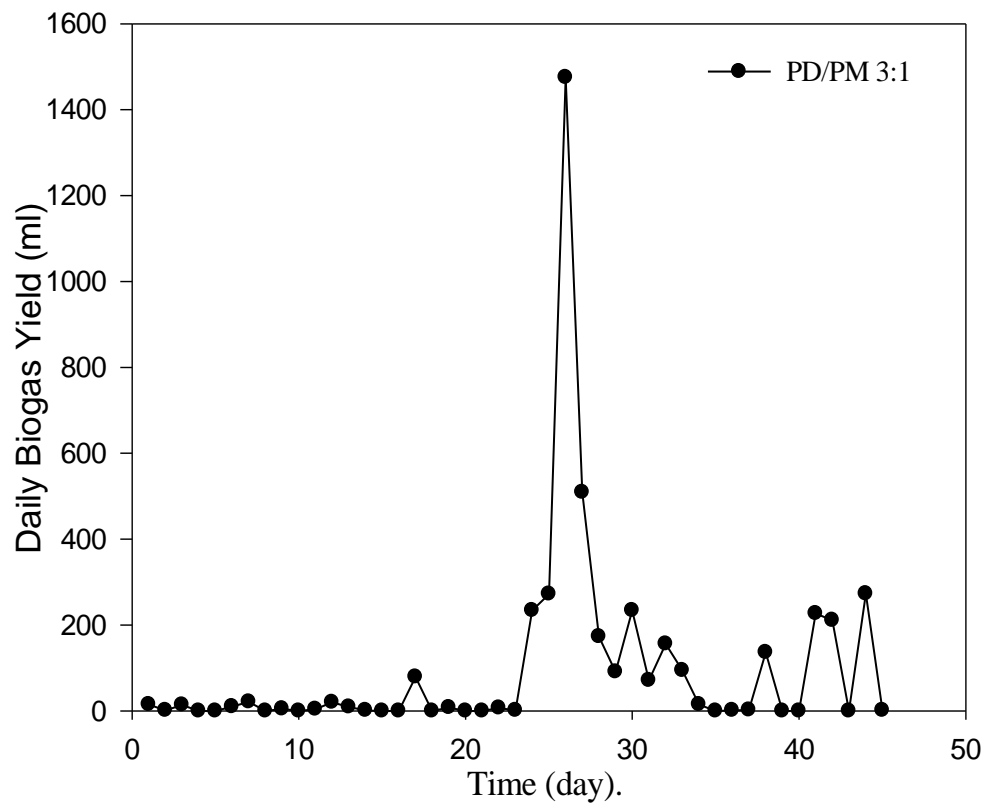
**Figure 4.8:** Profile of daily biogas production from mixtures of PD/CD 3:1.



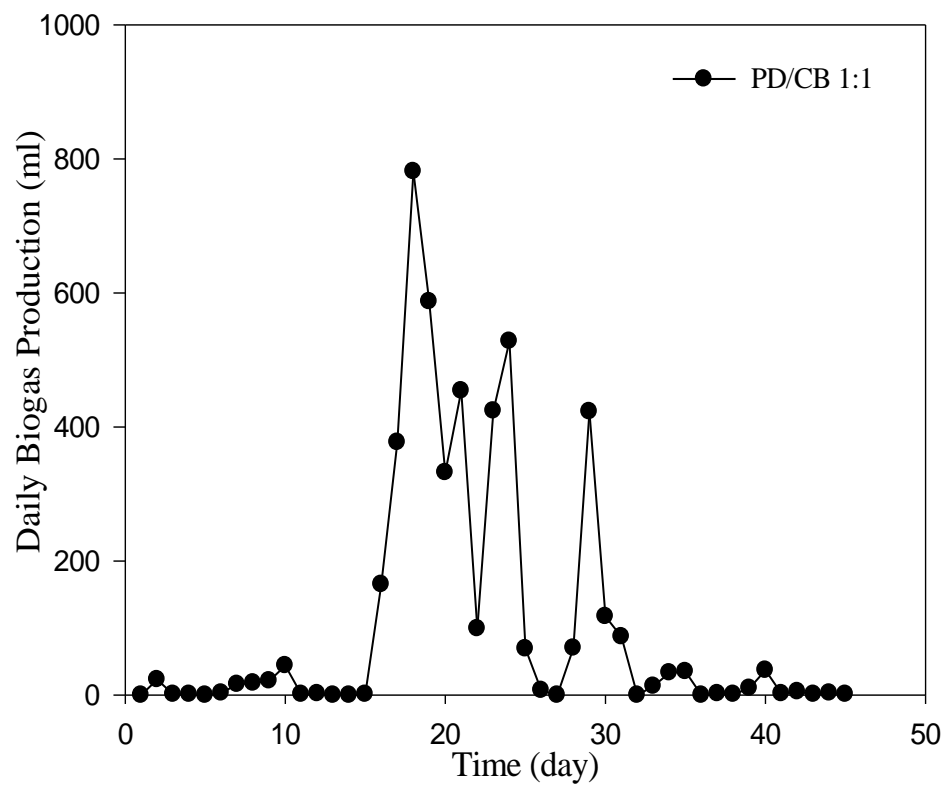
**Figure 4.9:** Profile of daily biogas production from mixtures of PD/PM 1:1.



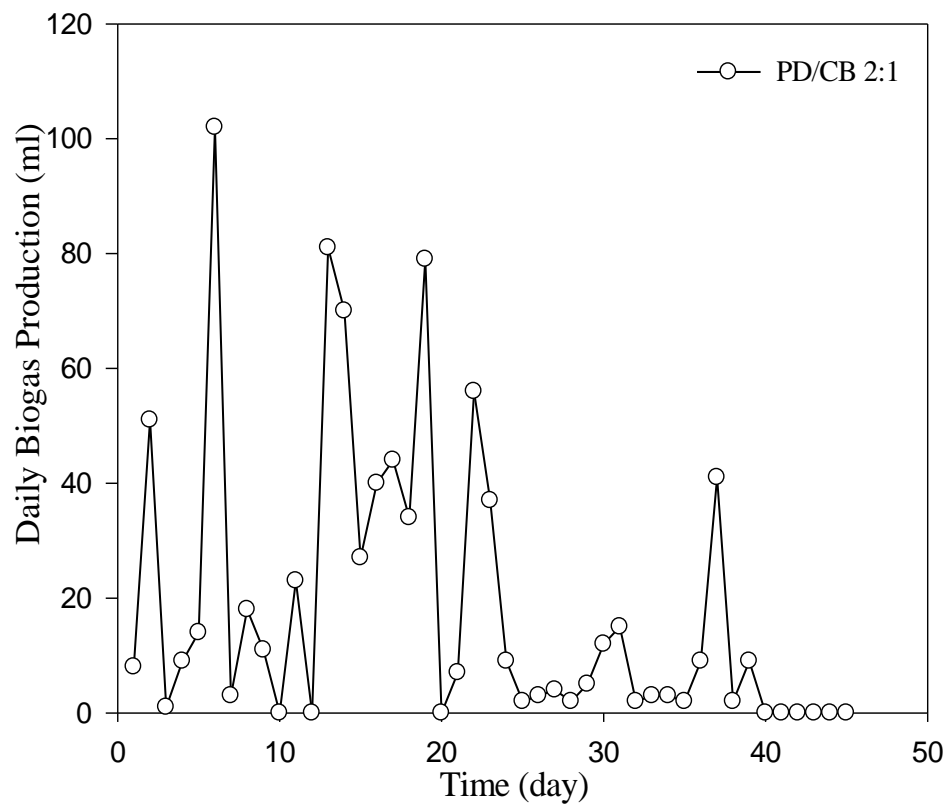
**Figure 4.10:** Profile of daily biogas production from mixtures of PD/PM 2:1.



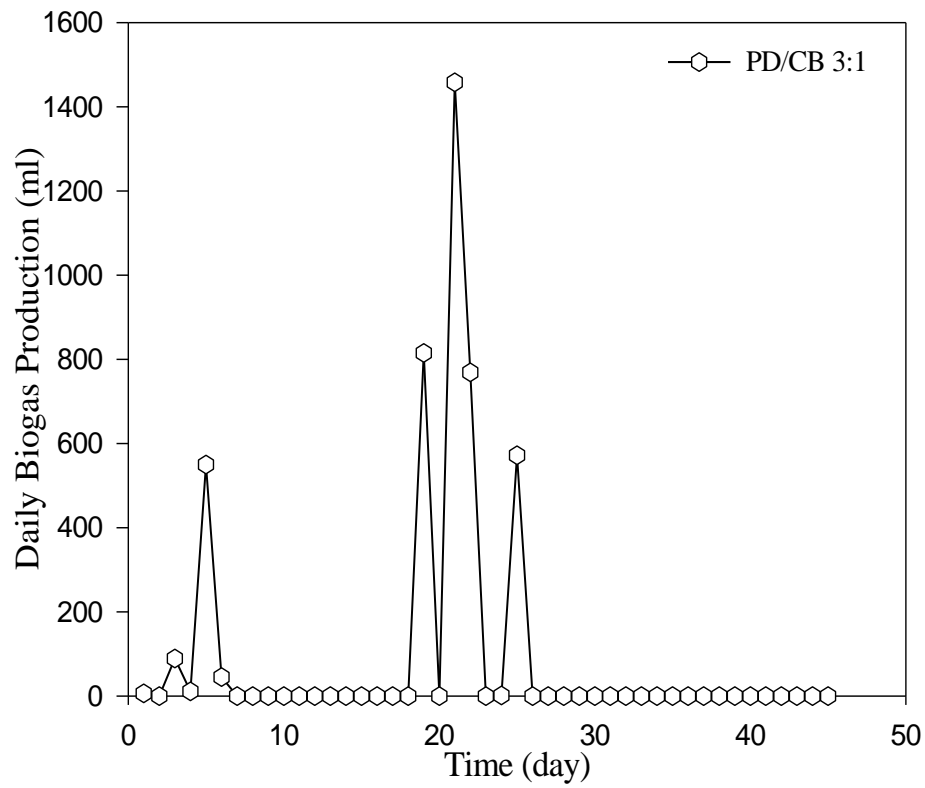
**Figure 4.11:** Profile of daily biogas production from mixtures of PD/PM 3:1.



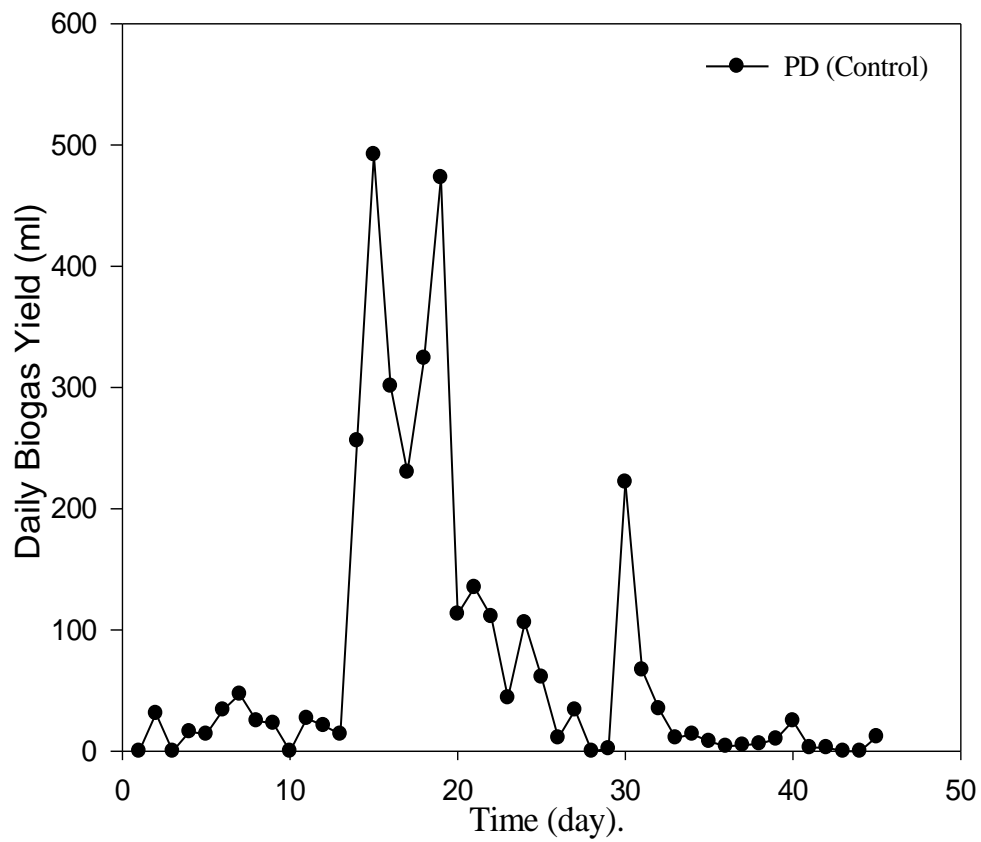
**Figure 4.12:** Profile of daily biogas production from mixtures of PD/CB 1:1.



**Figure 4.13:** Profile of daily biogas production from mixtures of PD/CB 2:1.



**Figure 4.14:** Profile of daily biogas production from mixtures of PD/CB 3:1.



**Figure 4.15:** Profile of daily biogas production from PD alone.

**4.5 Anaerobic digestion and biogas production from cassava peels as a single substrate and co-digestion of cassava peels with pig dung, cow dung and poultry manure.**

Cassava peels was anaerobically digested as a single substrate at ambient temperature for 45 days and this served as the control for other experimental studies. Then it was co-digested in triplicate with pig dung (CP/CD), cow dung (CP/PD) and poultry manure (CP/PM) at the ratios of 1:1, 2:1 and 3:1. The co-digestion of CP/CD, CP/PD and CP/PM in the ratio of 1:1, 2:1, 3:1 respectively had cumulative biogas yield of 6.631 dm<sup>3</sup>, 6.652 dm<sup>3</sup>, 12.347 dm<sup>3</sup>; 6.317 dm<sup>3</sup>, 5.780 dm<sup>3</sup>, 9.300 dm<sup>3</sup> and 2.762 dm<sup>3</sup>, 2.678 dm<sup>3</sup>, 3.491 dm<sup>3</sup> respectively, then cassava peels alone had cumulative biogas yield of 8.69 dm<sup>3</sup> (Table 4.9).

The bioreactor co-digested with CP/CD, 1:1, 2:1 and 3:1 ratios started biogas production on the 1<sup>st</sup> day. Flammability test indicated that 3:1 ratio started on the 2<sup>nd</sup> day while 1:1 and 2:1 ratios flammable biogas started on the 3<sup>rd</sup> day. CP/CD 3:1 ratio which started biogas production on the 1<sup>st</sup> day, flammable biogas production on the 2<sup>nd</sup> day recorded the peak on the 8<sup>th</sup> day with biogas yield of 1.822 dm<sup>3</sup> and cumulative biogas yield of 12.347 dm<sup>3</sup> as shown in Table 4.9.

The bioreactor co-digested with CP/PD, 1:1 and 2:1 ratios started biogas production on day the 2<sup>nd</sup> day, while 3:1 ratio biogas production started on the 5<sup>th</sup> day. Flammability test indicated that 1:1, 2:1 and 3:1 ratios started on the 7<sup>th</sup> day. CP/PD 3:1 ratio started biogas production on the 2<sup>nd</sup> day, flammable gas production on the 7<sup>th</sup> day and the peak recorded on the 5<sup>th</sup> day with biogas yield of 1.539 dm<sup>3</sup> and cumulative biogas yield of 9.300 dm<sup>3</sup> as shown in Table 4.9.

The bioreactor co-digested with CP/PM, 1:1, 2:1 and 3:1 ratios biogas production started on the 2<sup>nd</sup> day. Flammability test indicated that 1:1 and 3:1 ratios flammable biogas production started on the 3<sup>rd</sup> day while 2:1 ratio flammable biogas production started on the 4<sup>th</sup> day. CP/PM 3:1 ratio biogas production started on the 2<sup>nd</sup> day, recorded the peak of gas production on the 26<sup>th</sup> day with biogas yield of 5.69 dm<sup>3</sup> and cumulative biogas yield of 3.491 dm<sup>3</sup> as shown in Table 4.9.

The bioreactor with cassava peels alone (520g) control, started its biogas production on the 3<sup>rd</sup> day, flammable biogas production on the 4<sup>th</sup> day. The peak was observed on the 9<sup>th</sup> day with biogas yield of 1.969dm<sup>3</sup> and cumulative biogas yield of 8.690dm<sup>3</sup>.

#### **4.5.1 Gas Chromatograms of produced biogas**

The percentage biomethane content obtained in the study were 78.05% for CP/CD 3:1, 64.05% for CP/PD 3:1, 55.77% for CP/PM3:1, and 49.39% for cassava peels (control). From this study, the mixing ratio of CP/CD proved to be the most suitable mixture to enhance biogas production as well as biomethane content in all the co-digested substrates (Table 4.10).

#### **4.5.2 Statistical Analysis**

Post-Hoc Duncan test was used to determine the means of maximum cumulative biogas yield and it was observed that there is no statistical difference between CP/PM 1:1, 2:1 and 3:1 but they are statistically different from all other treatments and significantly lower than all other treatments. There is no statistical difference between CP/PD 1:1, CP/PD 2:1, CP/CD 1:1 and CP/CD 2:1 but they are statistically different from all other treatments. Also there is no statistical difference between CP/PD and PD alone but they are statistically different from all other treatments. CP/CD is statistically different from all other treatments.

The cumulative biogas produced in the study is shown in Tables 7, 9 and 11 and the result of compositional analysis of the biogas is shown in Tables 8, 10 and 12. The percentage methane content of 78.05% (CP/CD) is an indication that cassava peels co-digested with cow dung efficiently enhanced biogas yield hence a very good substrate for biogas production and was then used for optimization of the biogas.

**Table 4.9: Cumulative biogas yield from the different substrate ratios (dm<sup>3</sup>) from cassava peels co-digested with other substrates.**

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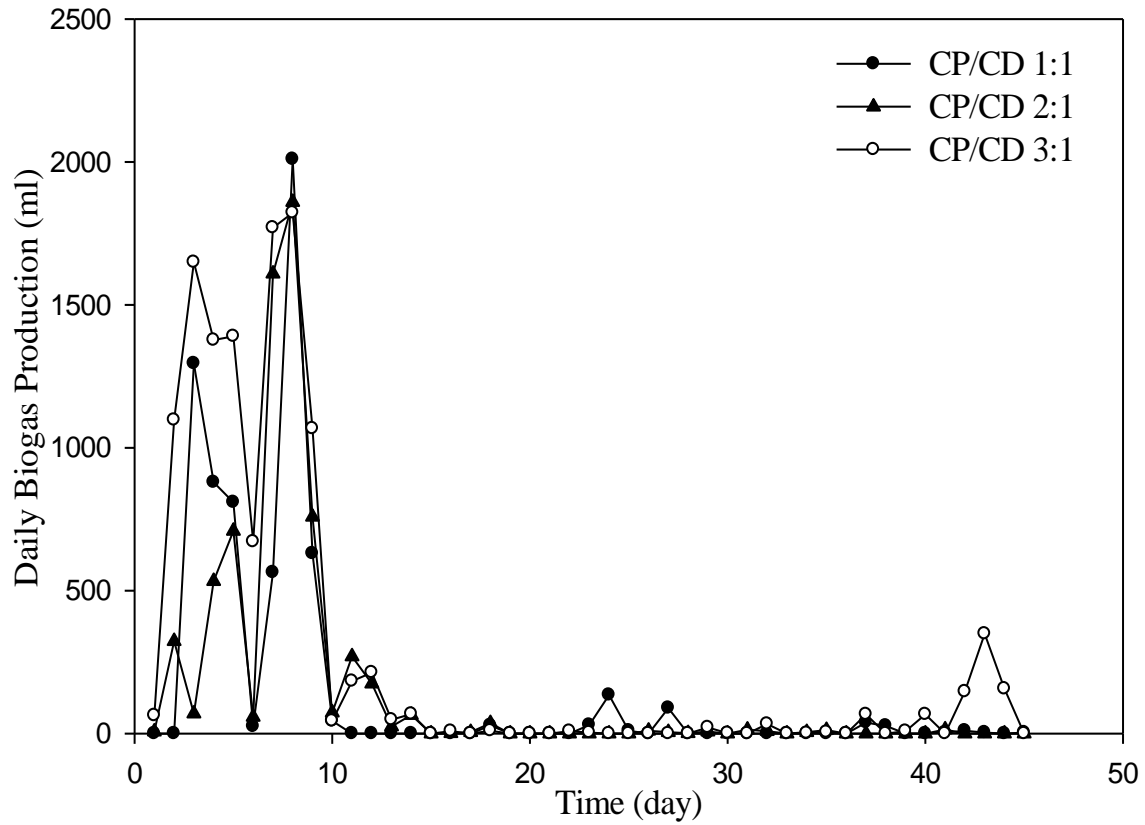
<b>Treatment</b>	<b>1:1</b>	<b>2:1</b>	<b>3:1</b>
<b>CP/CD</b>	6.631	6.652	12.347
<b>CP/PD</b>	6.317	5.780	9.300
<b>CP/PM</b>	2.762	2.678	3.491
<b>CP alone</b>	8.69		

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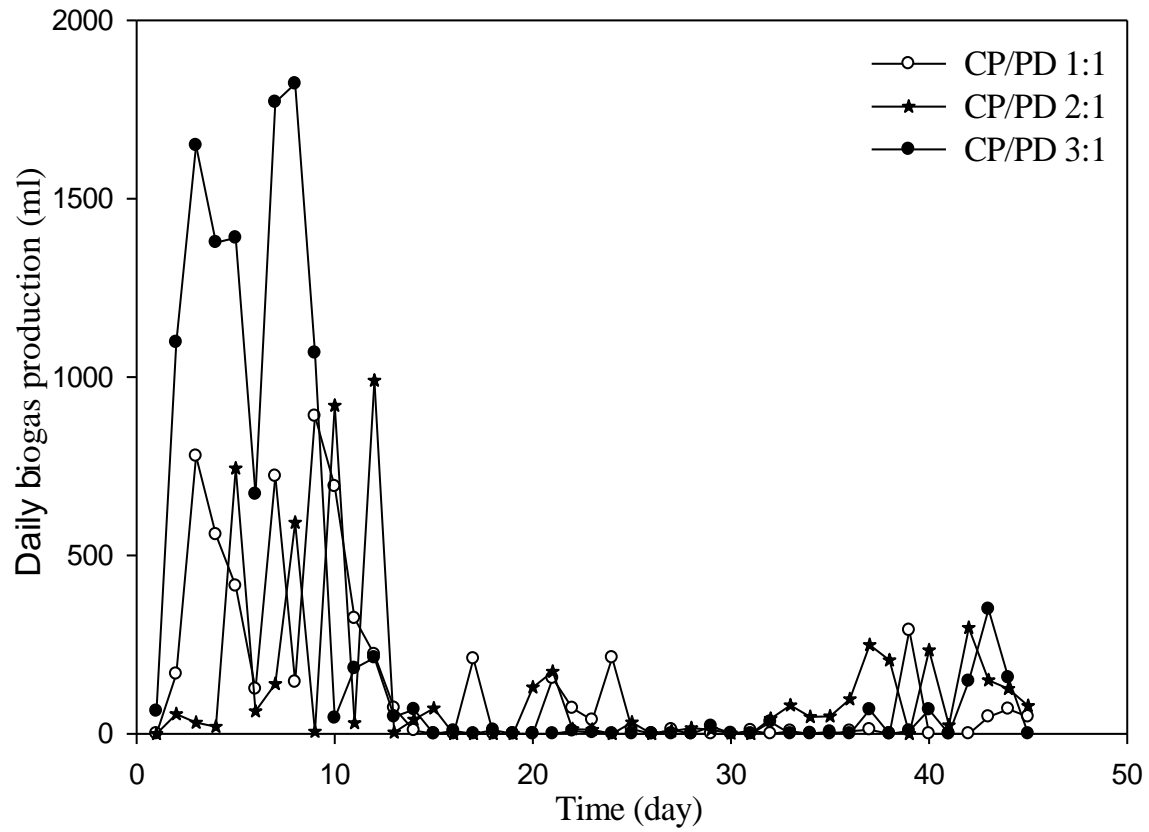
**Table 4.10: Percentage biogas composition of bioreactors with the highest methane yield.**

Component	CP		CP/PD, 3:1		CP/CD, 3:1		CP/PM, 3:1	
	Ppm	% Comp	Ppm	% Comp	Ppm	% Comp	Ppm	% Comp
<b>control</b>								
<b>CO<sub>2</sub></b>	5.7788	37.599	2.0820	12.18927	3.4228	13.34128	58.508	32.869
<b>CO</b>	0.1111	0.7229	1.2289	7.194712	0.5518	2.150789	3.7768	2.128
<b>Acetic acid</b>	-	-	0.7719	4.519162	1.0450	4.073169	4.4382	2.493
<b>Methanol</b>	0.9281	6.0385	1.8261	10.69108	0.6004	2.340221	2.8723	1.613
<b>Ethylene</b>	0.2937	1.9109	-	-	-	-	0.2545	0.143
<b>Methane</b>	7.5826	49.390	10.9406	64.05279	19.9844	78.05065	9.3290	55.7716
<b>Ethanol</b>	0.6580	4.281	-	-	-	-	8.6992	4.887
<b>Phenol</b>	-	-	0.2311	1.352997	-	-	0.2215	0.124
<b>Total</b>	15.3523		17.0806		25.6044		178.0995	

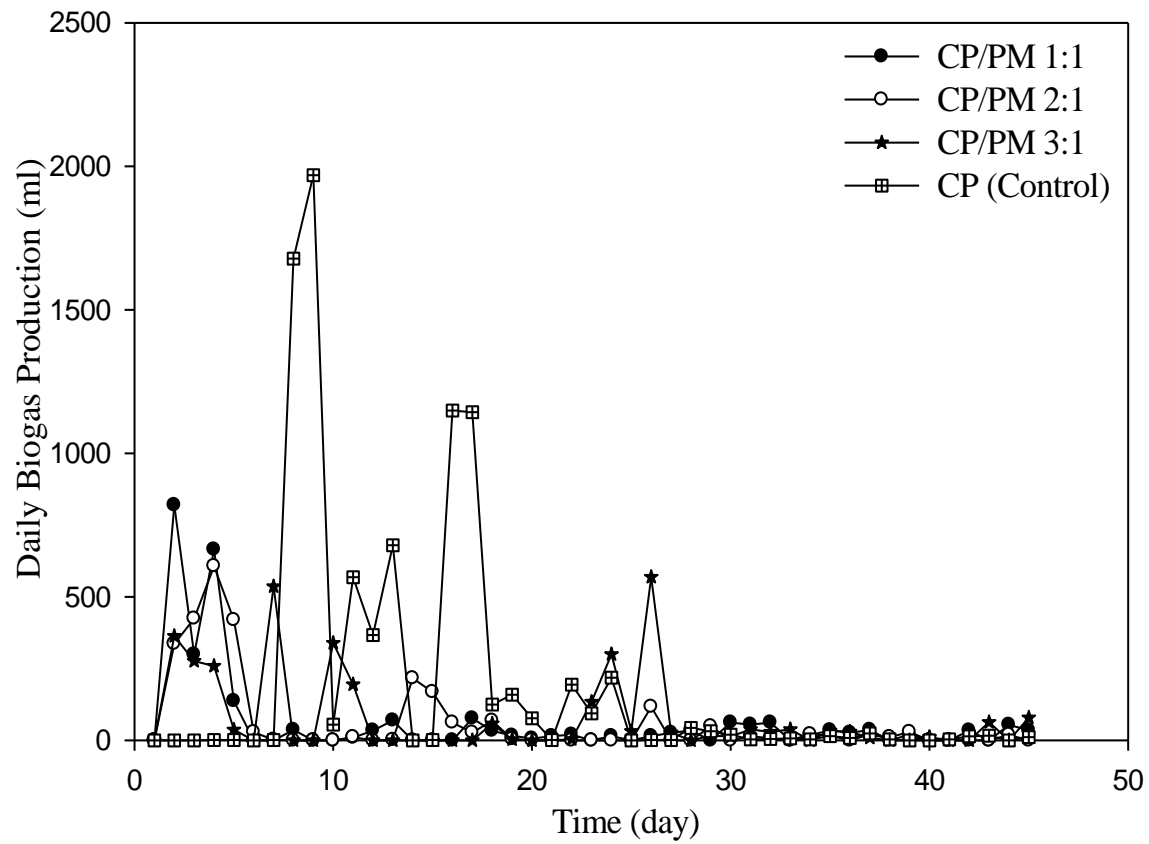
The mean values of biogas produced were plotted against the hydraulic retention time (HRT) as shown in Figures 4.16 - 4.18.



**Figure 4.16:** Profile of daily biogas production from mixtures of CP/CD.



**Figure 4,17:** Profile of daily biogas production from mixtures of CP/PD.



**Figure 4.18:** Profile of daily biogas production from mixtures of CP/PM and CP alone.

#### 4.6 Optimization of Biogas

**Anaerobic digestion of cassava peels co-digested with cow dung using the values gotten from the Box Behnken's design of response surface methodology.**

Cassava peels co-digested with cow dung which had the highest biogas yield was anaerobically digested in triplicate at the ratios of 1:1, 2:1 and 3:1, pH of 5, 7, and 9 and Hydraulic Retention Time of 15, 30 and 45days at ambient temperature using the values obtained from the design. The co-digested substrates (CP/CD (pH5) 2:1, CP/CD (pH7) 3:1 and CP/CD (pH9) 2:1) that flamed early with the highest biogas production were selected for GC compositional biogas analysis.

The bioreactor co-digested with CP/CD (pH5) 2:1 ratio, 15days hydraulic retention time started biogas production on the 1<sup>st</sup> day, CP/CD (pH7) 3:1 ratio, 15days hydraulic retention time started biogas production on the 1<sup>st</sup> day, also CP/CD (pH9) 2:1 ratio 45dayshydraulic retention time started biogas production on the 1<sup>st</sup> day.

Flammability test indicated that CP/CD (pH7) 3:1 ratio, 15days hydraulic retention time biogas production started on the 1<sup>st</sup> day, CP/CD (pH5) 2:1 ratio, 15days hydraulic retention time flammable biogas production started on the 3<sup>rd</sup> day while CP/CD (pH9) 2:1 ratio, 45days biogas production started on the 2<sup>nd</sup> day.

The GC compositional biogas analysis shows that CP/CD (pH7) 3: 1 ratio, 15days hydraulic retention time had the highest percentage of methane (79.1431%). This is shown in Table 4.11.

The percentage methane content of 79.1431% of the biogas from the substrate is an indication that CP/CD (pH7) 3:1 ratio, 15dayshydraulic retention time have proved to be most suitable combination with favourable conditions acceptable to enhance biogas production and biomethane content as well.

**Table 4.11: Percentage biogas composition of bioreactors with the highest methane yield.**

Component	CP/CD, pH7, 3:1		CP/CD, pH9 2:1		CP/CD, pH5 2:1	
	Ppm	%Comp.	Ppm	%Comp.	Ppm	%Comp.
<b>Carbondioxide</b>	2.3376	10.92525	5.0208	4.164	2.8214	14.248
<b>Carbonmonoxide</b>	0.0375	0.17526	0.6853	2.854	0.7440	3.757
<b>Acetic acid</b>	1.0062	4.70268	1.7342	7.222	-	-
<b>Methanol</b>	0.9489	4.43488	2.0611	8.583	3.3574	16.954
<b>Ethylene</b>	0.1324	0.61880	0.5226	2.176	0.7911	3.995
<b>Methane</b>	16.9337	79.1431	13.9875	58.253	10.8610	54.847
<b>Ethanol</b>	-	-	-	-	1.2270	6.196
<b>Phenol</b>	-	-	-	-	-	-
<b>Total</b>	21.3963		24.0115		19.802	

**Key: CP= Cassava peels, CD= Cow dung**

**Table 4.12: Optimization Response**

---

<b>StdOrder</b>	<b>RunOrder</b>	<b>PtType</b>	<b>Blocks</b>	<b>pH</b>	<b>Substrate</b>	<b>HRT</b>	<b>RESPONSE</b>
<b>11</b>	1	2	1	7	1	45	109.62
<b>6</b>	2	2	1	9	2	15	116.11
<b>2</b>	3	2	1	9	1	30	57.09
<b>3</b>	4	2	1	5	3	30	47.08
<b>13</b>	5	0	1	7	2	30	70.84
<b>1</b>	6	2	1	5	1	30	46.91
<b>5</b>	7	2	1	5	2	15	178.49
<b>12</b>	8	2	1	7	3	45	134.01
<b>8</b>	9	2	1	9	2	45	127.29
<b>4</b>	10	2	1	9	3	30	60.3
<b>9</b>	11	2	1	7	1	15	84.89
<b>10</b>	12	2	1	7	3	15	214.69
<b>14</b>	13	0	1	7	2	30	151.66
<b>15</b>	14	0	1	7	2	30	156.03
<b>7</b>	15	2	1	5	2	45	59.5

---

Optimization response was obtained from the optimization of biogas result using the values obtained from the design (Table 2). The co-digestion was carried out in 15 runs as shown in Table 14. The highest response was observed in CP/CD 3:1, pH7 for 15 days hydraulic retention time. This was used in response surface plots of pH, substrate ratio and hydraulic retention time.

#### **4.6.1 Response Surface Plots of pH, Substrate ratio and HRT (Cassava Peeis co-digested with Cow Dung)**

The surface plot displays a three-dimensional view that provides a clearer picture of the response surface plots modeled from the yield of biogas as a function of HRT, pH and Substrate ratio as shown in figure 4.19- 4.24.

Figure 4.19 shows that the biogas yield started increasing from the beginning of the anaerobic digestion to about 6.8-7.5 range, then the yield decreased drastically as the pH increased to 9.0. This implies that the pH value had some effects on the biogas yield. Also there was a corresponding decrease in biogas yield as the HRT was increasing.

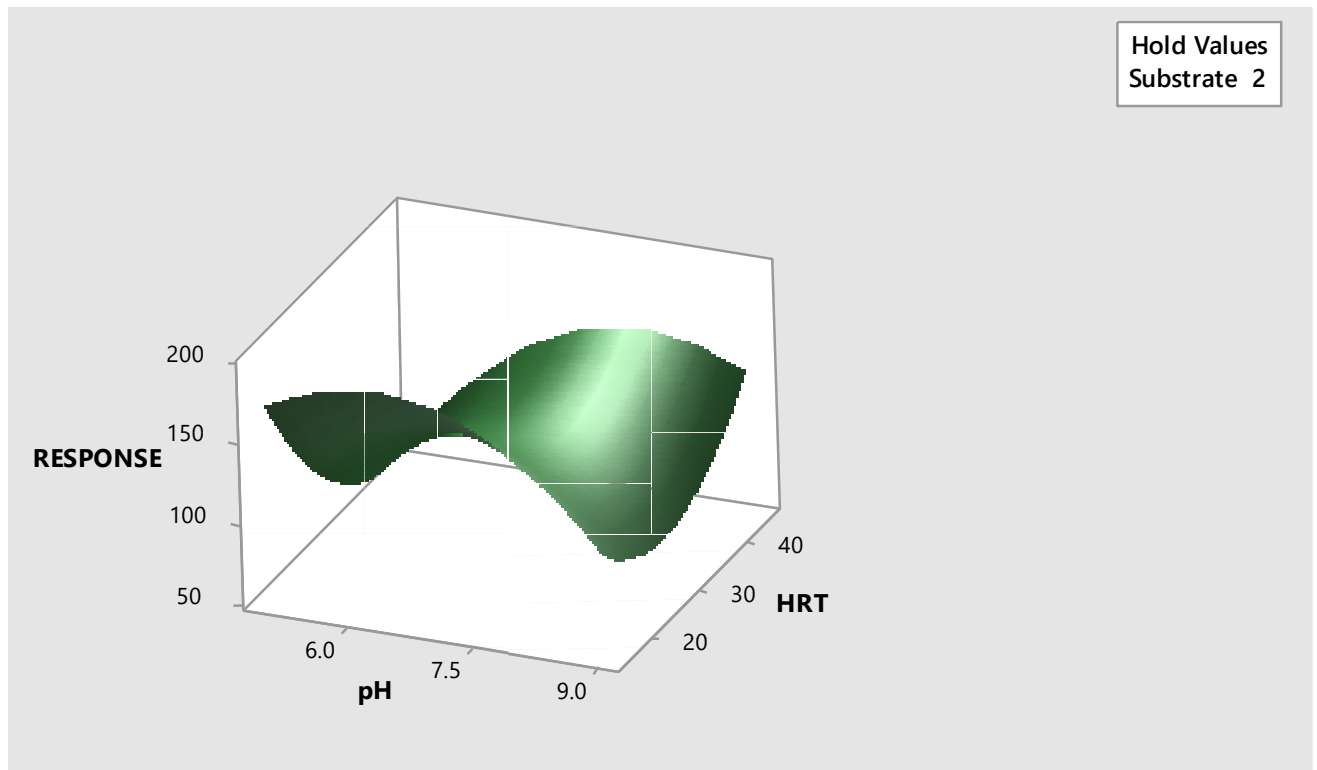
Figure 4.20 shows that as the substrate ratio was increasing, the biogas yield was also increasing, also there was a high yield at the start of the study but as the pH value increased, the biogas yield decreased drastically.

Figure 4.21 shows that as the substrate ratio was increasing, the biogas yield was also increasing. The biogas yield started increasing from the beginning of the anaerobic digestion and started decreasing when it was close to 15 days.

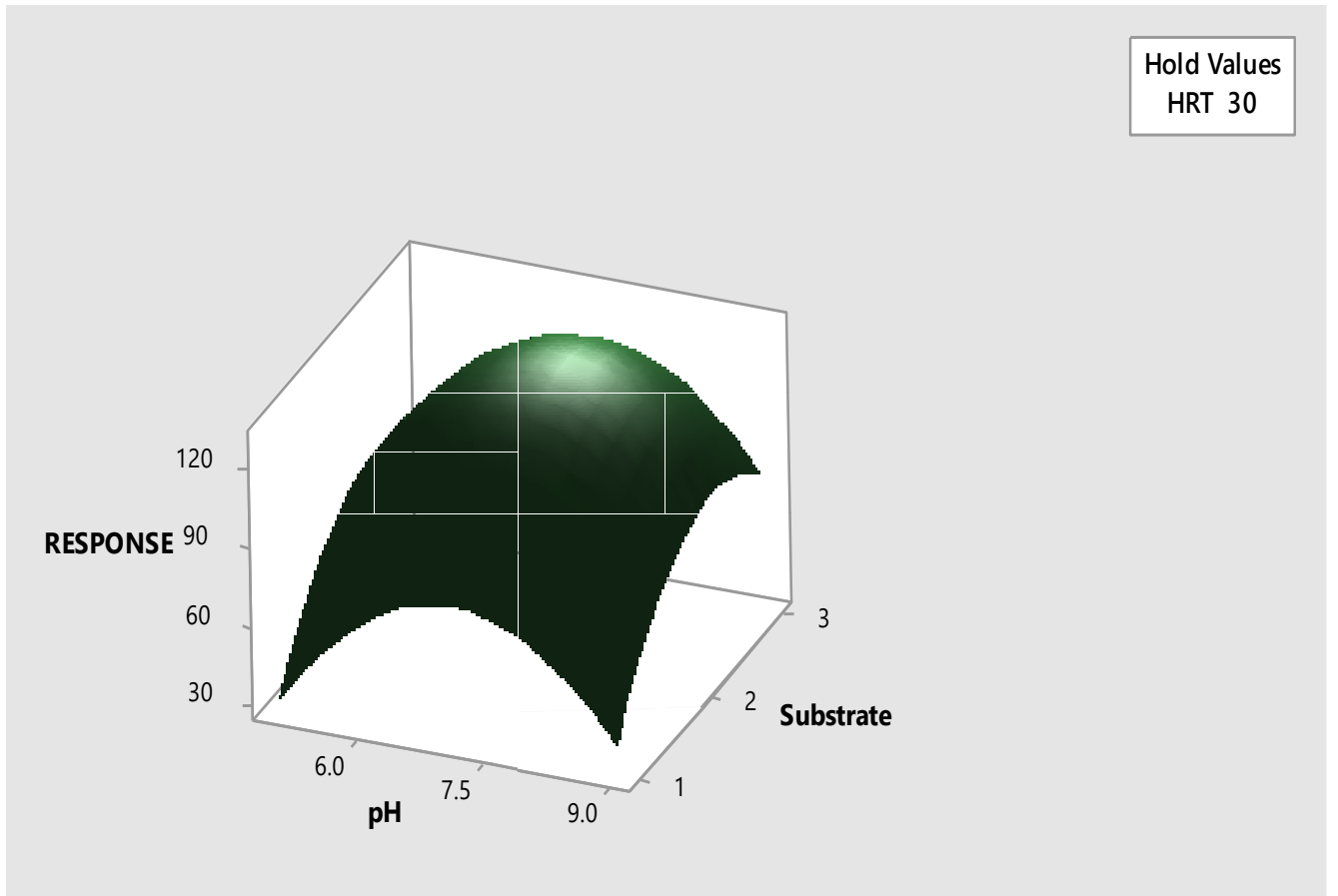
Figure 4.22 shows that there was an exponential increase in biogas yield with increase in substrate ratio and a decrease in yield at the highest substrate ratio. At the same time, as the pH was increasing, the biogas yield was increasing but very high increase in the pH resulted to a decrease in biogas yield.

Figure 4.23 shows that the biogas yield increased from the beginning of the anaerobic digestion, decreased a little at about the 25<sup>th</sup> day but there was increase in biogas yield with increase in substrate ratio.

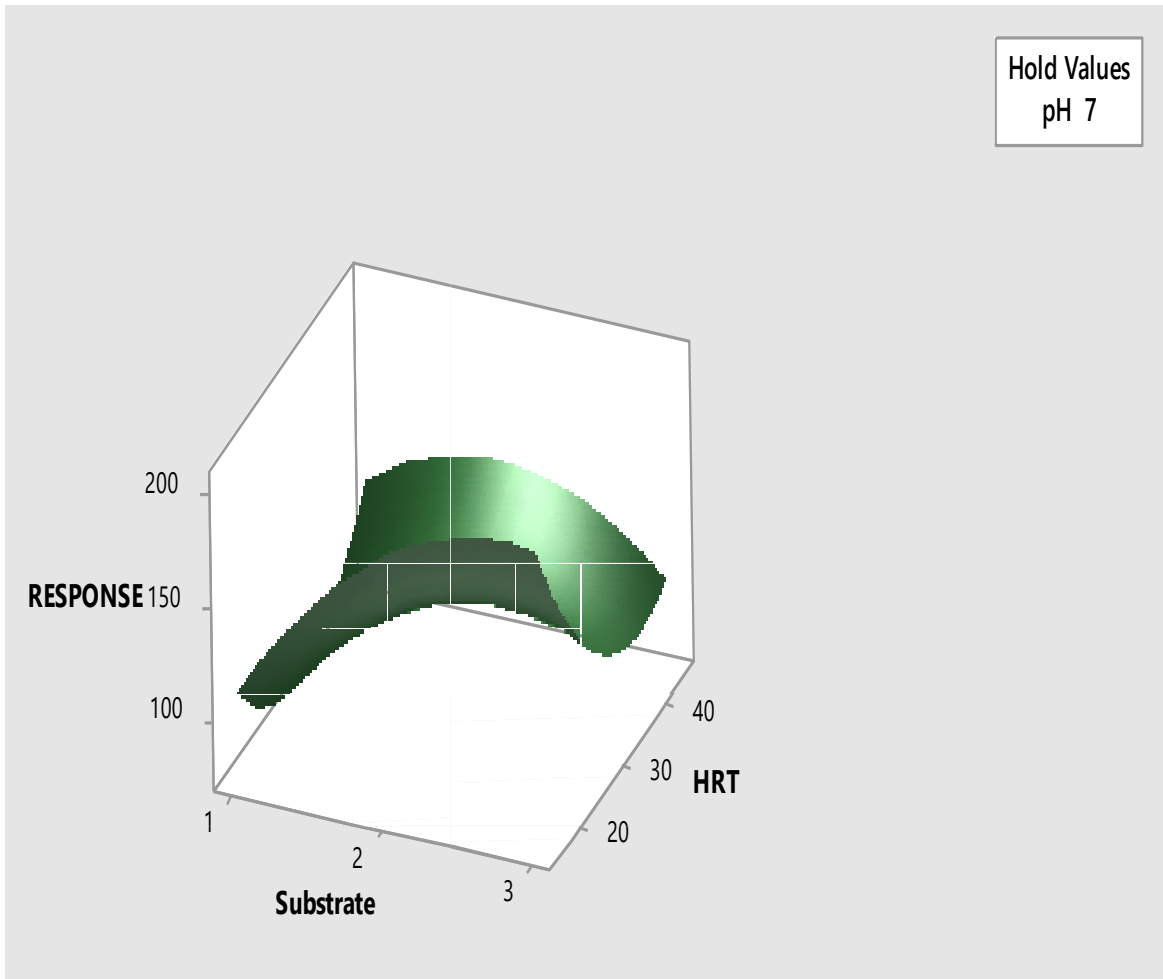
Finally figure 4.24 shows that the biogas yield was high at the beginning of the study but started reducing at the end of the digestion process. Also the biogas yield was increasing as the pH value was increasing but started decreasing at the highest pH value.



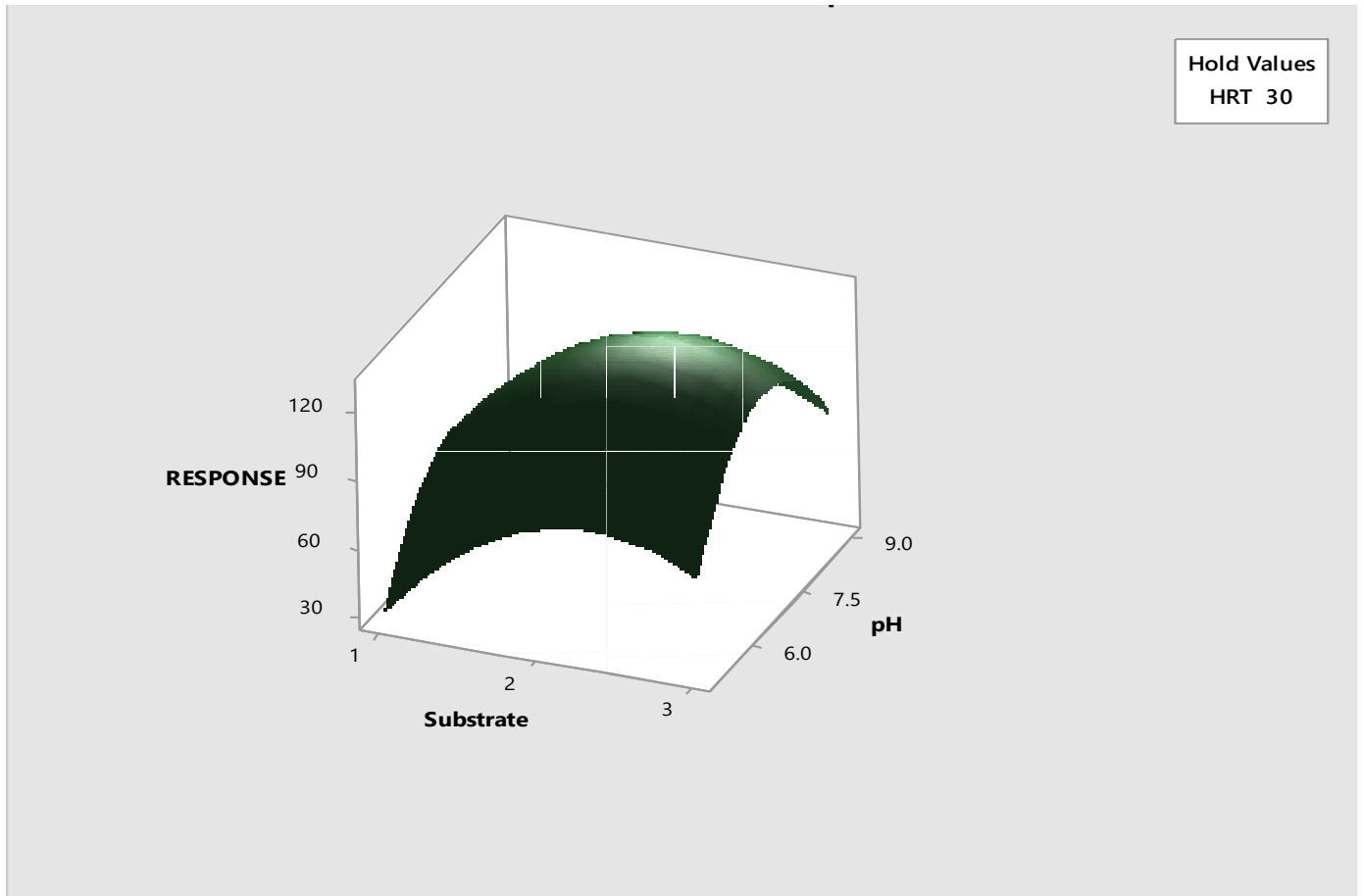
**Figure 4.19: Response surface plot of biogas yield against Hydraulic Retention Time and pH**



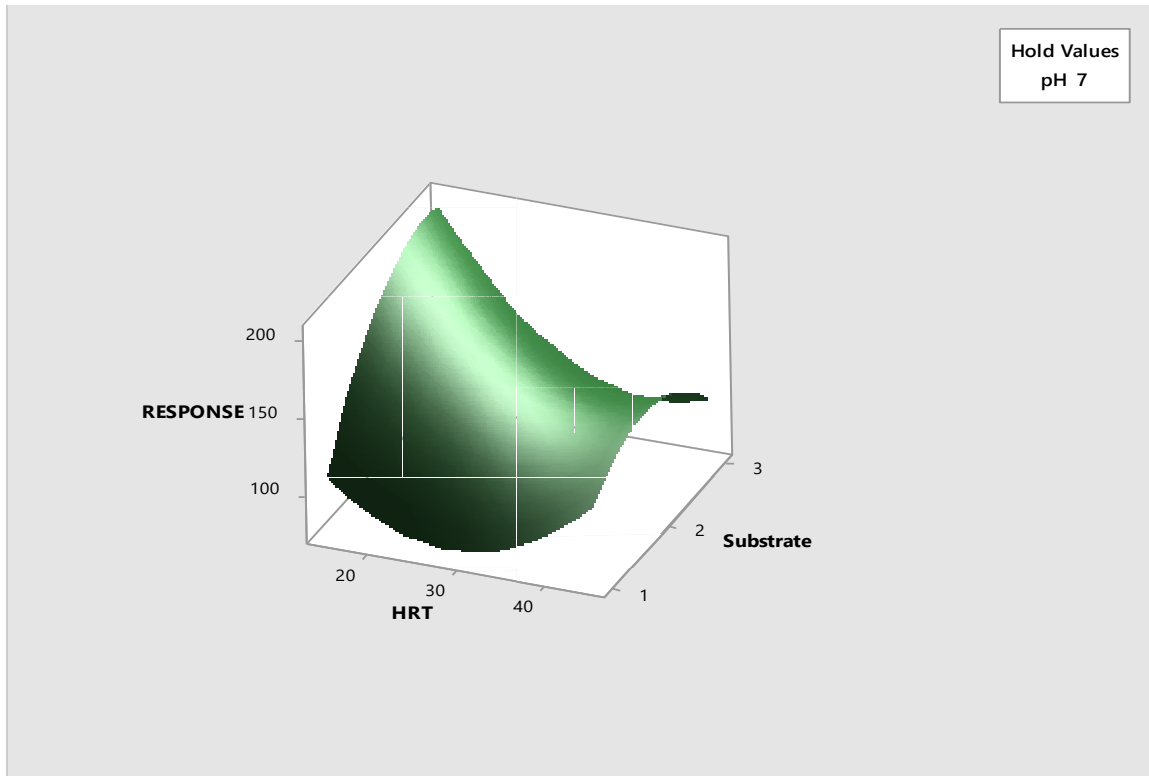
**Figure 4.20: Response surface plot of biogas yield against Substrate ratio and pH**



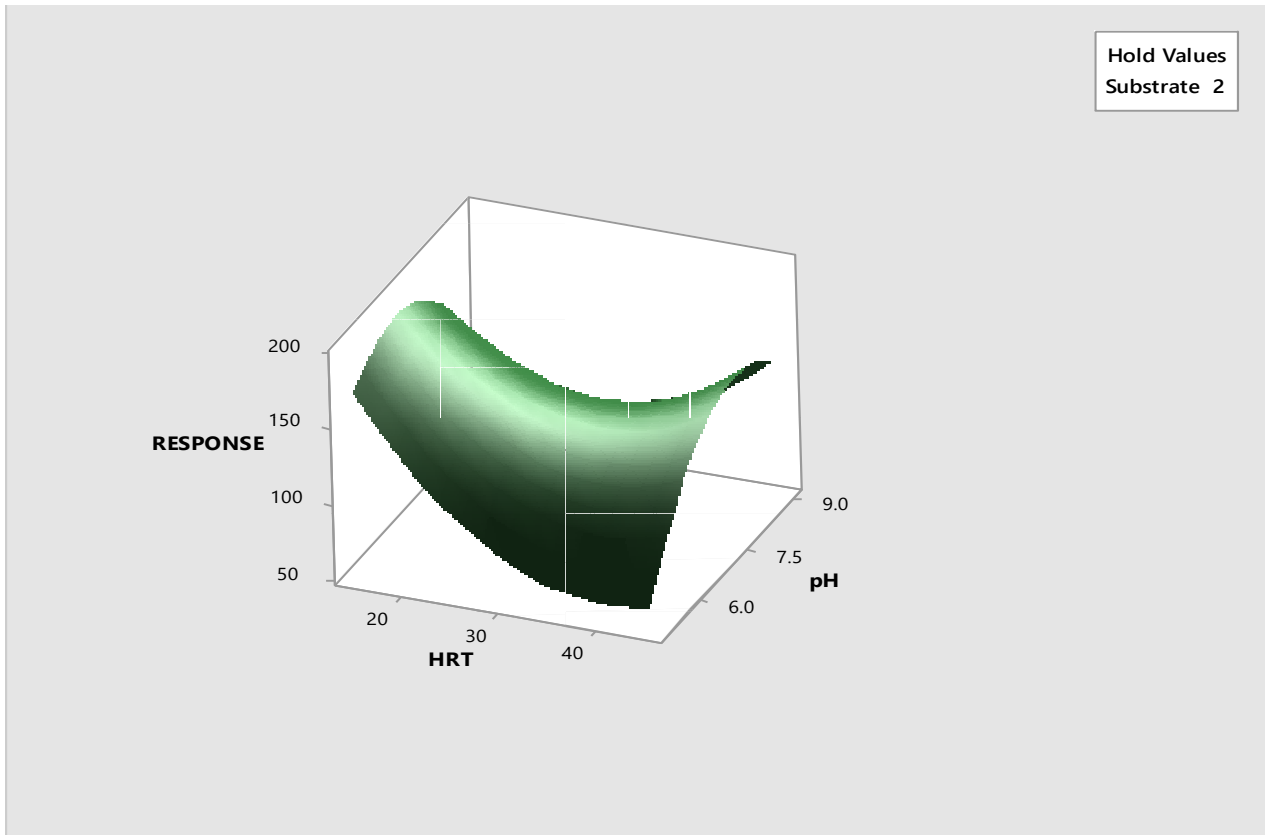
**Figure 4.21: Response surface plot of biogas yield against Hydraulic Retention Time and Substrate ratio.**



**Figure 4.22: Response surface plot of biogas yield against pH and Substrate ratio.**



**Figure 4.23: Response surface plot of biogas yield against Substrate ratio and Hydraulic Retention Time.**



**Figure 4.24: Response surface plot of biogas yield against pH and Hydraulic Retention Time.**

#### **4.6.2 Interaction plot for Response (Cassava peels co-digested with cow dung)**

The interactions of factors subjected to the biogas production are shown in figure 4.25. The result shows that there was no observed interaction between pH and substrate ratio. On the hand, there was interaction between pH and HRT, as well as Substrate ratio and HRT in all the values.

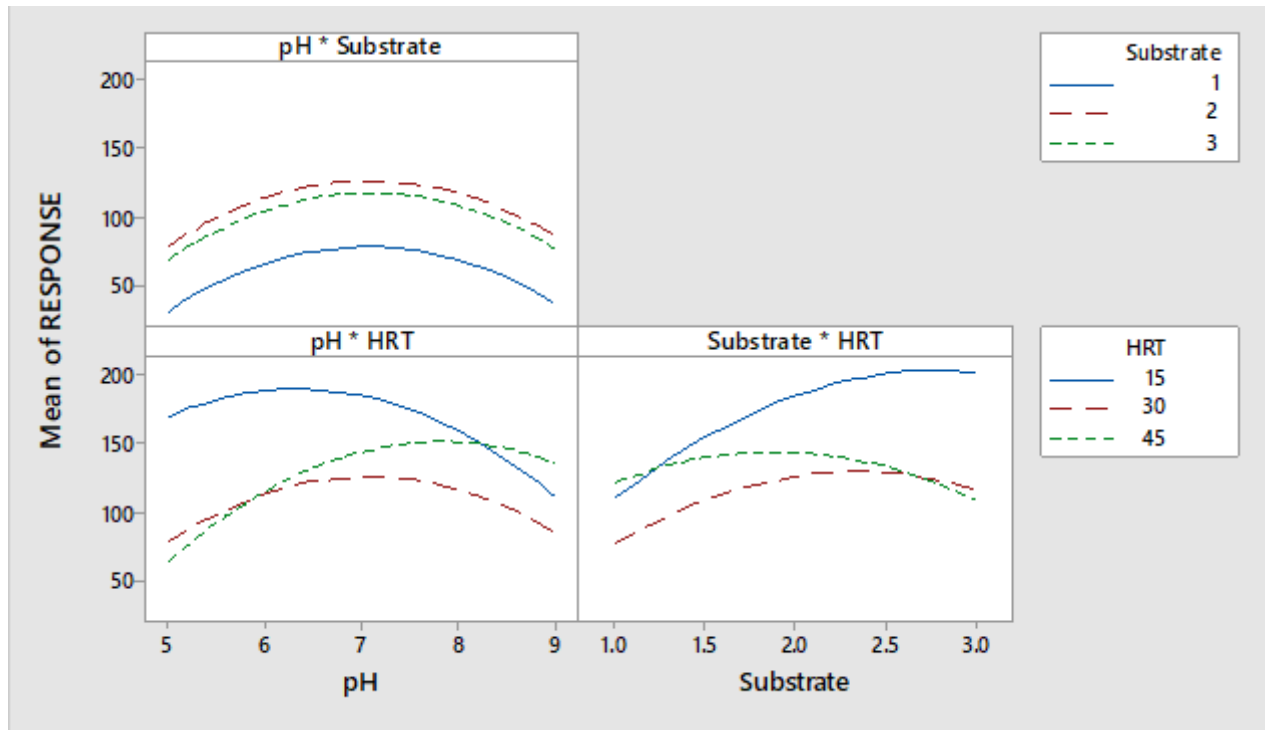


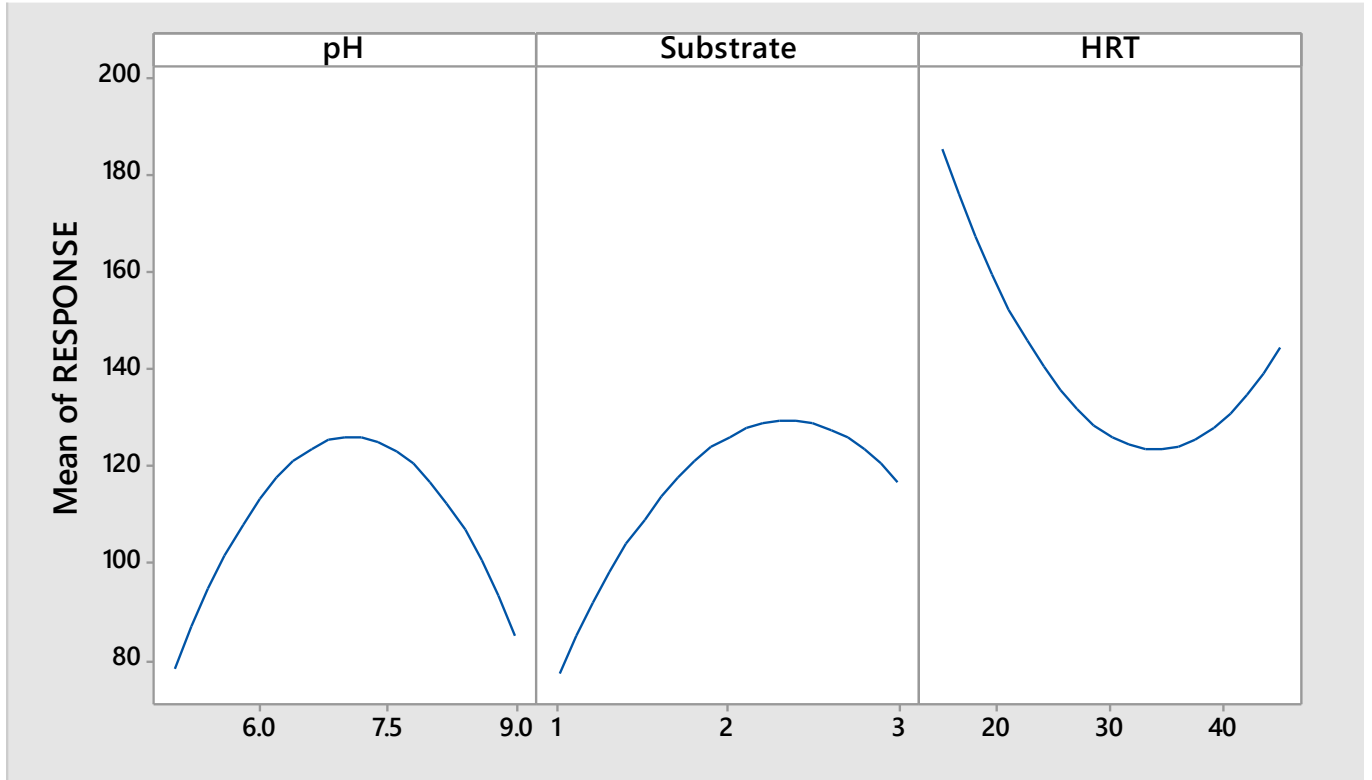
Figure 4.25: Interaction of the factors affecting the biogas yield.

### **4.6.3 Main effects plot for Response**

The effect of pH, Substrate ratio and Hydraulic retention time on biogas yield is shown in figure 4.26. The results show that there was a gradual increase in biogas yield from the beginning of the anaerobic digestion till at about pH value 6.8-7.5, followed by a drastic decrease in yield as the pH increased.

There was an increase in biogas yield from the beginning of the study but started decreasing gradually with increase in Hydraulic retention time although a little increase in yield was observed beyond 40days HRT.

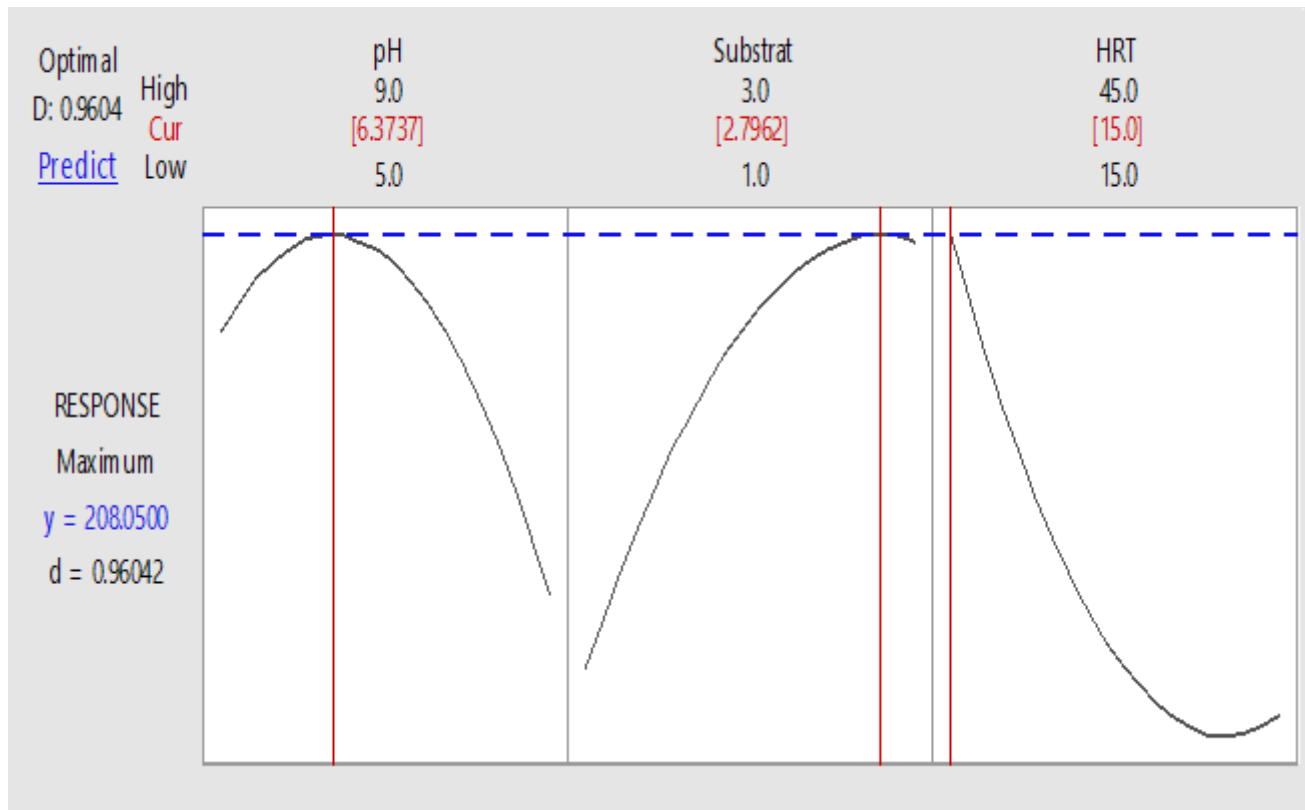
On the other hand, the biogas yield increased as the substrate ratio was increased. As explained earlier, the pH, Substrate ratio and Hydraulic retention time have significant roles to play in biogas yield.



**Figure 4.26: Observed effects of pH, Substrate ratio and Hydraulic retention time on biogas yield.**

#### **4.6.4 Optimization of the factors affecting biogas production**

Figure 4.27 shows the optimization plot of biogas yield obtained from co-digestion of Cassava peels and cow dung. Results show that the optimum conditions for the production of biogas are pH, Substrate ratio and Hydraulic retention time values of 6.37, 2.8: 1 and 15 days respectively. The results also show that the maximum yield predicted from this data is 208.05. Repeating the experiment under optimum conditions, a yield of 277.56 was obtained.



**Figure 4.27: The Optimization plot of biogas yield obtained from co-digestion of cassava peels and cow dung.**

#### **4.7: Anaerobic digestion of Cassava peels co-digested with cow dung using the predicted values**

Cassava peels co-digested with cow dung using the predicted values of pH 6.3737, substrate ratio 2.7962:1 for 15 days HRT showed the same trends in result as the experimental result. The maximum yield predicted from the data was 208.05 while the experimental result using the optimum conditions had a higher biogas yield of 277.56.

**Table 4.13: Anaerobic digestion of Cassava peels co-digested with cow dung using the predicted values of daily biogas yield**

<b>Days</b>	<b>Replicates</b>		
	<b>D1</b>	<b>D2</b>	<b>D3</b>
<b>1</b>	110	140	180
<b>2</b>	1374	1224	1684
<b>3</b>	1120	1160	1115
<b>4</b>	790	400	610
<b>5</b>	276	276	275
<b>6</b>	290	370	222
<b>7</b>	200	170	276
<b>8</b>	0	60	22
<b>9</b>	60	22	0
<b>10</b>	0	0	42
<b>11</b>	0	22	0
<b>12</b>	0	0	0
<b>13</b>	0	0	0
<b>14</b>	0	0	0
<b>15</b>	0	0	0
<b>Mean of replicates</b>	<b>281.333</b>	<b>256.267</b>	<b>295.067</b>
<b>Mean ± standard deviation</b>			<b>277.56</b>

#### **4.11: Discussion**

Agricultural wastes are widely used nowadays as sources of feedstock for anaerobic digestion and biogas production and have a very high yield in large scale production of biofuels. Proximate analysis of the substrates used in the study; Palm oil mill effluent, cow dung, pig dung, poultry manure, cassava peels and cabbage were conducted. Results revealed that none of the substrates had the optimum C: N ratio, some of the substrates had low C: N ratios while cassava peels had a C: N ratio of 46:1 which is slightly higher than the optimum range of 20-30:1 (Sawyer, Trois & Workneh, 2019). The C: N ratio of POME (10.13:1) supports the report of Adela, Muzzammil Loh & Choo, (2014) with C/N ratio of 10.58:1%.

Carbon to nitrogen ratio (C: N) is one of the important factors that influence biogas production from different substrates and this makes it a vital parameter to be given due consideration in enhancing biogas production from feedstocks (Nuhu, Mujahid, Aminu, Abbas, Babangid, Tsunatu, Aminu, Mustapha, Ahmed & Onuka, 2013). When the C: N ratio of the substrate is very high, nitrogen will be rapidly used up by the methanogenic bacteria to meet up with their protein needs and there will be no more reactions on the remaining carbon content of the material resulting to low biogas production. On the other hand, if the C: N ratio is very low, microbial metabolism will result in excessive liberation of nitrogen which will accumulate in the form of ammonia and this causes drastic increase in the pH value of the digesting slurry above 8.5, consequently exerting toxic effect on the methanogens (Dioha et al., 2014); Ganiyu & Oloke, 2012).

The proximate composition of digester feeds has a very big influence on the quality and biogas yield. Agricultural wastes of plant origin such as crop residues are not easily digestible as animal wastes because of difficulty in the hydrolysis of cellulose, hemicelluloses and lignocellulosic

constituents (Bolaji & Adebayo,2018).Also the quality of biogas (methane content) and its cumulative yield is highly dependent on the characteristics of the feedstock as it is not advisable to use one agricultural waste singly as biomass in biogas production (Sawyer et al., 2019; Nsair,Cinar, Alassali, Qdais & Kucht, 2020). Therefore to achieve optimum microbiological activities, in anaerobic digestion generally and enhance biogas production, improving the characteristics of the feedstock and other vital parameters of the digester is a necessity. The improvement in the cumulative yield in biogas production by co-digested substrates could be attributed to the factors mentioned above (Aragwa et al., 2013); Tetteh, Amano, Denis, Asante-Sackey and Edward,(2018);Bhatnagar,Ryan, Murphy& Enright 2018).

The total solids (TS) and volatile solids content of POME is low (13.93 and 13.39%) and this may be attributed to be the cause of the low biogas yield. This is because the proximate composition of substrates has very great influence on biogas yield. Also Ibrahim et al. (1984) reported that the methanogenesis process which is a very important terminal step of producing biogas in anaerobic digestion of POME alone was affected due to its physicochemical properties resulting to reduction in the amount of methanogens hence low biogas yield.

In this study, eleven (11) bacterial species were isolated. These were *Bacillus*, *Pseudomonas*, *Enterococcus*, *Staphylococcus*, *Micrococcus*, *Erwinia*, *Vibrio*, *Escherichia coli*, *Enterobacter*, *Samonella* and *Shigella* species. Also isolated were five fungal species; *Saccharomyces*, *Aspergillus*, *Rhizopus*, *Penicillium*, *Geotrichium* species.The microorganisms isolated from the digesting slurry are in agreement with Asikong,Udensi, Ekpoke, Eja & Antai, (2014).

Under anaerobic and ambient temperature range of 25<sup>0</sup>C to 36<sup>0</sup>C and hydraulic retention time of 45days, palm oil mill effluent was co digested with cow dung, pig dung, poultry manure, cassava peels and cabbage waste. Within the period of digestion, the biogas produced in the anaerobic

digestion process was flammable in the following bioreactors co-digestion setup POME/CD, POME/PD, POME/CP, POME/CB (3L: 520g, 3L: 600g and 3L: 680g), POME/PM (3L: 680g) only, and POME alone. POME/PM 3L: 520g and POME/PM 3L: 600g did not produce flammable biogas. The delay in flammable biogas production of POME/PM (3L: 680g) which started on the 33<sup>rd</sup> day and the inability of POME/PM 3L: 520g and POME/PM 3L: 600g to produce flammable biogas may be attributed to the production of ammonia due to imbalance in C/N ratio. This delay period was also reported by Ofoefule & Uzodinma, (2009) in the blend of cassava peels waste with pig dung in anaerobic digestion process.

The cumulative biogas yield of the co-digested substrates POME/CD (3L: 520g, 3L: 600g and 3L: 680g), POME/PD (3L: 520g, 3L: 600g and 3L: 680g), POME/PM (3L: 520g, 3L: 600g and 3L: 680g), POME/CP (3L: 520g, 3L: 600g and 3L: 680g), POME/CB (3L: 520g, 3L: 600g and 3L: 680g) had higher cumulative biogas yield of 8.035dm<sup>3</sup>, 10.344dm<sup>3</sup>, 7.570dm<sup>3</sup>; 6.529dm<sup>3</sup>, 6.171dm<sup>3</sup>, 9.306dm<sup>3</sup>; 4.601dm<sup>3</sup>, 6.462dm<sup>3</sup>, 7.995dm<sup>3</sup>; 7.082dm<sup>3</sup>, 5.182 dm<sup>3</sup>, 9.055dm<sup>3</sup> and 9.127dm<sup>3</sup>, 9.284dm<sup>3</sup>, 8.332dm<sup>3</sup> respectively than POME alone that had cumulative biogas yield of 4.635dm<sup>3</sup>, showing that co-digestion of these substrates was capable of improving the efficiency of biogas production. It also shows that improvement of the characteristics of the substrates and other factors of the bioreactor is required for optimum microbial activities in anaerobic digestion and effective biogas production (Tetteh et al. (2018). Similar result was observed by Murtoet al. (2004) who reported that co-digestion can improve biogas production by 50-200% depending on the operating condition and the substrates used in the anaerobic digestion. Aragwa et al. (2013) also reported that co-digestion of different feedstock substantially enhanced the biogas yields by 24 to 47% over the control (organic kitchen waste and dairy manure only).

The result also shows that the highest percentage methane yield was achieved from co-digestion of POME/CD (3L: 600g). The methane level achieved was 77.89% for POME/CD (3L: 600g). Other mixtures were 76.66% for POME/PD (3L: 680g), 44.14% for POME/PM (3L: 680g), 65.28% for POME/CP (3L:680g), 68.80% for POME/CB (3L: 600g) and 56.53% for POME alone (control). From this study, the mixing ratio of POME/CD (3L: 600g) has been recognized as suitable mixture for biogas production as well as methane content. In this study also, only 56.53% of methane was produced from the anaerobic mono-digestion of POME. The potency of co-digestion is once more proven as the production level was elevated from 56.53% to as much as 77.89% in terms of methane content (Murto et al., 2004).

Studies on anaerobic digestion of pig dung (PD) as single substrate and its co-digestion with cow dung (CD), poultry manure (PM) and cabbage (CB) was conducted. All the substrates were anaerobically digested at the ratios of 1:1, 2:1, and 3:1, at ambient temperature range of 25<sup>0</sup>C to 36<sup>0</sup>C and hydraulic retention time of 45days. The biogas produced in the anaerobic digestion process was flammable in bioreactors co-digested with PD/CD 1:1, PD/PM 2:1, PD/PM 3:1, PD/CB 1:1, PD/CB 2:1 and PD alone while PD/CD 2:1, PD/CD 3:1, PD/PM 1:1 and PD/CB 3:1 did not produce flammable biogas. A similar observation was reported by Oporum et al.(2019) in which goat manure was co-digested with poultry dropping and plantain peels for biogas production. This may be attributed to a lot of factors like the production of ammonia due to imbalance in C/N ratio or slow rate of hydrolysis of complex polysaccharides and lignocellulosic constituents that makes plant residues indigestible (Bolaji & Adebayo, 2018).

The cumulative biogas yield of the co-digested substrates, PD/CD(1:1, 2:1 and 3:1), PD/PM (1:1, 2:1 and 3:1) and PD/CB (1:1, 2:1 and 3:1) had higher cumulative biogas yield of 5.777dm<sup>3</sup>, 1.591dm<sup>3</sup>, 1.792dm<sup>3</sup>; 1.189dm<sup>3</sup>, 0.862dm<sup>3</sup>, 4.417dm<sup>3</sup> and 4.794dm<sup>3</sup>, 0.838dm<sup>3</sup>, 4.316dm<sup>3</sup>

respectively than PD alone that had cumulative biogas yield of 3.370dm<sup>3</sup>. This shows that co-digestion of these substrates was capable of improving the efficiency of biogas production.

The result also shows that the highest percentage methane yield was achieved from co-digestion of PD/CD (1:1). The methane level achieved was 65.24% for PD/CD (1:1), then other mixtures was 63.20% for PD/CB (1:1), 61.39% for PD/PM (3:1), and 44.14% for PD alone (control). The remaining percentages were constituted by the following: Carbon-dioxide, Carbon-monoxide, and trace elements namely; hydrogen (H<sub>2</sub>), oxygen (O<sub>2</sub>), nitrogen (N<sub>2</sub>) and water (H<sub>2</sub>O). The potency of co-digestion is once more proven as the production level was also elevated from 44.14% to 65.24 % methane content. Biogas production was sluggish till the end of the hydraulic retention time. This observation may also be associated with a number of factors; pH, nutritional composition of the substrate, and C: N ratio (Tetteh et al., 2018). For biogas to be useful and effective for cooking and lighting, it must be combustible and the methane content must be more than 45%, but if it doesn't flame, it means that the methane content is less than 45% and has more of CO<sub>2</sub> and other trace gases. This implies that it may not be useful for the purpose of energy utilization (Ofoefule, Onyeoziri & Uzodinma, 2011).

Furthermore the anaerobic digestion of cassava peels (CP) as single substrate and its co-digestion with cow dung (CD), pig dung (PD) and poultry manure (PM) were anaerobically digested at the ratios of 1:1, 2:1, and 3:1, at temperature range of 25<sup>0</sup>C to 36<sup>0</sup>C and hydraulic retention time of 45days. The total solid (TS) content of cassava peels used is 88.52% and the moisture content of 11.48%. This supports the report of Nkodi, Taba, Kayembe, Mulaji & Mihigo (2016). The proximate composition of feedstocks also affect the quality and yield of the biogas and this could be attributed to the increase in biogas yield of cassava peels alone as similar result was reported by Oporum et al. (2019). The biogas produced in the anaerobic digestion process was flammable

in all the bioreactors with CP/CD 3:1 having both the highest biogas yield and percentage methane content. The increase in biogas yield from CP/CD 3:1 ratio could be due to the synergistic effect of the nutrient contents of the feedstocks. Esposito, Frunzo, Liotta, Panico & Pirozzi (2012), reported that there is synergistic effect on co-digestion substrates as microbial degradation level of the mixture increases more than that of single substrate.

The cumulative biogas yield of the co-digested substrates CP/CD(1:1, 2:1 and 3:1) and CP/PD(1:1, 2:1 and 3:1), had higher cumulative biogas yield of 6.631dm<sup>3</sup>, 6.652dm<sup>3</sup>, 12.347dm<sup>3</sup> and 6.317dm<sup>3</sup>, 5.780dm<sup>3</sup>, 9.300dm<sup>3</sup> respectively and CP/ PM (1:1, 2:1 and 3:1) had a low cumulative biogas yield of 2.762dm<sup>3</sup>, 2.678dm<sup>3</sup>, 3.491dm<sup>3</sup> CP alone (control) had cumulative biogas yield of 8.690dm<sup>3</sup>. The result shows that the highest percentage methane yield was achieved from co-digestion of CP/CD (3:1). The methane level achieved was 78.05% for CP/CD (3:1), others were 64.05% for CP/PD (3:1), 55.77% for CP/PM (3:1), and 49.390% for CP alone (control). The remaining percentages were constituted by the following: Carbon-dioxide, Carbon-monoxide, and trace elements namely; hydrogen (H<sub>2</sub>), oxygen (O<sub>2</sub>), nitrogen (N<sub>2</sub>) and water (H<sub>2</sub>O). The potency of co-digestion is once more proven as the production level was also elevated from 49.390% to 78.05 % methane content.

Cassava peels co-digested with cow dung (CP/CD) which had the highest biogas yield was optimized by anaerobic digestion in triplicate at the ratios of 1:1, 2:1 and 3:1, pH of 5, 7, and 9 and Hydraulic Retention Time of 15, 30 and 45 days at ambient temperature using the values obtained from the design. The biogas produced in the anaerobic digestion process was flammable in all the bioreactors, but the best bioreactors that flamed early with highest percentage methane content were selected. These were the bioreactors charged with CP/CD 2:1 substrate ratio, pH5 for 15 days HRT, CP/CD 3:1 substrate ratio, pH7 for 15 days HRT and CP/CD 2:1 substrate ratio,

pH9 for 45days HRT. The result shows that the highest percentage methane yield was achieved from co-digestion of CP/CD (3:1), pH7 for 15days HRT. The methane level achieved was 79.14%, others were 54.85% for CP/CD (2:1), pH9 for 45days HRT and 54.85% for CP/CD (2:1), pH5 for 15days HRT.

Optimization response also proved that digester that was digested with 3:1 substrate ratio at pH7 for 15days HRT had the highest response of 214.69. This was in the same trend with the report of Rachadaporn et al. (2016) that carried a research on modeling of anaerobic co-digestion of pig manure and domestic organic waste and Ogiehor & Ovueni (2014) that evaluated the effect of temperature, pH and solid concentration on biogas production from poultry wastes.

The response surface plots results revealed that increase in substrate ratio increased the biogas yield while increase in pH and hydraulic retention time decreased the biogas yield.

The interaction plot result shows that there was no interaction between pH and substrate ratio while there was interaction between pH and hydraulic retention time as well as substrate ratio and hydraulic retention time in all the values.

The result of the main effects plot for response shows that there was a decrease in biogas yield at low pH value but increased drastically as the pH increased to 6.8 - 7.5 followed by a decrease in yield as the pH increased to the highest pH value.

The biogas yield was high at the start of the study but started decreasing with increase in HRT. On the hand, the biogas yield increased with increase in substrate ratio. They study therefore proves that pH, substrate ratio and hydraulic retention time have significant roles to play in biogas yield.

Optimization of the factors using the predicted values of pH 6.37, substrate ratio 2.8:1 for 15days hydraulic retention time showed the same trends in result as the experimental result (pH7,

3:1 substrate ratio for 15 days HRT) which supports the work of Ogiehor & Ovueni (2014). The results also show that the maximum yield predicted from this data is 208.05 m<sup>3</sup>kg<sup>-1</sup>VS per kg of TS. Repeating the experiment under optimum conditions gave a higher yield of 277.56 m<sup>3</sup>kg<sup>-1</sup>VS per kg of TS.

## CHAPTER FIVE

### CONCLUSION AND RECOMMENDATION

#### 5.1 Conclusion

This research work shows that co-digestion of agrowastes which are abundantly available was capable of improving the efficiency of biogas production, as they are very good substrates that can be used as feedstock for biogas production to convert trash to treasure and to reduce greenhouse gas emission. It has also shown that co-digestion of cassava peels and cow dung (CP/CD 3:1) at pH7 for 15days hydraulic retention time is statistically different from other treatments hence is capable of improving biogas yield and could be adopted in large scale biogas production for domestic use, proper waste management and prevention of greenhouse effect. All these substrates used in the study and other agrowastes that are disposed indiscriminately, causing environmental pollution and health hazards can also be converted through anaerobic digestion and biogas production to biofuel to bring solution to the energy crisis in the country and the digestate can be used as soil conditioner.

#### 5.2 Recommendation

Production of agrowastes in the society is inevitable. They are produced by several sectors including the agricultural, industrial and municipal sectors. Agrowastes like palm oil mill effluent, cow dung, pig dung, poultry manure, cassava peels and cabbage, especially cassava peels, are good substrates for biogas production.

For effective and adequate utilization of cassava peels and other agrowastes in biogas production,

- Further research has to be carried out in large scale using the observed parameters from the study to enhance biogas production.

- Higher institutions in the nation should have a biogas plant which can be used as a study plant for the institution to facilitate the knowledge of biogas technology.
- Higher institutions should encourage Interdisciplinary research collaborations involving all the departments so that the biogas produced can be purified, compressed into a cylinder and can now be used to cook, generate electricity and in automobile engines.
- Government agencies should help in providing mini and affordable biodigesters for researchers and household use as it is done in other countries like China, India etc.

### **5.3 Contribution to Knowledge**

- Results obtained from this research work show that we can generate wealth from wastes through biogas production.
- The experimental design helped us to obtain the optimal conditions needed to enhance biogas production.
- The problem associated with the use of fossil fuel is reduced.
- Clean and green environment is maintained by reduction of greenhouse gas emission which causes global warming.
- Finally the digestate can be used as soil conditioner.

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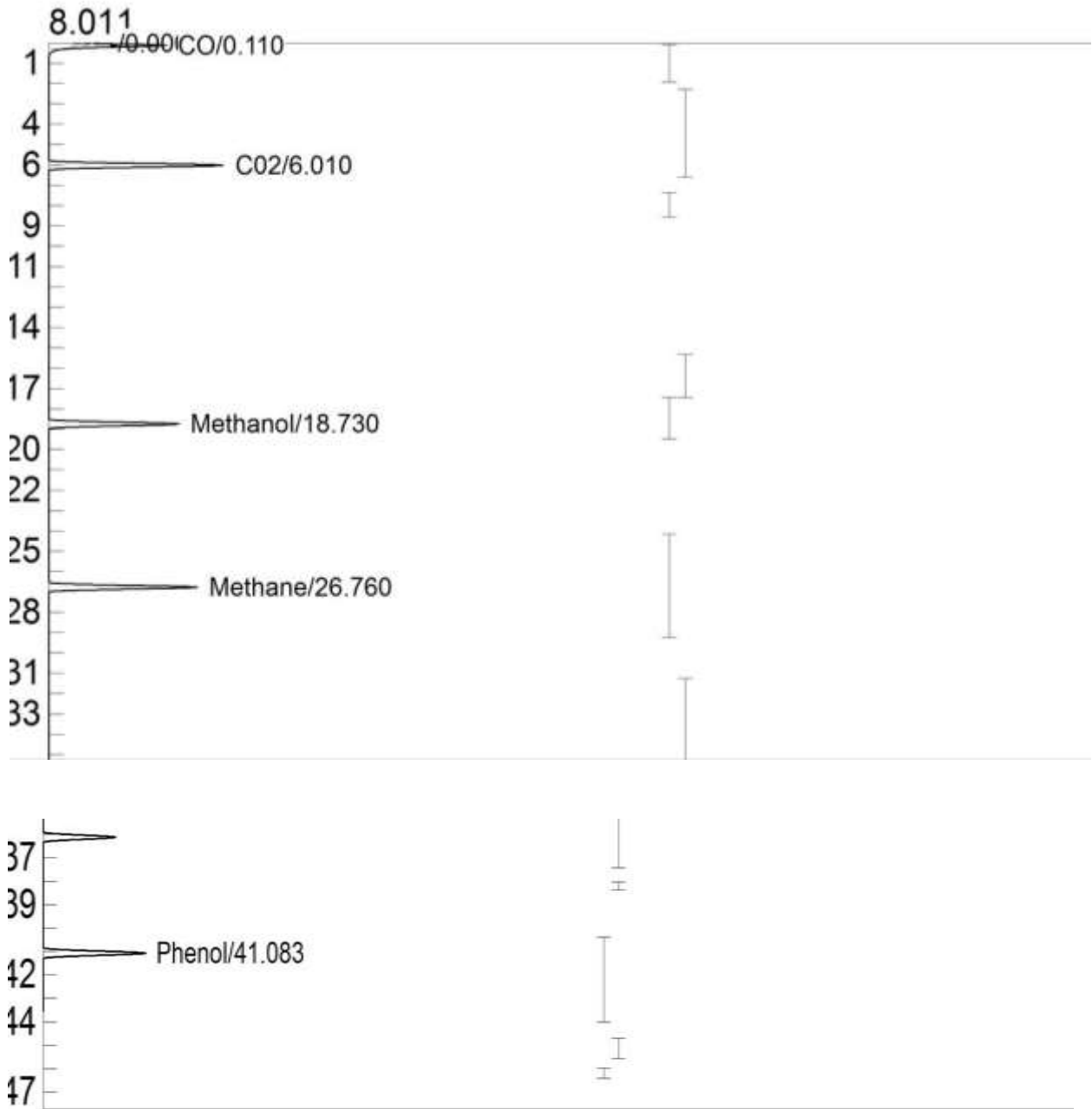
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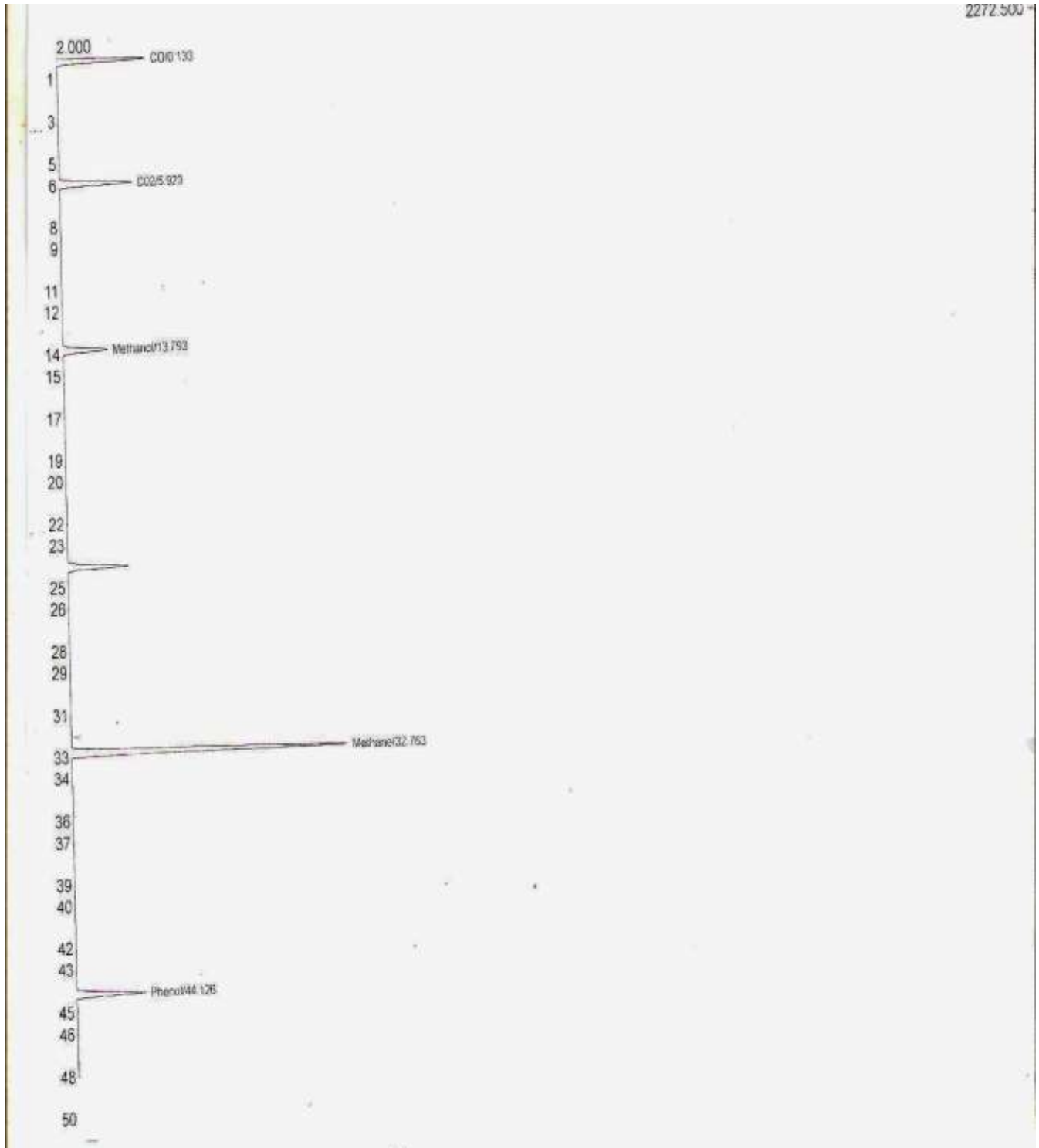
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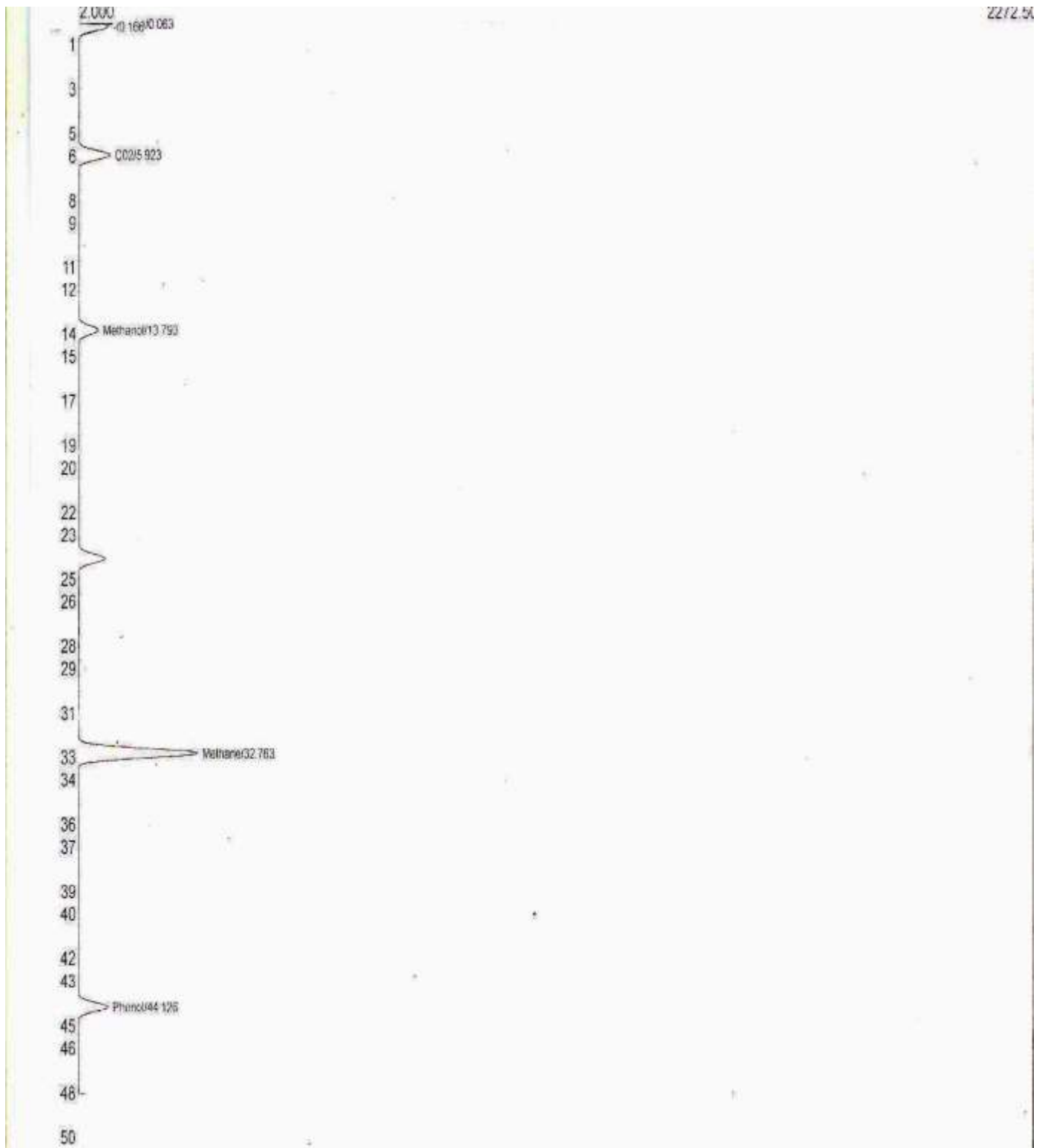
APPENDIX



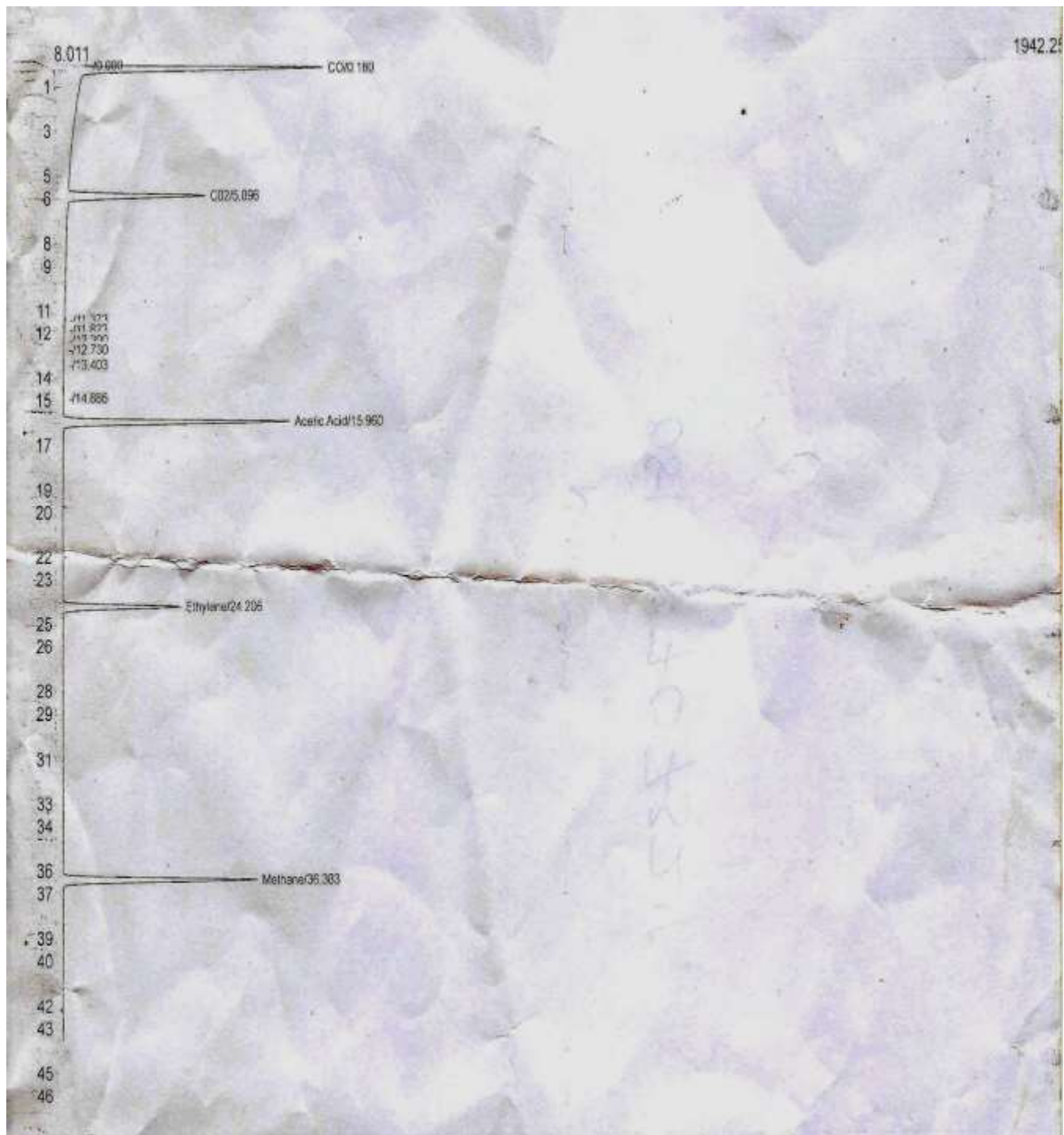
Chromatogram of POME control (3L of POME)



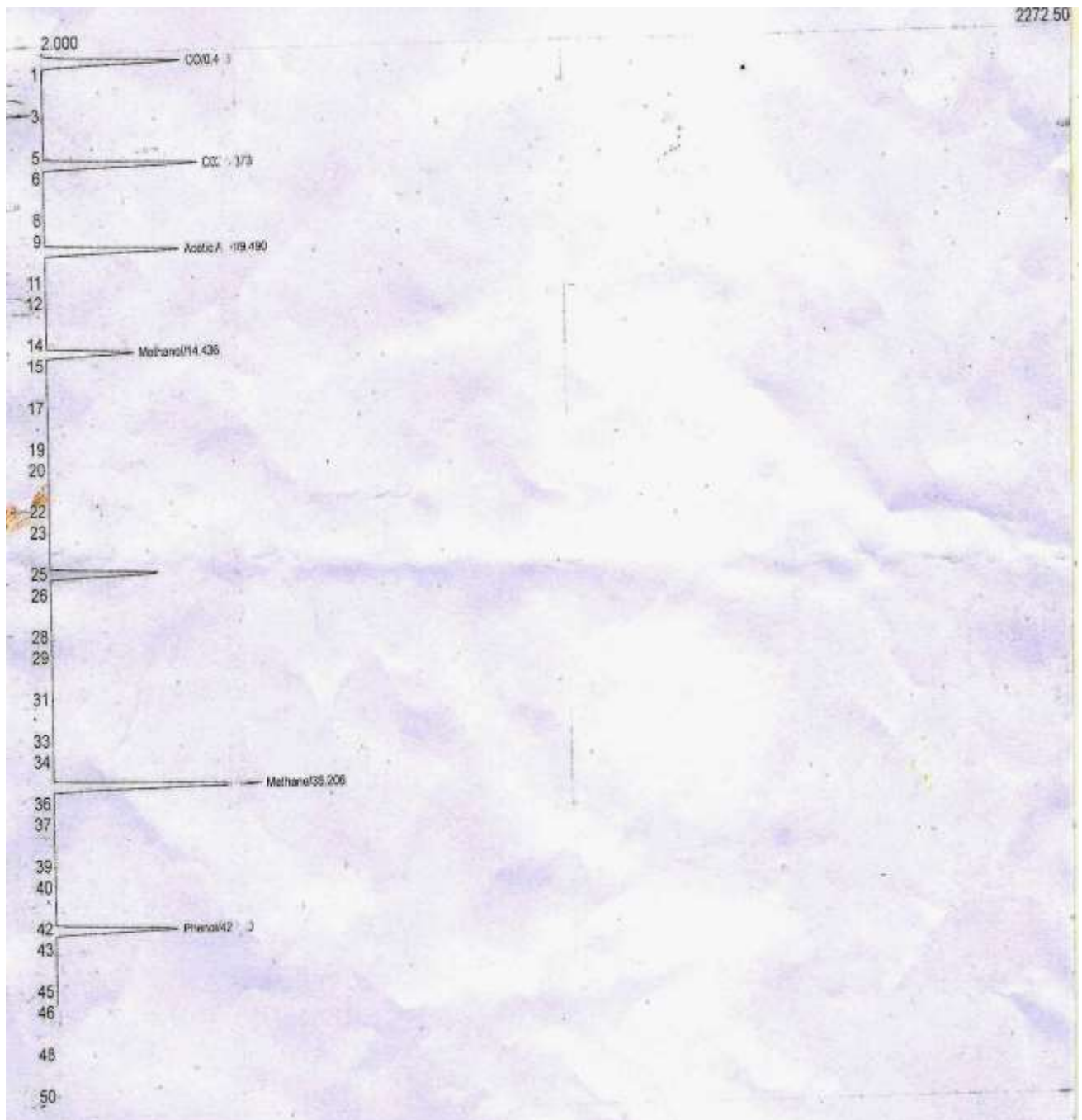
Chromatogram of POME co-digested with pig dung 3L:680g



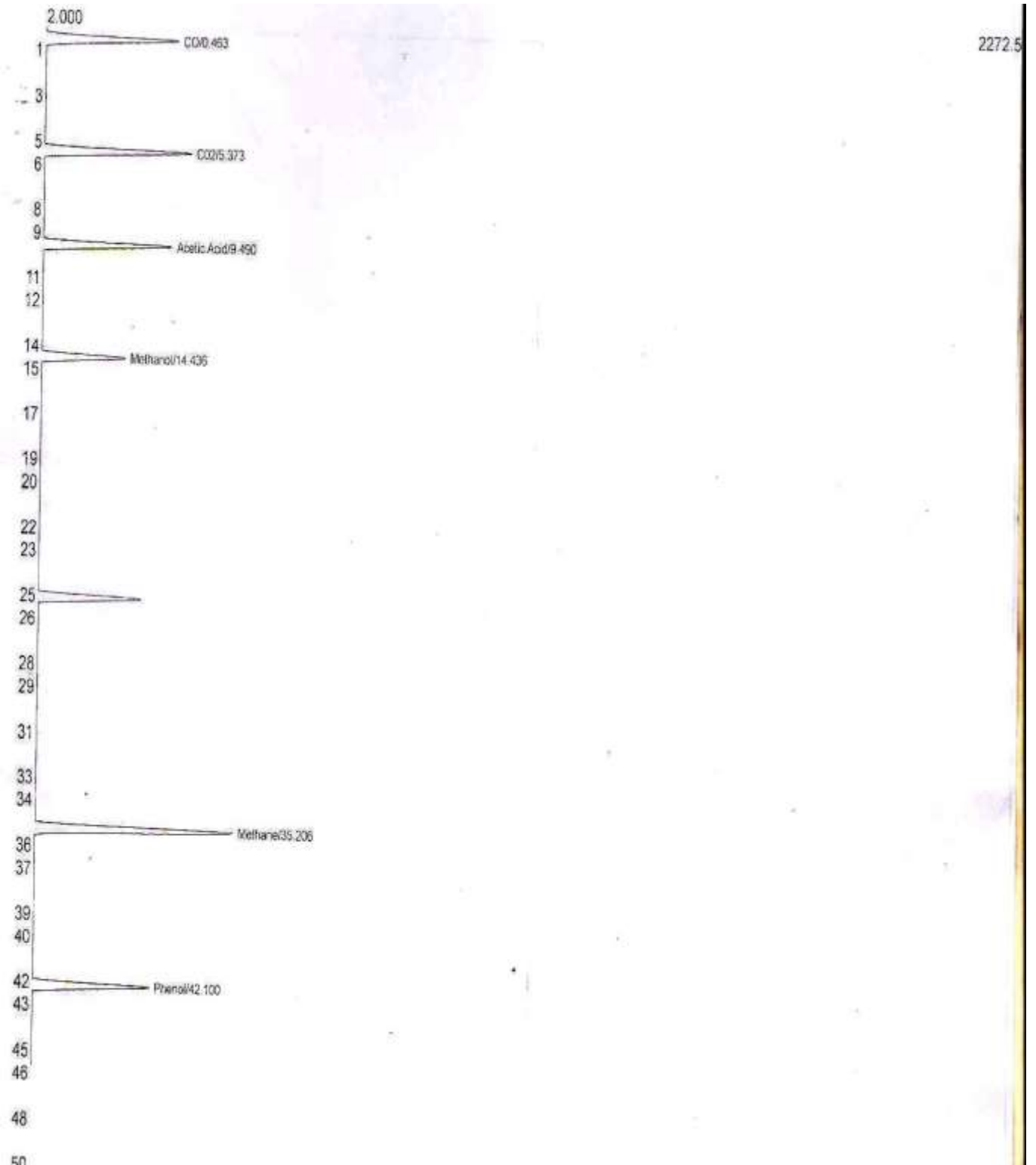
Chromatogram of POME co-digested with cow dung 3L:520g



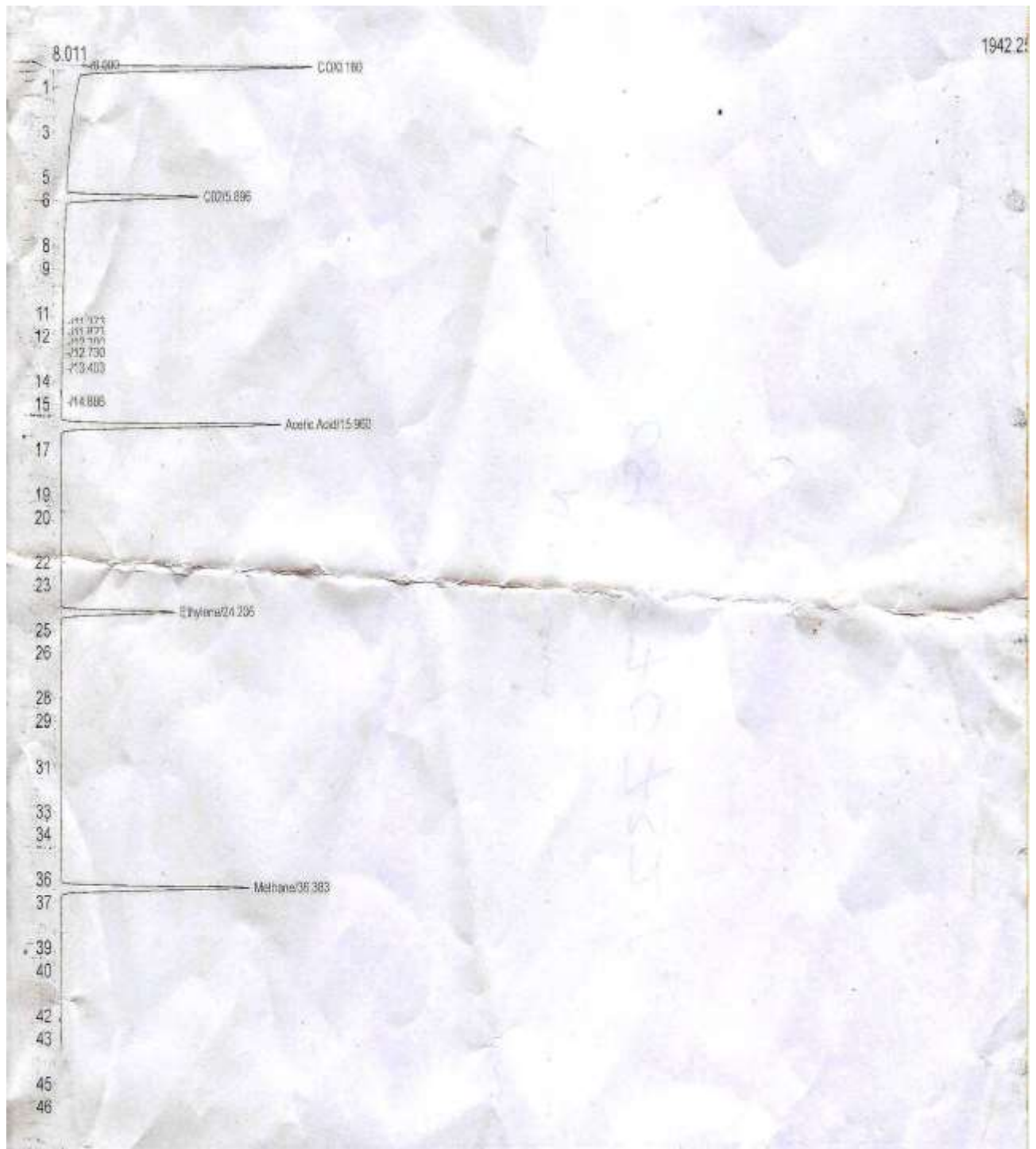
Chromatogram of POME co-digested with poultry manure 3L:680g



Chromatogram of POME co-digested with cassava peels 3L:680g



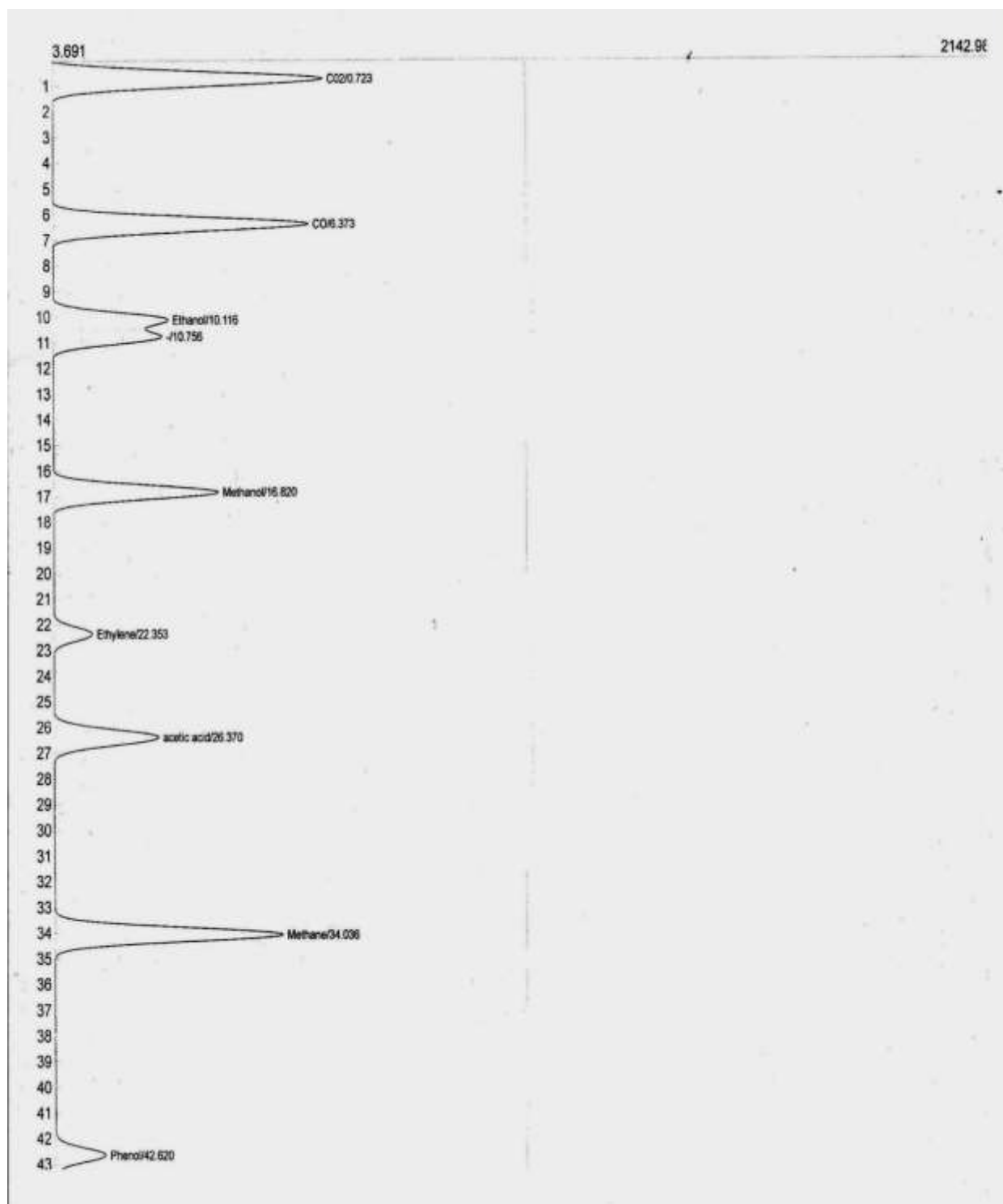
Chromatogram of POME co-digested with cabbage 3L:600g



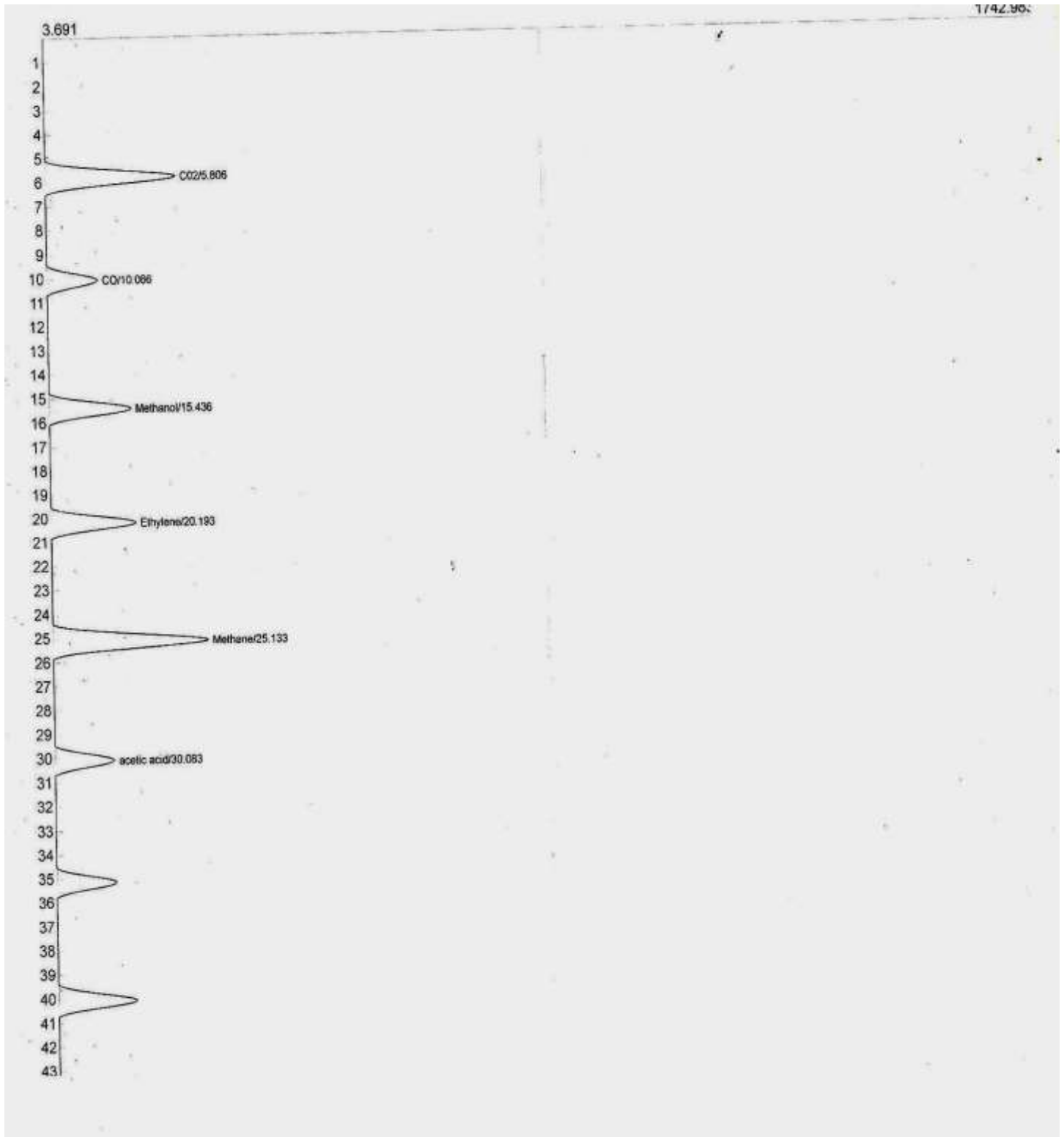
Chromatogram of pig dung control 520g



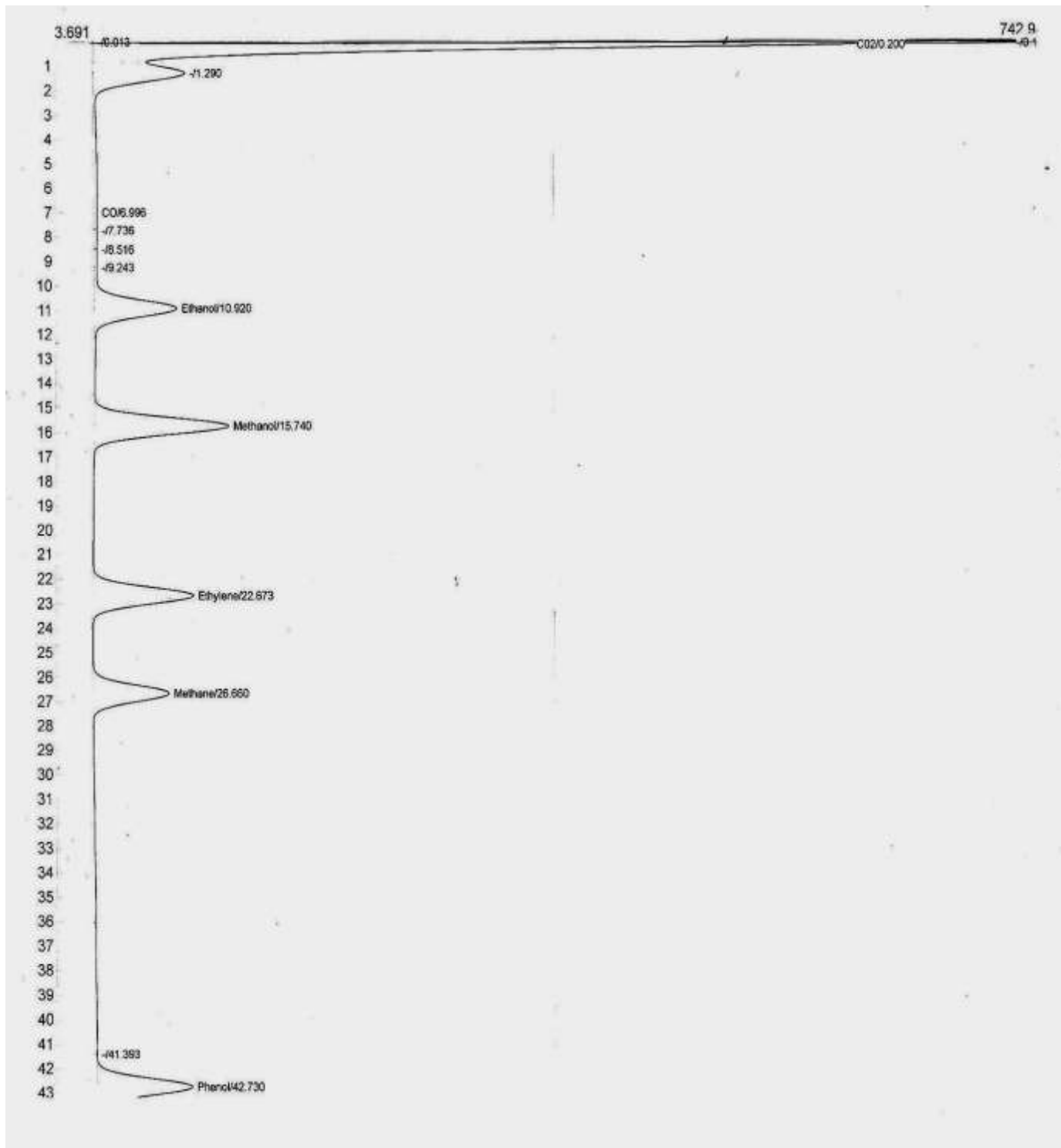
Chromatogram of pig dung co-digested with cow dung 1:1



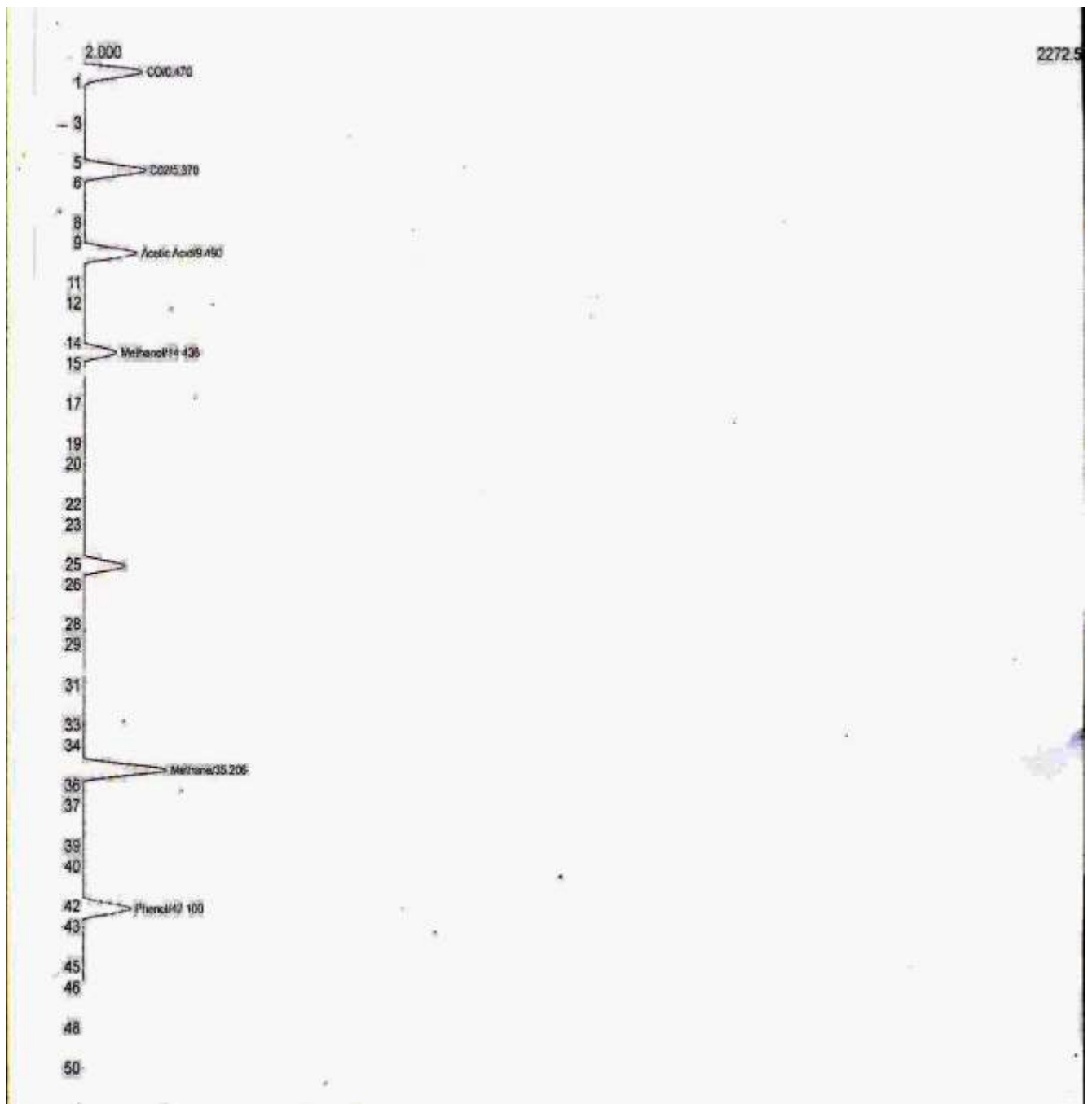
Chromatogram of pig dung co-digested with poultry manure 3:1



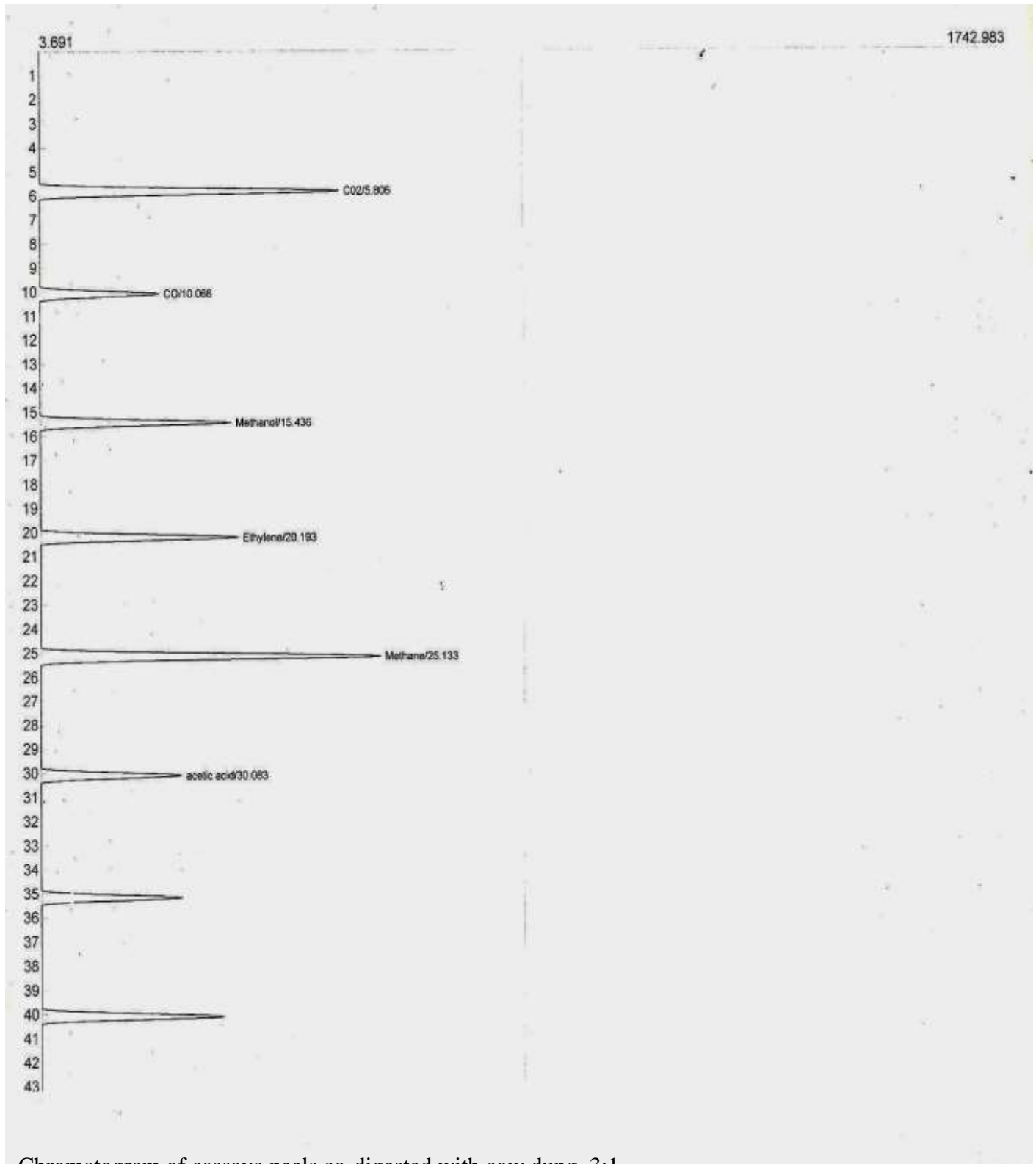
Chromatogram of pig dung co-digested with cabbage 1:1



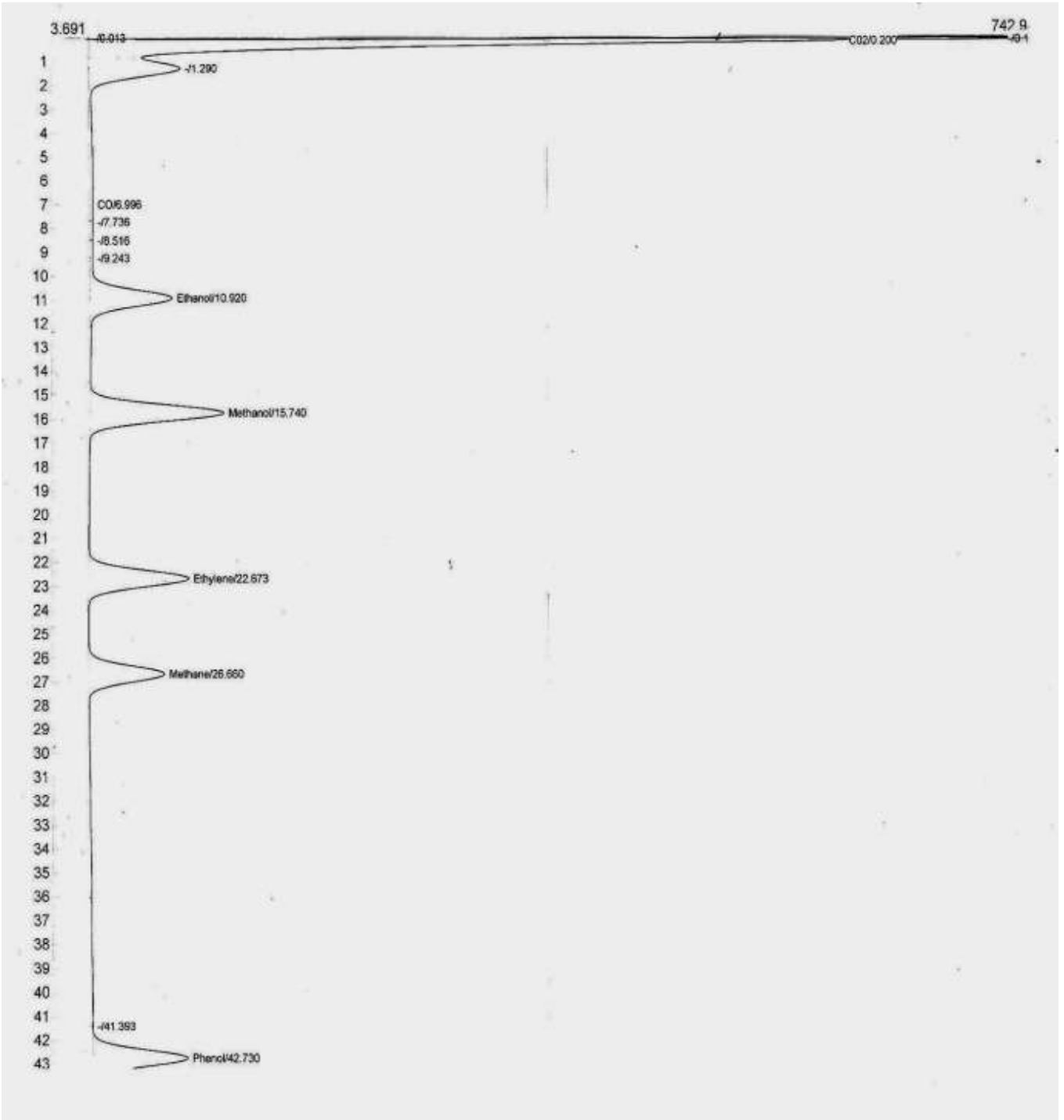
Chromatogram of cassava peels control (520g)



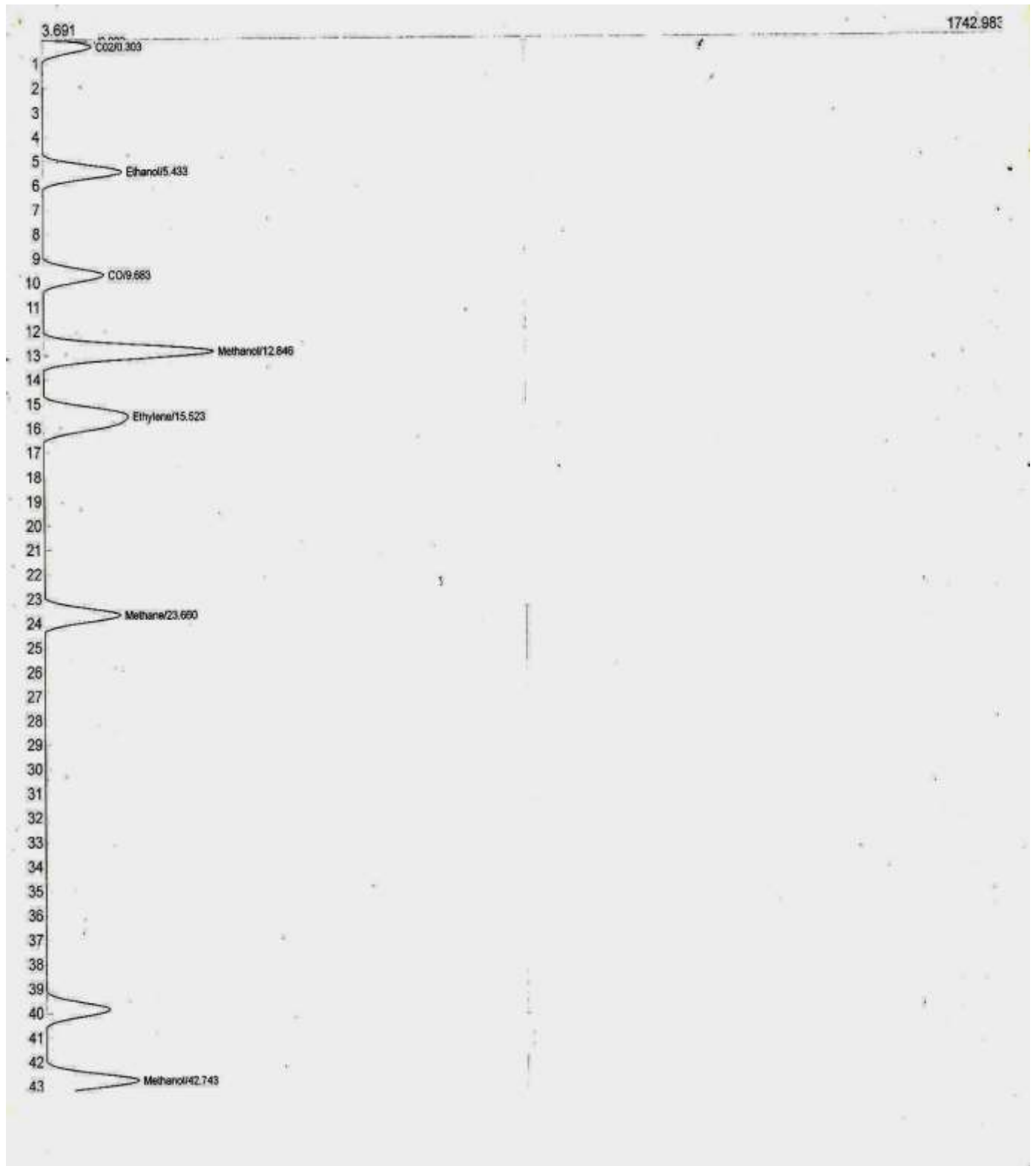
Chromatogram of cassava peels co-digested with pig dung, 3:1



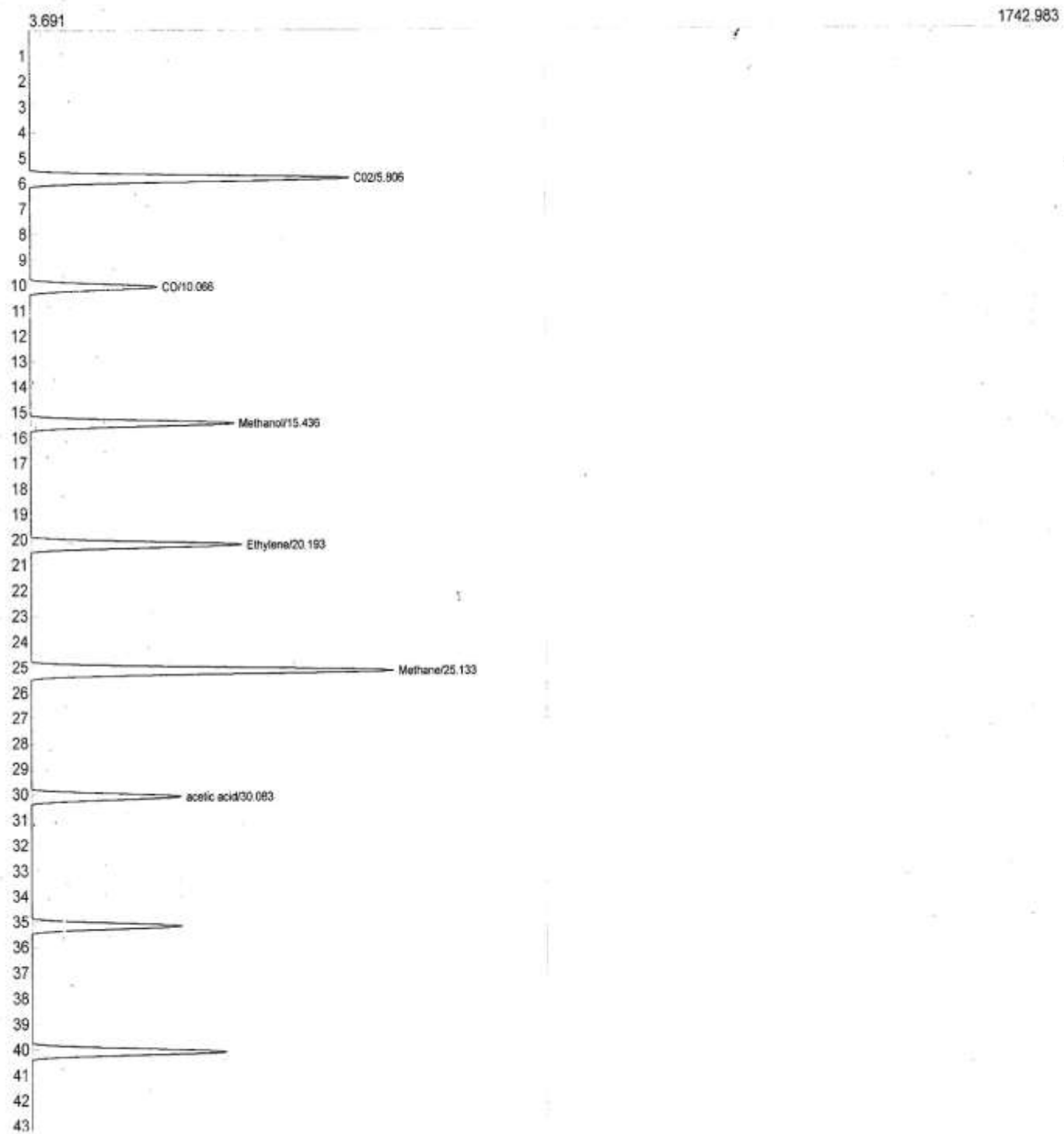
Chromatogram of cassava peels co-digested with cow dung, 3:1



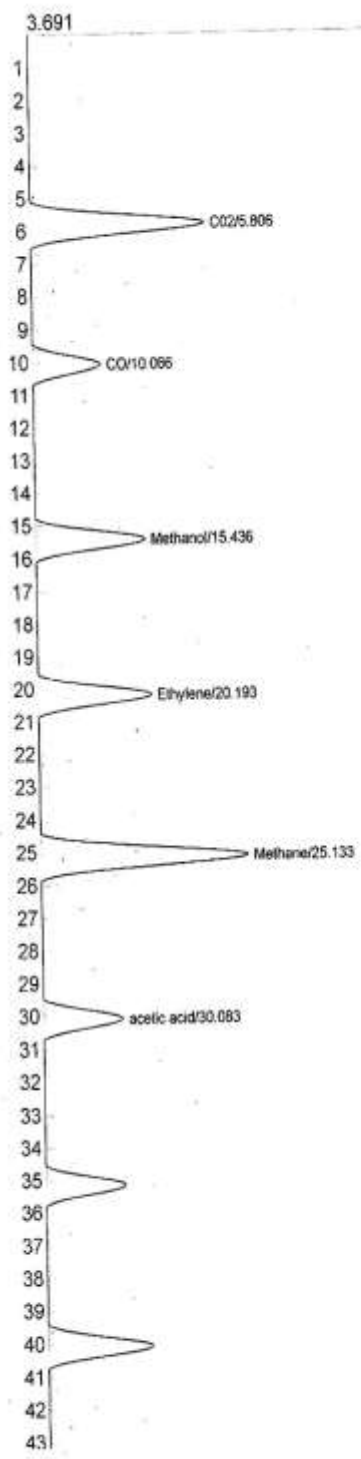
Chromatogram of cassava peels co-digested with poultry manure, 3:1



Chromatogram of cassava peels co-digested with cow dung, 2:1, pH5



Chromatogram of cassava peels co-digested with cow dung, 3:1 and pH7



Chromatogram of cassava peels co-digested with cow dung, 2:1, pH9

**Table 1: Means±standard deviation of daily biogas yield for digesters 2, 7, 11 And 12, anaerobically digested for 15 Days**

<b>MEAN OF REPLICATES</b>					
<b>DIGESTERS</b>		<b>D1</b>	<b>D2</b>	<b>D3</b>	<b>MEAN OF MEANS</b>
2	49.8	180.5	117.9	116.11	
7	230.6	124.0	180.7	178.49	
11	14.0	153.0	87.6	84.89	
12	337.2	99.0	207.8	214.69	

**Table 2: Mean± standard deviation of daily biogas yield for digesters 3, 4, 5, 6, 10, 13 and 14 anaerobically digested for 30 days.**

<b>MEAN OF REPLICATES</b>				
<b>DIGESTERS</b>	<b>D1</b>	<b>D2</b>	<b>D3</b>	<b>MEAN OF MEANS</b>
3	72.2	1.3	99.5	57.09
4	56.3	29.9	55.0	47.08
5	113.9	88.2	10.3	70.84
6	8.0	85.4	47.3	46.91
10	7.2	164.8	8.8	60.3
13	127.8	165.9	161.2	151.66
14	134.7	192.6	140.7	156.03

**Table 3: Mean± standard deviation of daily biogas yield for digesters 1, 8, 9 and 15 anaerobically digested for 45 days**

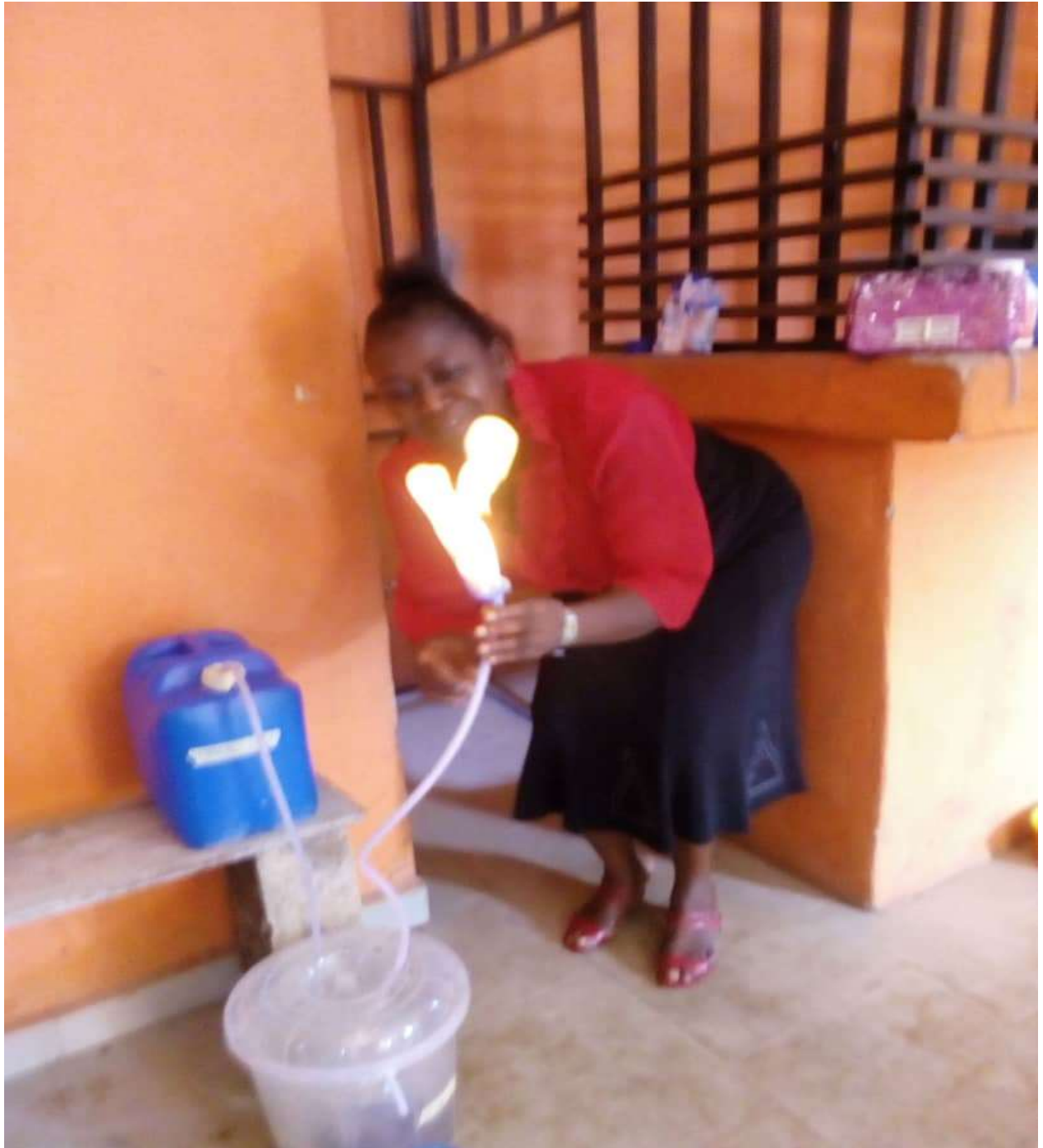
<b>MEAN OF REPLICATES</b>				
<b>DIGESTERS</b>	<b>D1</b>	<b>D2</b>	<b>D3</b>	<b>MEAN OF MEANS</b>
1	20.8	195.7	112.2	109.62
8	155.6	104.3	142.3	134.01
9	98.8	155.6	127.3	127.29
15	76.8	41.3	60.2	59.5



**Measuring the Substrates used in the anaerobic digestion**



**Taking the Daily pH Reading**



**Biogas Flamability Test**