POTENTIALS OF SELECTED CROPS FOR BIOFUEL PRODUCTION

BY

UMEGHALU, IFEANYICHIM CHINWUBA EDWIN

REGISTRATION NUMBER 2010217001P

A DISSERTATION SUBMITTED TO THE DEPARTMENT OF AGRICULTURAL AND BIORESOURCES ENGINEERING, FACULTY OF ENGINEERING, NNAMDI AZIKIWE UNIVERSITY AWKA, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF DOCTOR OF PHILOSOPHY (Ph. D) IN AGRICULTURAL AND BIORESOURCES ENGINEERING

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DEDICATION

This work is dedicated to God the creator of the universe and all in it.

To my late parents, Chief Francis Anaduaka Umeghalu (Ikpo-aku) and Ezinne Mrs. Elizabeth Nwudeafor Umeghalu (Lawyer) who saw the value of education and supported my quest.

To His Excellency General Olusegun Obasanjo (rtd) Former President and Commander-in-Chief of Nigerian Armed Forces, General Dr. Ambrose Amadu Ali and Late E.C. Unachukwu. The men who lit the candle of education in me.

CERTIFICATION

This Disertation titled Potentials of Selected Crops for Biofuel Production by UMEGHALU, Ifeanyichim Chinwuba Edwin, a postgraduate student in the Department of Agricultural and Bioresources Engineering, Faculty of Engineering, Nnamdi Azikiwe University, Awka, with Registration Number 2010217001P meets the requirement for the award of the Degree of Doctor of Philosophy (Ph.D) in Agricultural and Bioresourses Engineering of Nnamdi Azikiwe University, Awka, and is approved for its contribution to knowledge and literary presentation.

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ABSTRACT

Presently, man relies on fossil fuels as his major source of energy. However, the negative effects of fossil fuels usage have provided the pedigree to consider other alternative energy sources. Bio-fuel offers option as alternative energy source due to its environmental friendliness, its sustainability and it is renewable. Traditional crops used as dedicated energy crops for bio-ethanol production seems to have violated the technical and ecological reliance standard in terms of sustainability, by interfering with food chain. The aims and objectives of this study is to investigate the potentials of wild cocoyam, jackfruit seeds, water yam and yellow yam for bio-fuels production in terms of: (i) Characterization of the crops; (ii) Optimizing the enzymatic hydrolysis for ethanol production; (iii) Produce ethanol from the starches; (iv) Produce biogas from the fermentation brutes; (v) Study the kinematics of the biogas production. The starches of the crops were extracted by wet milling method. Reducing sugar yield was optimized using the Central Composite Design (CCD) and the result analyzed using Design Expert 8.0.7.1 Trial Version where time, enzyme concentration, water quantity and temperature are the variables. *Saccharomyces cerevisiae* yeast was used for starch fermentation. Distillation of fermentation wort was done using distillation apparatus. The broths were used for biogas production. Optimization of cumulative biogas production was studied using CCD and the optimized result analysis was investigated using Design Expert 8.0.7.1 Trial Version where major variables are time, inoculums concentration, water quantity and substrate concentration. Starch yields from the crops were: wild cocoyam-65.42%, jackfruit -51.65%, water yam-41.65%, and yellow yam26.17%. Optimal reducing sugar yield of 166.15mg/mg, 136.44mg/mg and 122.23mg/mg respectively were obtained for wild cocoyam, water yam, and yellow yam at the temperature of 70^{o}C, 0.3g/g enzyme concentration, 3ml/g water quantity and at 3hrs while yield of 142.58mg/mg was obtained for jackfruit at temperature 70^{o}C, 0.3g/g enzyme concentration, 3ml/g water quantity and at 2hrs. Ethanol yields were: wild cocoyam (79.6%); jackfruit (72.6%); water yam (63.1%), and yellow yam (53.6%). The cumulative biogas yields of 136.44ml, 122.23ml were obtained for wild cocoyam and jackfruit seed wastes respectively. Results from the study showed that wild cocoyam, jackfruit seeds and water yam are very good primary feedstock while yellow yam can be supplementary feedstock for bio-fuels production.

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SYMBOLS

AD- Anaerobic Digestion

AOAC - Association of Official Analytical Chemists

BOD - Biochemical Oxygen Demand

CBN – Central Bank of Nigeria

CD – Cow Dung

CGF - Corn Gluten Feed

COD – Chemical Oxygen Demand

CSTR --Completely Stirred Reactor

CS - Corn Syrups

DDGS -Distillers Dried Grains with Soluble

EREC - European Renewable Energy Commission

EU – E uropean Union

FAO - Food and Agricultural Organization

GHG - Greenhouse Gas

HRT - Hydraulic Retention Time

HSW - Heavy Steep Water

HFCS - High Fructose Corn Syrup

HFW-A - Hydrolysis and fermentation of worts alone

HFW - Waste from the process of hydrolysis and fermentation of worts

IEA - International Energy Agency

IITA - International Institute of Tropical Agriculture

OLR - Organic Loading Rate

RED - Renewable Energy Directive

RES - Renewable Energy Sources

LSW - Light Steep Water

MMT - Million Metric Tons

SRT - Sludge Retention Time

VFA - Volatile Fatty Acids

CHAPTER ONE

INTRODUCTION

1.1 Background of study

There is no activity of man that does not require energy. Presently, man relies on fossil fuels as the main energy source which has resulted to several environmental and economical challenges. Therefore, the search for environmentally friendly materials that have potential to substitute fossil oils in various industrial applications and domestic uses is currently being considered a top priority research topic in the fuel and energy sector. The emphasis is largely due to the rapidly depletion of world fossil fuel reserve (Mayer, 2001). This has made sustainable energy to become a critical national economic and security issue.

Transportation fuels make up a large portion of energy consumption in the world. One way to partially replace fossil based products is by the use of fuels derived via biochemical (Brown *et al*., 1994). The goal of these efforts is to safeguard the future of the planet and the natural resources which our children will need to secure a decent and beneficial future. Thus, the use of bio-fuels is increasing in many countries throughout the world.

At present, a total of approximately 30 billion litres of bio-fuel are used annually in Europe, North America, and South America (Don, 2004). This amount is expectedly increasing significantly as the demand for sustainable transportation fuels increase. Bio-fuels are fuels produced from biomass for either transportation or combustion purposes. They include biogas (from organic waste materials), biodiesel (from plant and animal oils) and bio-ethanol (from plant sugar sources). Bio-fuels are environmental friendly, renewable and the raw materials for their production are locally available. Its use will overcome energy poverty, reduce negative fossil fuels environmental impact, global warming and pollution, improve

sanitation, reduce demand for wood and charcoal and provide substrates which are efficient fertilizer for growing crops.

Globally, Nigeria is the sixth largest producer of petroleum products (Nwokoji, 2012), but this position has not fully solved the energy needs of the country. Presently, Nigeria produces over 2.4 million barrels of crude oil daily with estimated crude oil reserve of over 40 billion barrels and over176 trillion cubic feet of natural gas reserve. However, despite these huge deposits of crude oil and natural gas, the country is experiencing enormous scarcity of petroleum products especially in the rural areas where the price and supply of the products are fluctuating.

Traditionally, bio-energy derived mainly from the combustion of wood and agricultural residues has negative impacts which include severe health consequences on women and children who are the prime users of these products. Combustion of wood in confined spaces produce indoor pollution of green house gases (CO and CO_2) which cause respiratory illness and premature deaths. The use of this type of biomass also increases pressure on local natural resources as communities must satisfy increasing demand for energy services.

Nigeria is an agrarian country with more than 70% of her work force employed in agricultural sector (Nwokoji, 2012). This has given rise to production of millions of tons of biomass which is the major raw materials for bio-fuel production. These biomasses are usually plentiful in rural areas where they are treated as wastes (Mattocks, 1980). Bamikole (2012) stated that efforts to expand petroleum oil production by way of expanding the refineries and upgrading of the existing ones will not cut down the country's dependence on oil, will not create the mix, will not be cheap, and will not create the employment envisioned by the year 2020. Nigeria therefore, must invest in alternative and renewable fuel drive.

It is clear that the development of bio-fuels is vital for possible diversification of the country's economy, growth in industrial production of goods and services, generation of employment, and possible eradication of poverty as well as saving the country's foreign exchange that could have otherwise be spent on importation of fuels.

1.2 Statement of Problem

A country's economic growth and developmental aspiration is highly dependent on the cost of its energy supply sector. An investor makes maximum profit when all the economic indices point to low cost of production. Since Nigerian independence in 1960, her energy supply has been characterized with rising prices of fossil fuels, low reliability of electricity provision from national grids with persistent risk of power cuts and vulnerability of hydro power to drought (Chukwuma and Chukwuma, 2014). Presently, power generation in the country fluctuates between 3800 and 4400 MW which has adversely affected the country's economic growth. Effort by successive government to break the jinx is yet to be applauded (Nwokoji, 2012).

Nigeria becoming one of the 20 largest economies by the year 2020 as predicted by Energy Commission of Nigeria (ECN) would require the country to generate about 40,000 mega watts (MW) of electricity necessary to drive the economy (Aneke, 2012). Also, with the country's population of over 160 million people (NPC, 2006), where about 70% live in the rural area with their occupation mainly subsistence agriculture resulting to poor farm yields, the use of energy crops like corn, potato, rice they meet about 94% of their energy needs with biomass, particularly by burning wood. This dependency on fuel wood has led to a rapid deterioration of Nigerian's ecosystems. Moreover, collecting fuel wood is difficult and time consuming work primarily done by women and children. Smoke from burning fuel wood also lead to respiratory and eye diseases.

Bio-fuels can be used to replace fossil fuels otherwise used for heating, electricity and transport in addition digestate is one of the by-products obtained from production of them. Production of good quality digestate for use as bio-fertilizer is the result of careful control of all aspects of the process, from feedstock to field (Chukwuma and Chukwuma, 2014). Thus, biogas is a feasible option for the domestic needs of the rural dwellers.

Anaerobic digestion (AD) is a green technology involving the generation of methane-rich biogas via the biological degradation of biomass like agricultural and municipal solid wastes and wastewaters. AD processes have for many years been used to treat and sanitize sewage sludge waste from aerobic wastewater and animal manure, reduce its odour and volume, and produce useful biogas. Biogas in turn is a first generation, renewable bio-fuel that offers the prospect of replacing fossil fuels in the transportation sector and limiting the net greenhouse gas emissions implicated in climate change (Magnusson and Alvfors, 2012).

However, anaerobic digestion systems are rather complex processes that unfortunately often suffer from instability (Lyberators and Skiads, 1999). The task is further complicated by the lack of a valid and reliable method for quantifying microbial cell biomass in digesters containing insoluble substrates.

In anaerobic digestion of substrates, a variety of micro-organisms perform together to bring about conversion of organic fraction of substrates. Therefore, understanding the process mechanisms and kinetics are the requirements for good reactor design where operating conditions, methane (CH_4) production, system stability, and effluent quality can be predicted or specified. Various models have been developed to provide greater in depth understanding of the mechanisms influencing the bio-chemical anaerobic digestion process. Since the initial dynamic mathematical digester models of the late 1960s by (Sanchez *et al*., 2006), additional and more complex models have been developed to account for

significant microbial interactions and inhibitions (Angelidaki *et al*., 1999; Nwabanne *et al*., 2012; Budiyono *et al*., 2010; Igoni *et al*., 2008). Some researchers in Nigeria have also studied the kinetics of biodegradability of organic material in order to characterize the biodegradability process. But the task of obtaining valid required kinetic constants is complicated by the fact that anaerobic digestion in itself is a complicated multi-stage dynamic process that entails actions of several groups of bacteria. The composition of such groups varies in an unknown manner with changes in retention time, feedstocks, temperature, reactor type, and other operating conditions. The task is further complicated by the lack of valid and reliable method for quantifying microbial cell biomass in digesters containing insoluble substrates. The predictive power of AD models is limited by the lack of knowledge regarding the specific bacteria involved and their metabolism and physiological limitations.

1.3 Aim and Objectives of study

The aim of this study is to study the potentials of wild cocoyam, jackfruit seed, water yam, and yellow yam starches for bio-fuel production, while the general objective is to investigate their potentials for bio-fuel production. The specific objectives of this research project are:

1. To characterize wild cocoyam, jackfruit seed, water yam, and yellow yam for bio-fuel production.
2. To optimize the enzymatic hydrolysis of the crops.
3. To produce ethanol from the starches of wild cocoyam, jackfruit seed, water yam, and yellow yam.
4. To produce biogas from the fermentation broths of the feedstocks in batch mode
5. To study the kinetics of batch process anaerobic digestion of the biogas production from the crops.

1.4 Justification of study

Nigeria is facing serious energy crisis despite its position in the global petroleum production. Moreover, like all other natural resources, supplies of fossil fuels are limited; they are also non-renewable, and are bound to be depleted sometime in future. More so, rising cost of fossil-based petroleum products have made the products unaffordable to the rural dwellers that constitute about 70% of the population. Fortunately, most parts of Nigeria are suitable for energy crop cultivation and so the country cannot afford to be left behind in the recent quest by even the highly industrialized nations of the world such as United States of America, France, China, Germany, Brazil, for renewable sources of energy.

Most of the energy crops currently employed for bio fuels production such as tubers and grains compete with human and animal food. However, numerous feedstock abound locally which would not necessarily compete with staple food, and some of them have not been employed in bio-ethanol production. Therefore, research on the fermentability of this locally available feedstock in ethanol production could provide more choices when availability of materials is limited.

1.5 Scope of Study

This work seeks to investigate the potentials of wild cocoyam, jackfruit seed, water yam, and yellow yam for bio-fuel production. Response Surface Methodology was used for optimization of the enzyme hydrolysis of the starches for bio-ethanol production. Also, the optimization of biogas production using the feedstocks wastes in co-digestion with cow paunch in batch mode was studied using Central Composite Design (CCD). The kinetics of biogas production was also studied.

CHAPTER 2

LITERATURE REVIEW

2.1 Bio-ethanol

Ethanol, which is known as pure alcohol, ethyl alcohol or bio-ethanol, is a colourless, flammable, volatile liquid with a strong odour. The melting point of ethanol is 114.1°C, whereas it boils at 78.5°C. Due to the low freezing point of ethanol, it has been used in thermometers for temperatures below –40°C, and automobile radiators as antifreeze. The properties of ethanol are given in Table 2.1. The chemical formula of ethanol is C_2H_5OH, containing a –OH group bonded to carbon. Ethanol can be produced synthetically and naturally by yeasts. Ethanol fermentation has been used for the production of alcoholic beverages, and for the rising of bread dough for centuries; recently, it has been produced to use industrially. Since 1908, fuel ethanol has found use for transportation gasoline and today, 73% of ethanol production is consumed as fuel worldwide. Bio-ethanol has become an attractive fuel because it is renewable and oxygenated (Balat *et al*., 2008). Sanchez and Carlos (2008) indicate that oxygenated ethanol reduces the emission of carbon dioxide and aromatic compounds. Ethanol is also non-toxic and is a non-contaminant to water sources. Bio–ethanol is being used purely or blended with gasoline for transportation in Brazil and in some states of the U.S. (Balat *et al*., 2008). Although bio-ethanol has been introduced as an alternative to petroleum-derived fuels, corrosiveness, low flame luminosity, low vapour pressure (compared to gasoline), miscibility with water, and low energy density are some of the disadvantages of bio-ethanol (Balat *et al*., 2008). Aside from fuel, ethanol has other applications in various industry branches such as: personal care products, cleaning agents, pharmaceuticals, and beverages.

Table 2.1: Properties of ethanol

Description	Values
Chemical Formula	C_2H_5OH
Molecular weight (g/mol)	46
Density at 20°C (kg/m³)	789
Calorific value (MJ/kg)	26.9
Calorific value of stoichiometric mixture (MJ/m³)	3.85
Heat of evaporation (kJ/kg)	840
Temperature of self-ignition (K)	665
Stoichiometric air/fuel ratio (kg air/kg fuel)	9
Lower flammability (λ_1)	2.06
Higher flammability (λ_h)	0.3
Kinematic viscosity at 40°C (mm²/s)	1.4
Motor octane number /research octane number	89/107
Cetane number	8
Flame temperature (K)	2235
Molecular composition (by mass)	
C (%)	52.2
H (%)	13

Source: Ofoefule (2012)

Bio-ethanol is an alternative fuel to substitute for petrol. Alcohol production by fermentation has received special attention because the world energy crisis has enhanced the interest in renewable energy sources. There is a growing interest in the utilization of starch for the production of alcohol as starch is renewable and globally available in large quantities. In recent years, largely in response to uncertain fuel supply and efforts to reduce carbon dioxide emissions, bio-ethanol (along with bio-diesel) has become one of the most promising bio-fuels today and is considered as the only feasible short to medium alternative to fossil transport fuels in Europe and in the wider world. The current European Union (EU) commitment under Directive 2003/30/EC on the promotion of bio-fuels for transport set a target of 5.75% of all transport fuels by 2010. The recent European Commission energy roadmap has now increased this to 10% by 2020. Bio-ethanol is seen as a good fuel alternative because the source crops can be grown renewably and in most climates around the world. In addition the use of bio-ethanol is generally CO_2 neutral. This is achieved because in the growing phase of the source crop, CO_2 is absorbed by the plant and oxygen is released in the same volume that CO_2 is produced in the combustion of the fuel. This creates an obvious advantage over fossil fuels which only emit CO_2 as well as other poisonous emissions. In the 1970s, Brazil and the USA started mass production of bio-ethanol -grown from sugarcane and corn respectively. Smaller scale production started more recently in Spain, France and Sweden mostly from wheat and sugar beet. In recent years the concept of the bio-refinery has emerged, whereby one integrates biomass conversion processes and technology to produce a variety of products including fuels, power, chemicals and feed for cattle. In this manner one can take advantage of the natural differences in the chemical and structural composition of the biomass feed stocks. The Commission's document (An EU Strategy for Bio-fuels 1) reports on this concept

of the "bio-refinery" within the Seventh Framework Programme (FP7) and it will give it high priority support. In the framework of the RESTMAC project ("Creating Markets for Renewable Energy Technologies EU–RES Technology Marketing Campaign") which aims to develop and employ a comprehensive and well thought-out thematic approach to encourage the uptake of selected RES technologies in the market, this brochure will present information about the production of bio-ethanol and its co-products, but will also focus the use of bio-ethanol and on some political issues.

Bio-ethanol is usually obtained from the conversion of carbon-based feedstocks. Ethanol is colorless, volatile, flammable liquid that is an intoxication agent in liquors. It is also used as a solvent called ethyl alcohol and has the chemical formula C_2H_5OH. Bio-ethanol from biomass sources is the principal fuel used as a petrol substitute for road transport vehicles. The high price of crude oil makes bio-ethanol fuel attractive (Bryner and Scott, 2006). Bio-ethanol is mainly produced by the sugar fermentation process although it can also be manufactured by the chemical process of reacting ethylene with steam.

$$C_2H_6 + H_2O \underset{500 - 600^0C}{\overset{80 - 100\ atm}{\rightleftharpoons}} H_2sO_4C_2H_5OH \text{ ---------- (2.1)}$$

Glucose (a simple sugar) is created in plants by the process of photosynthesis.

$$6CO_2 + 6H_2O + light \longrightarrow C_6H_{12}O_6 + 6O_2 \text{---------- (2.2)}$$

During ethanol fermentation, glucose is decomposed into ethanol and carbon dioxide. $C_6H_{12}O_6 \longrightarrow 2C_2H_5OH + 2CO_2 + heat$ ---- (2.3)

After doubling the combusting reaction because two molecules of ethanol are produced for each glucose molecule, and adding all three reaction together, there are equal numbers of each type of molecule on each side of the equation, and the net reaction for the overall [production and consumption of ethanol is simply put as;

Light ⟶ heat

The heat of the combustion of ethanol is used to drive the piston in the engine by expanding heated gases. It can thus be said that sunlight is used to run the engine and in this instance any renewable energy source from sunlight is the only way energy enter the planter (Wikipedia, 2010).

2.2 Production of Bio-ethanol

In 2006, worldwide bio-ethanol production was approximately 51.3 billion liters (Balat et al., 2008). An increase in fuel ethanol production resulted from the fact that many countries want to reduce dependency on foreign oil and enhance air quality. Two leaders of ethanol production in the world are Brazil and the United States.

Bio-ethanol can be produced from different feedstock, such as corn, sugar cane, cellulose, potato, etc. Sugar cane, as a raw material, is used for 60% of global ethanol production, while 40% of global production of ethanol comes from other crops. Corn grain is the main raw material of ethanol production in the United States (90%) where as in Brazil; sugar cane is the major source (Balat *et al.*, 2008). Desirable raw materials for ethanol fermentation should have applicable sugars that can be fermented by microorganisms. Sucrose containing feedstock, starchy feedstock, and lignocelluloses biomass can be used as raw materials for ethanol production. Ethanol fermentation is summarized with the chemical equations below:

$$C_6H_{12}O_6 \rightarrow 2CH_3COCOO^- + 2H^+ \quad \text{.....(2.4)}$$

$$CH_3COCOO^- + H^+ \rightarrow CH_3CHO + CO_2 \quad \text{.....(2.5)}$$

$$CH_2CHO + NADH \rightarrow C_2H_5OH + NAD \quad \text{.....(2.6)}$$

2.3 Ethanol use in appliances

Ethanol can be used in a variety of ways viz: cooking, heating and lighting appliances. In some cases, ethanol can be used in modified appliances designed for

conventional fuels. In other cases, appliances designed specifically for ethanol fuel are required.

2.3.1 Chemicals

A number of chemicals are produced in the ethanol industry and potentially even more in the 2nd generation bio-ethanol industry, serving a wide range of uses in the pharmaceuticals, cosmetics, beverages and medical sectors as well as for industrial uses. The market potential for bio-ethanol is therefore not just limited to transport fuel or energy production but has potential to supply the existing chemicals industry. SEKAB co-produce the following chemicals along with fuel ethanol:

1. Acetaldehyde (raw material for other chemicals e.g. binding agent for paints and dyes)
2. Acetic acid (raw material for plastics, bleaching agent, preservation) Ethyl acetate (paints, dyes, plastics, and rubber)
3. Ethanol 95% (foods, pharmaceuticals, fuel ethanol, detergents)
4. Thermol (cold medium for refrigeration units and heat pumps) (SEKAB, 2007) KWST also provide a range of chemicals mixed into marketable compounds such as:

a. Ethyl alcohol (ethanol) (spirits industry, cosmetics, print colours and varnish)

b. Isopropyl alcohol (IPA), Ethyl acetate (EAC), WABCO-antifreeze (disinfectant, cleaning agent for electronic devices, solvents)

c. Vinasse, Potassium Sulphate (feeding stuffs, fertilizer) (KWST, 2007)

2.3.2 Transport fuel

Bio-ethanol has mostly been used as a bio-fuel for transport, especially in Brazil. Indeed it was in Brazil where the first bio-ethanol fuelled cars emerged on a large-scale. Although generally unknown to the average consumer, a large volume of bio-ethanol is already used in Europe as it is blended with petrol at 5%.

It is used as a substitute for lead as an oxygenating additive and has a high octane rating, which improves performance. Although the eventual target is the private consumer, few are aware of bio-ethanol's potential to, at least, partly replace petrol as a transport fuel in Europe.

Stakeholders in the bio-ethanol fuel market:

- bio-ethanol producers
- fuel suppliers
- car manufacturers
- the government - support is also extremely important as was the case in Brazil in the late 1970s and in the USA today bio-ethanol has been endorsed by the President and helped by subsidies and tax breaks
- transport users

In addition supermarkets who provide petrol stations to customers are seeing the opportunity to provide petrol/ethanol blends from 5-85% (E5 -E85). Even though most experts agree that up to a 10% mix will not damage modern car engines, the manufacturer warranty for standard cars is set at 5%. Above this level to maintain the warranty, the car engines need to be modified or one has to buy a fuel flexible vehicle (FFV).

Sweden is the strongest in the bio-ethanol transport market with over 792 E85 fuel stations and 15,000 Ford Focus FFVs have been sold there since it's debut on the market in 2001. By May 2006, 15% of all newly sold cars were either bio-ethanol or biogas fuelled vehicles. E85 is being sold at prices substantially less than petrol, between 75 and 85 € cents per liter compared to 1.11 € and 1.19 € for petrol. An important consideration when marketing the price of bio-ethanol is the fact that ethanol contains around 30% less energy per liter than petrol which means you have to fill up more frequently. Therefore the

sale price will have an important impact on take-up of bio-ethanol as a transport fuel.

Research into renewable energy technologies is still relevant, especially in view of the often very high costs of fossil fuels worldwide. Another reason for their relevance is the fact that the rampant use of firewood for domestic and industrial heating in low income countries invariably necessitates the destruction of forests and this is harmful to the environment. Also, it had been pointed out that the use of firewood, kerosene and charcoal in households had adverse effects on human health (Adelekan and Adelekan, 2004). Furthermore, using biomass to produce energy can reduce the use of fossil fuels, reduce pollution and waste management problems and show environmental advantages in terms of life-cycle energy use and greenhouse gas (GHG) emissions (Marshall, 2007; Rettenmaeir *et al*., 2010; Fernando *et al*., 2010). Overall, these reasons are compatible with the aims and objectives of the Kyoto Protocol (KP) which are tailored towards the reduction of greenhouse gases. According to International Energy Agency (2010), bio-energy currently provides about 10% of global primary energy supply, 1.3% of electricity production, and 1.5% of transport fuels. Driven by increasing concern over energy security and greenhouse gas mitigation, the global demand for liquid bio-fuels more than tripled between 2000 and 2007. Production costs are uncertain and vary with the feedstocks available, but are currently estimated to be USD 0.80 – 1.00 per litre of gasoline equivalent. Prasad *et al*. (2007) and Balat et al. (2008) observed that with world reserves of petroleum is fast depleting, ethanol has in recent years emerged as the most important alternative source of liquid fuel and has generated a great deal of research interest in ethanol fermentation. The global annual production of fuel ethanol is around 40 to 50 billion litres, of which 90 per cent is produced by the USA and Brazil from maize and sugarcane respectively (World Bank, 2008).

According to estimates of the European Renewable Energy Commission about 40% of electricity demand will be generated from renewable energy sources by 2020. Furthermore, the new Renewable Energy Directive (RED) will undoubtedly stimulate the renewable energy heating and cooling market, according to EREC's projections, up to 25% of heating and cooling consumption can come from renewable energy by 2020. Moreover, the Renewable Energy Sources (RES) Directive provides a strong incentive to significantly reduce oil dependence in the transport sector over the coming years by setting a minimum target of 10% renewable energy in transport. The RES Directive set an important framework for the future growth of the renewable energy industry and paved the way for a stable investment climate, thereby not only increasing the security of Europe's energy supply, contributing to abating climatic change, but also providing high quality jobs and sustainable economic recovery. EREC published its 'RE-thinking 2050 – A 100% renewable energy vision for the European Union's report in April 2010. 'RE-thinking 2050' outlines a pathway towards a 100% renewable energy supply system by 2050 for electricity, heating and cooling as well as transport for the European Union, examining the effects on Europe's energy supply system, on CO_2 emissions as well as outlining economic and social benefits of a fundamental change towards a sustainable energy system. Similar policies are also being established in other regions. For instance in 2009, India announced a national bio-fuel policy with a mandate to achieve 20% blend of bio-ethanol and bio-diesel by 2017 (Das and Priese, 2011).

Bio-fuels can be defined as fuels produced from biomass for either transportation or combustion purposes. Biomass includes all plant and plant-derived materials, including animal manure, not just starch, sugar, and oil crops already used for food and energy, the biomass resource base is composed of a wide variety of forestry and agricultural resources, industrial-process residues, municipal-solid and urban-

wood residues. Bio-fuels produced from biomass are generally classified to three generations of processing technology.

Sugar, starch, oil crops or animal fats have most commonly been used to make first generation Bio-fuels: biodiesel, bio-alcohols, biogas, and syngas biodiesel, the most common bio-fuel in Europe, is produced from oil crops or fats (eg. animal fats, soy, rapeseed, jatropha, mahua, mustard, sunflower, palm oil, hemp) using transesterification. Bio-alcohol (eg. ethanol), the most common bio-fuel worldwide and especially in Brazil – is produced by fermentation of sugars derived, for example, from sugar crops (eg. sugarcane and sorghum) and starch crops (eg. corn, wheat and potato). Most feedstocks for first-generation bio-fuels could also be used for animal or human food and have thus, caused concern, which could lead to a global food crisis. This concern has led to second-generation bio-fuel. Cellulose ethanol, which is produced from lignocelluloses biomass, is a representative of second-generation bio-fuel. Lignocellusic biomass consists of the residual, non-edible parts of food crops (eg. stems, leaves, and husks) as well as other non-food crops (eg switch, grass, fuel wood) and industrial wastes. Recently, algae fuel, which is a bio-fuel produced by algae, was spot-lighted as the third –generation bio-fuel.

Bio-fuels are renewable energy resources, unlike other natural resources such as petroleum, coal and nuclear fuels. Bio-fuels can also be defined as any fuel with 80% minimum content by volume of materials derived from living organisms harvested within the ten years preceding its manufacture (Isoun, 2007).

2.4 Comparing the alternatives

2.4.1 Fuels competing with ethanol

Methanol, butanol and some types of vegetable oil are three alternatives to ethanol. Both methanol and butanol can be used to replace or extend gasoline or diesel fuel. Vegetable oils, however, are limited to replacing only diesel fuel until further

research proves otherwise. Methanol is the most important alternative. It is a liquid alcohol containing one carbon atom (CH_3OH). Like ethanol, it is used to replace or be blended with gasoline. Methanol is produced by a chemical process that uses methane as the primary feedstocks. Methanol can also be produced from coal or biomass. On a world wide scale, the methanol production industry is relatively large and it uses natural gas for feedstocks. Methanol production requires high temperature, high pressure and special catalysts. This process is much more complex than ethanol production and is generally economical in only very large industrial plants.

Butanol is a four carbon alcohol. It has two possible chemical structures, depending on the position of the hydroxyl: N-butanol ($CH_3CH_2CH_2CH_2OH$) and 2 butanol ($CH_3OH_1CH_2CH_3$). Fermentation produces N butanol. Unlike ethanol or methanol, butanol can substitute for or be blended with diesel fuel in compression ignition engines. It is produced by bacterial fermentation of starch or sugar containing feedstocks and purified by distillation. The bacteria produce ethanol and acetone in addition to the principal product, butanol. The production of butanol has two disadvantages: (1) the fermentation of butanol is difficult compared with that of ethanol; and (2) butanol fermentation produces less useful fuel per unit of feedstocks than ethanol fermentation with yeast. Butanol has been produced commercially under wartime conditions. Today, however, butanol is no longer produced commercially for use as fuel.

2.5 Cost/economics

It is difficult to provide general information about ethanol fuel economics because production costs and product value depend on plant location, feedstocks, production scale and end use. Ethanol production includes both capital and operating costs. Two important factors in capital costs for small batch plants are starch hydrolysis systems and boiler capacity. In large plants, engineering,

distillation systems and process control are relatively more significant. Generally, capital costs for alcohol plants range $0.50 to $1 (U.S) per litre of annual production capacity. Based on figures from U.S. plants, capital costs per litre of annual production capacity for very small and very large plants are generally greater than those for intermediate scale plants.

2.6 Biomass resources

There are various forms of biomass resources in the world, which can be grouped into four categories. Wood residues are by far the largest current source of biomass for energy production. It comes from the wood product industry which includes paper mills, sawmills, and furniture manufacturing. Municipal solid waste is the next largest, followed by agricultural residues and dedicated energy crops. Among these biomass resources including short-rotation woody crops and herbaceous crops, primarily tall grasses, dedicated energy crops seem to be the largest most promising future resources of biomass. This is because of the ability to obtain numerous harvests from a single planting, which significantly reduces average annual costs for establishing and managing energy crops, particularly in comparison with conventional crops (Monique *et al*., 2003). Fermentation processes from any material that contains sugar could derive ethanol. The varied raw materials used in the manufacture of ethanol via fermentation are conveniently classified into three main types of raw materials: sugars, starches, and cellulose materials.

Sugars (from sugarcane, sugar beets, molasses, and fruits) can be converted into ethanol directly. Starches (from corn, cassava, potatoes, and root crops) must first be hydrolyzed to fermentable sugars by the action of enzymes from malt or molds. Cellulose (from wood, agricultural residues, waste sulfite liquor from pulp, and paper mills) must likewise be converted into sugars, generally by the action of mineral acids. Once simple sugars are formed, enzymes from microorganisms can

readily ferment them to ethanol. The most widely used sugar for ethanol fermentation is molasses which contains about 50 wt% of sugar and about 50 wt% of organic and inorganic compounds, including water. It is thick, dark-colored syrup produced during refinement of sugar. Since molasses contains microorganisms which can disturb the fermentation, the molasses is taken first to the sterilizer and then to the fermentor. Then it is diluted with water to the mass fraction of 10±18% to reduce its viscosity in the pipeline. In addition, a very high concentration of sugar can give too much ethanol and results in a prolonged fermentation time and an incomplete sugar conversion. After the pH of the mash is adjusted to about 4–5 with mineral acid, it is inoculated with yeast or bacteria, and the fermentation is carried out nonaseptically at 20–32°C for about 1–3 days. Most agricultural biomass containing starch can be used as a potential substrate for the ethanol fermentation by microbial processes. These substrates include corn (maize), wheat, oats, rice, potato, and cassava. On a dry basis, corn, wheat, sorghums (Milo), and other grains contain around 60–75% (wt/wt) of starch, hydrolysable to hexose with a significant weight increase (stoichiometrically the starch to hexose ratio is 9:10), and these offer a good resource in many fermentation processes (Jackman, 1987).

Fermentation of starch is somewhat more complex than fermentation of sugars because starch must first be converted into sugar and then into ethanol. Starch is first hydrolyzed by adding α-amylase to avoid gelatinization; it is then cooked at high temperature (140–180°C). Next, the liquefied starch is hydrolyzed to glucose with glucoamylase. The resulting dextrose is fermented to ethanol with the aid of microorganisms producing CO_2 as a co-product. During the process currently employed for industrial-scale ethanol fermentation from starchy materials, high-temperature cooking (140–180°C) is very effective for fermentation of starchy materials because it raises starch saccharification efficiency and achieves high

levels of ethanol production under complete sterilization of harmful microorganisms. However, production costs are high due to the high energy consumption in the cooking process and the addition of large amounts of amylolytic enzymes. So processes to reduce the high production costs are required. To resolve these difficulties, noncooking and low-temperature cooking fermentation systems have been developed (Matsumoto *et al*., 1985). Industrial ethanol production has been reported using various starchy materials such as corn, wheat, starch and potatoes, cassava root (Lindeman and Rocchiccioli, 1979), corn Stover (Kadam and McMillan, 2003; Wilke *et al*., 1981), and starch (Maisch *et al*., 1979). Among many starchy materials, cassava starch is an inexpensive fermentable source. It is a tropical root crop produced in more than 80 countries (Sasson ,1990). Fresh cassava has very high starch content, up to 30%. The content of sucrose is about 4%. Dried cassava has 80% fermentable substrate. However, cassava waste processing is difficult because it is high in toxic materials. The potential toxicity of cassava is due to the presence of cyanogenic glycosides, linamarin, and lotaustralin, which on hydrolysis yield hydrogen cyanide on its peel. Traditional methods of cooking like boiling and decanting remove cyanoglycosides to a certain extent, but even then a certain amount of residual toxicity remains in it (Westley, 1980). Moreover, since starch particles in cassava are bigger and there are some branched structures, more glucoamylase has to be added into the reactor. Furthermore, the nitrogen content of the cassava is very low, so during the fermentation, nutrient has to be added into the reactor to maintain the normal growth of the microorganisms. Among the three main types of raw materials, cellulose materials represent the most abundant global source of biomass and have been largely unutilized. However, the effective utilization of the lignocellulosic feedstocks is not always practical because of its seasonal availability, scattered stations, and the high costs of transportation and storage of such large amounts of

organic then cooked at high temperature (140–180°C). Next, the liquefied starch is hydrolyzed to glucose with glucoamylase. The resulting dextrose is fermented to ethanol with the aid of microorganisms producing CO_2 as a co-product. During the process currently employed for industrial-scale ethanol fermentation from starchy materials, high-temperature cooking (140–180°C) is very effective for fermentation of starchy materials because it raises starch saccharification efficiency and achieves high levels of ethanol production under complete sterilization of harmful microorganisms. However, production costs are high due to the high energy consumption in the cooking process and the addition of large amounts of amylolytic enzymes. So processes to reduce the high production costs are required. To resolve these difficulties, non-cooking and low-temperature cooking fermentation systems have been developed (Matsumoto *et al*., 1985).

Among the three main types of raw materials, cellulose materials represent the most abundant global source of biomass and have been largely unutilized. Recently, the enzymatic hydrolysis of biomass cellulose is considered to be the most promising technology available (Ogier *et al*., 1999; Yu and Zhang, 2004). However, despite the work done, the industrial scale up of this process appears to be still hindered by technological issues or by the lack of a biomass refinery approach in which ethanol is one of several products. In fact, because raw material cost comprises more than 20% of the production cost (Kaylen *et al*., 2000; Zhuang *et al*., 2001), the optimization of the cellulose conversion should be accomplished by correct management and utilization of all process streams. A consequence of this situation is that even limited government intervention is still crucial to maintaining ongoing research. Furthermore, lignocellulose is a more complex substrate than starch. It is composed of a mixture of carbohydrate polymers (cellulose and hemicellulose) and lignin. The carbohydrate polymers are tightly bound to lignin mainly by hydrogen bonds but also by some covalent bonds. The

biological process for converting the lignocellulose to fuel ethanol requires the following: delignification to liberate cellulose and hemicellulose from their complex with lignin, depolymerization of the carbohydrate polymers to produce free sugars, and fermentation of mixed hexose and pentose sugars to produce ethanol. Among the key processes described above, the delignification of lignocellulosic raw materials is the rate-limiting and most difficult task to be solved. Another problem is that the aqueous acid used to hydrolyze the cellulose in wood to glucose and other simple sugars destroys much of the sugars in the process. Extensive research has been carried out in this field for decades (Yu and Zhang, 2004), and the first demonstration plant using lignocellulosic feedstocks has been in operation in Canada since April 2004 (Tampier *et al*., 2004). It is expected that the cost of lignocellulosic ethanol can undercut that of starch-based ethanol because low-value agricultural residues can be used.

2.7 Generations of bio-ethanol production

The production of bio-ethanol from traditional means, or 1st Generation bio-fuels is based upon starch crops like corn and wheat and from sugar crops like sugar cane and sugar beet. However, the cultivation of alternative sugar crops like sweet sorghum opens up new possibilities in Europe, especially in hotter and drier regions, such as Southern and Eastern Europe. Sweet sorghum requires less water or nutrients and has a higher fermentable sugar content than sugar cane as well as a shorter growing period which means that in some regions like in Africa you can get two harvests a year from the same crop.

In addition to this, the development of lingo-cellulosic technology has meant that not only high energy content starch and sugar crops can be used but also woody biomass or waste residues from forestry. This development is seen as the 2nd Generation of bio-fuels.

2.7.1 Bio-ethanol from sugar feedstocks

Both raw juice and molasses from sugar cane and sugar beets can be used for ethanol production. The juice is extracted from sugar cane by either squeezing (roll mills) or diffusion (diffuser). Part of the juice is used for sugar manufacture while the remaining is used for ethanol production. Molasses, which is a low-value by-product, is also used for ethanol production. The solid residue from the extraction step, which is referred to as biogases, is burned to generate energy for use in the plant. Ethanol is normally obtained by fermentation of cane juice or a mixture of cane molasses and juice. Before putting into the fermenters, the sugar solution must undergo purification and pasteurization. Purification normally involves treatment with lime, heating and later decantation similar to treatment use in sugar manufacture. Pasteurization involves heating and immediate cooling. The cooling typically includes two stages. In the first stage, the hot sugar solution is passed through a heat exchanger in counter- current flow to the cold solution. At the end of this stage, the hot solution is cooled to about 60^0C. In the second stage, the sugar solution is cooled further to 30^0C using water as the cooling fluid. The sugar concentration normally is adjusted to approximately 19^0C (Drapcho *et al*., 2008).

Today the processes of milling (cutting of cane into regular pieces) and raw sugar refining are usually done together on one site. During the milling the sugar cane is washed, chopped and shredded by revolving knives. The shredded cane (20-25cm) is fed into mill combinations which crush and extract the cane juice. The juice is filtered and pasteurized (treatment of heat to kill micro-bacterial impurities) along with chemicals.

2.7.2 Cereal crops

For starch (cereal) based crops the procedure is similar to sugar crops but with the added process of hydrolysis to break down the polymers into monomers which can then be broken down into simple C6 sugars. From the milling of the

grain to the release of the starch, it is then diluted into water to adjust the volume of sugar in the mash. The mixture is cooked with yeast and all the water soluble starches dissolve into the water. And through either acid hydrolysis or enzymes, the starch is converted into sugars. The unrefined fermented liquid known as "beer", is produced and through various evaporation and distillation stages fuel grade ethanol can be produced.

They can be naturally divided into cereal crops, sugar crops and woody/lignocellulosic biomass. Any sort of wood, crop residues or forestry waste like sawdust and chips can be used for 2nd Generation bio-ethanol. Miscanthus and the other examples below are some fast growing grasses which are proving more and more popular for heating fuel. They could also be used for lignocellulosic bio-ethanol.

2.7.3 Lignocellulosic bio-ethanol

Lignocellucosic feedstocks consists of three main components namely cellulose hemicelluloses and lignin. Lignocellucosic materials are more complex than starch. Generally, the percentage composition of lignocellucosic biomass is as follows: 40-60% cellulose, 20-40% hemicelluloses and 10-25% lignin depending on the biomass (Mattocks, 1987). Lignocellucosic biomass are cheap renewable resources and available in large quantity that can be used for the production of ethanol. They can be obtained at low cost from a variety of resources such as wood, grass, bagasse, waste paper, municipal solid waste and stalks of cereals (Kalman and Reczey, 2007). Technologies for conversion of these feedstocks to ethanol have been developed on two plant forms which can be referred to as the sugar platform and the synthesis gas (or syngas) platform.

Cellulosic materials are significantly more resistant to hydrolysis than starchy materials. Hemicellulose is a branched heteropolymer of not just glucose but multiple five and six carbon sugars; D-xylose, L-arabinose and hexose sugars; D-

galactose, D-glucose, D-mannose, L-rhamnose and L-fructose. The structure of hemicelluloses varies with the particular biomass, but generally xylose constitutes a relatively large percent of the composition. Lignin is not composed of sugars, but it is instead a complex aromatic polymer. As such, lignin cannot be used to make ethanol it can however be utilized as a fuel source (Zaldivar *et al*., 2001).

In the sugar platform, cellulose and hemicelluloses are first converted to fermentable sugars, which then are fermented to produce ethanol. The fermentable sugars include glucose, xylose, arabinose, galactose and mannose. Hydrolysis of cellulose and hemicellulose to generate these sugars can be carried out by using either acids or enzymes. Pre-treatment of the biomass are needed prior to hydrolysis. The main objectives of the pre-treatment process are to speed up the rates of hydrolysis and increase the yield of fermentable sugar. In all pre-treatment processes, these goals are accomplished by modifying the structure of the polymer matrix in the biomass thus making the carbohydrate fractions more susceptible to acid attack or more accessible to enzymes action.

The difference in process steps between starch and lignocellulosic feedstocks is that lignocellulosic biomass requires a more complicated hydrolysis stage. The reason for this is that cellulose in the wood containing carbohydrate polymers called cellulose. Cellulose is made up of long chains of glucose and a more complex set of enzymes are required to break the long chains. Therefore lignocellulosic bio-ethanol is technically more demanding and thus more expensive. Work at the moment ongoing to enhance the pre-treatment methods such as steam explosion, ammonia steam explosion, acid processing and synthesizing more efficient enzymes. Another area for development is fractionation technology so one can use more variable biomass, such as agriculture and forest crop residues and urban waste. The chemical structure

of the crop and forest residues are highly variable which creates added complexity compared to the homogeneity of starch or sugar crops.

2.7.4 Bagasse

Bagasse is the primary by-product from sugar cane production. Bagasse is commonly combusted in boilers or cogeneration systems in the sugar industry for the production of heat in the mill for sugar refining processes and for the production of electricity for either direct use by the plant or to sell to the national grid which can increase their overall profit. About 35% of the weight of sugar cane becomes bagasse. Brazil, India, China and Thailand are the largest producers and utilisers of bagasse.

Bagasse is also a straw like material left from cane sugar. It can also be used for making agro-pellets which can be exported as a feedstocks for home pellet boilers or co-firing.

2.7.5 Straw

Straw is another important co-product from cereals and has been used for centuries for various uses. Straw is the waste part of the plant that does not contain the grain and it makes up around 50% of the plants weight. Historical uses include use for rope, paper, packaging, hatching and bedding. It has mostly been used for animal feed although recent uses include bio-fuels in the lignocellulosic path to biogas production through anaerobic digestion. Straw has mainly been somewhat of a burden for farmers as they had to dispose of it some way but its application for bio-ethanol or biogas means they can sell this waste as a marketable by-product.

2.7.6 Fuel cells

Fuel cells are another potential area for ethanol use to produce heat and power. Fuel cells function by combining the fuel hydrogen with oxygen from the air to produce electrical energy, with water vapour and heat as by-products. Fuel

Cells have a typical electrical efficiency of between 30 and 60 % and an overall efficiency, if using the heat by-product, of 70-90 %. The units run with very low noise emissions and pollutant gas emissions are also reduced considerably. Its disadvantages are its relatively high cost and their short life span (regular replacement of components). They are however, regarded as very reliable for the duration of their lifespan and are often used for emergency power. Some uses of fuel cell systems include providing heat and power for hospitals, university campus', remote telecommunication stations as well as for transport, stationary power generation and residential buildings. The recent growth in small residential (0.5 to 10 kW) fuel cell CHP is based on natural gas fuelled units. A number of fuel cells can use bio-ethanol as well as fossil fuels, sometimes with, sometimes without the need for a reformer (to convert it to hydrogen). Acumentrics (USA) and Ceramic Fuel Cells (Australia) manufacture such fuel cells.

2.8 General process

Besides the initial removal of large and unsuitable items, key components of an integrated residual waste treatment system based on ethanol fermentation include recyclable materials, recovery and removal of contaminants via mechanical preprocessing, initial hydrolysis process (conversion to simpler compounds), fermentation of organics, post fermentation purification of ethanol (by distillation or filtration), gasification of solid residuals to provide process heat, and treatment and disposal of waste water. Nearly all of the ethanol fermentation technologies use an initial tipping floor removal of large or unsuitable materials, followed by mechanical preprocessing to remove recyclables and contaminants, and shredding of the material. Then the material is processed through vessels using various systems for the purpose of hydrolysis (breaking down to simpler compounds) of the material. Depending on the technology, this may include high temperature, acid

treatment, and/or materials (Polman, 1994). Recently, the enzymatic hydrolysis of biomass cellulose is considered to be the most promising technology available (Ogier *et al*., 1999; Yu and Zhang, 2004). However, despite the work done, the industrial scale up of this process appears to be still hindered by technological issues or by the lack of a biomass refinery approach in which ethanol is one of several products. In fact, because raw material cost comprises more than 20% of the production cost (Brown *et al*., 2001; Kaylen *et al*., 2000; Zhuang *et al*., 2001), the optimization of the cellulose conversion should be accomplished by correct management and utilization of all process streams. A consequence of this situation is that even limited government intervention is still crucial to maintaining ongoing research. Furthermore, lignocellulose is a more complex substrate than starch. It is composed of a mixture of carbohydrate polymers (cellulose and hemicellulose) and lignin.

The carbohydrate polymers are tightly bound to lignin mainly by hydrogen bonds but also by some covalent bonds. The biological process for converting the lignocellulose to fuel ethanol requires the following: delignification to liberate cellulose and hemicellulose from their complex with lignin, depolymerization of the carbohydrate polymers to produce free sugars, and fermentation of mixed hexose and pentose sugars to produce ethanol. Among the key processes described above, the delignification of lignocellulosic raw materials is the rate-limiting and most difficult task to be solved. Another problem is that the aqueous acid used to hydrolyze the cellulose in wood to glucose and other simple sugars destroys much of the sugars in the process. Extensive research has been carried out in this field for decades (Yu and Zhang, 2004), and the first demonstration plant using lignocellulosic feedstocks has been in operation in Canada since April 2004 (Tampier *et al*., 2004). It is expected that the cost of lignocellulosic ethanol can that of starch-based ethanol because low-value agricultural residues can be used in

phases, the slurred material is then fermented to produce alcohol, which is then purified through distillation and/or filtration to produce the desired fuel-grade quality ethanol. When cellulose was used as the raw material, the cellulose responsible for enzymatic hydrolysis of pretreated cellulosic biomass is strongly inhibited by hydrolysis products: glucose and short cellulose chains.

One way to overcome cellulose inhibition is to ferment the glucose to ethanol as soon as it appears in solution. Simultaneous saccharification and fermentation (SSF) combines enzymatic hydrolysis with ethanol fermentation to keep the concentration of glucose low. The accumulation of ethanol in the fermentor does not inhibit cellulose as much as high concentrations of glucose, so SSF is a good strategy for increasing the overall rate of cellulose to ethanol conversion. In comparison to the process where these two stages are sequential, the SSF method enables attainment of higher (up to 40%) yields of ethanol by removing end-product inhibition, as well as by eliminating the need for separate reactors for saccharification and fermentation (Bollók *et al*., 2000; Hari *et al*., 2001; Stenberg *et al*., 2000). Other advantages of this approach are a shorter fermentation time and a reduced risk of contamination with external micro flora, due to the high temperature of the process, the presence of ethanol in the reaction medium, and the anaerobic conditions (Emert and Katzen, 1980; Wyman, 1994)

2.9 Microorganisms related to ethanol fermentation

Ethanol fermentation is a biological process in which organic material is converted by microorganisms to simpler compounds, such as sugars. These fermentable compounds are then fermented by microorganisms to produce ethanol and CO_2. During the whole process of ethanol fermentation, there are mainly two parts for microorganisms. One is for the microorganisms which convert fermentable substrates into ethanol, and the other is to produce the enzyme to catalyze chemical reactions that hydrolyze the complicated substrates into simpler compounds.

Several reports and reviews have been published on production of ethanol fermentation by microorganisms, and several bacteria, yeasts, and fungi have been reportedly used for production of ethanol. There are some microorganisms which can accumulate high concentrations of ethanol. Historically, the most commonly used microbe has been yeast, among the yeasts. Saccharomyces cerevisiae, which can produce ethanol to give concentration as high as 18% of the fermentation broth, is the preferred one for most ethanol fermentation. This yeast can grow both on simple sugars, such as glucose, and on the disaccharide sucrose. Saccharomyces is also generally recognized as safe (GRAS) as a food additive for human consumption and is therefore ideal for producing alcoholic beverages and for leavening bread.

As with many microorganisms, S. cerevisiae metabolizes glucose by the Embden–Meyerhof (EM) pathway. Beside this, the Entner–Doudoroff (ED) pathway is an additional means of glucose consumption in many bacteria, such as Zymomonas. The high ethanol yield and productivity observed for Zymomonas are a consequence of its unique physiology. Zymomonas is the only microorganism that metabolizes glucose anaerobically using the ED pathway as opposed to the EM or glycolytic pathway (Matthew *et al*., 2005). The ED pathway yields only half as much ATP per mole of glucose as the EM pathway. As a consequence, Zymomonas produces less biomass than yeast, and more carbon is funneled to fermentation products. Also, as a consequence of the low ATP yield, Zymomonas maintains a high glucose flux through the ED pathway. All the enzymes involved in fermentation are expressed constitutively, and fermentation enzymes comprise as much as 50% of the cells' total protein (Sprenger, 1996). Zymomonas mobiles are an unusual Gram-negative microorganism that has several appealing properties as a biocatalyst for ethanol production. The microorganism has a homoethanol fermentation pathway and tolerates up to 120 g/l ethanol. It has a higher ethanol

yield (5–10% more ethanol per fermented glucose) and has a much higher specific ethanol productivity than *Saccharomyces sp*. (Sprenger, 1996).

2.10 Bio-ethanol production processes

Fermentation is the slow decomposition by micro-organisms of large organic molecules (such as starch) into smaller molecules such as ethanol. Ethanol fermentation can be described as the biochemical process by which sugar such as glucose; fructose and sucrose are converted into energy thereby producing ethanol and carbon dioxide as metabolic waste products. Yeasts carry out ethanol fermentation on sugar in the absence of oxygen. Because the process does not require oxygen, the fermentation is classified as anaerobic. Bio-ethanol can be manufactured from numerous sources (Mattocks, 1987). They can be produced from raw materials containing fermentable sugars such as sucrose- rich feed stock namely juices, sugar cane and beet etc. They can also be produced from some polysaccharides that can be hydrolyzed for obtaining sugars that can be converted to ethanol (Cardona and Sanchez, 2007). Starch contaminated in grains is the major polymer used for ethanol production. Starch is generally defined as a polymer consisting of long chains of alpha-glucose molecules linked together.

The structure of starch tends to be amorphous and thus readily hydrolyzed to the simpler glucose, disaccharide and maltose. Starch has been known to mankind for several thousand years. The Romans called it asylum, a word derived from the Greek amylon. It was first separated from wheat flour and other cereals known to the ancient world. History affirms that the ancients used starch and stiffening agent and adhesives as early as 3500 BC. Strips of Egyptian papyrus cemented together with starch adhesives have been notably dated to the late Neolithic period. The increased demand for starch soon brought the introduction of potato starch to supplement the wheat starch solely available up to that time. In 1811, the

discoveries of Kirchoff with respect to glucose and thinning of starches by enzymatic action gave great impetus to starch manufacture through the increased fields of application which they created. The use of roasted starch did not begin until 1821, its usefulness being discovered as far back as 1,300AD, heavily coated starch paper was in use and the application was subsequently extended to textile. The extensive use of starch was also recorded in the Middle Age and by 1744, the English were using it in sizing and warp glazing (Umeasalugo, 1988).

It is difficult to ascertain precisely when sugar was first known to mankind, however, it is generally agreed that it occurred in India many centuries dated back before Christ. Records are found of crude methods for purifying sugar that were brought from the East to Europe about 1400. The first preparation of dextrose in 1811 led to the development of the corn-sugar industry in the United State of America. The first manufacturing began in 1872, the product being liquid glucose. It was not until 1918 however, that appreciable quantities of pure crystalline dextrose were produced. The process flow chart of bio-ethanol production is shown in Fig, 2.1 below.

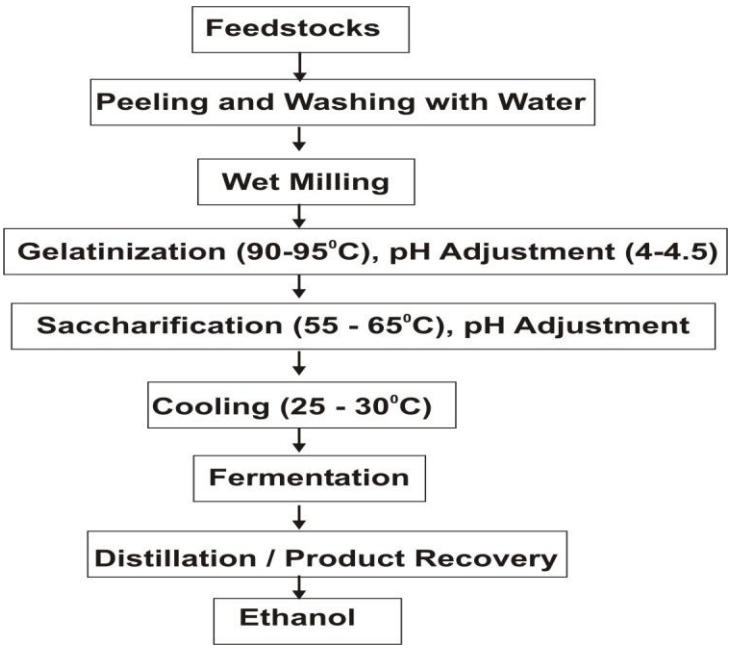

Fig.2.1. Process flow chart of bio-ethanol production from the feedstocks

2.10.1 Wet milling

Wet milling was developed more than 150 years ago for corn starch processing (Butzen and Hobbs, 2002). The first step in this process involves soaking of corn grains, which have been cleaned to remove foreign matters such as dirt and chaff in water containing 0.1 to $0.2^0/_0$ of SO_2 at 52^0C for 24 to 40 h. Steeping softens the kernel and breaks the disulfide bonds in the protein matrix of the endosperm to release starch granules. Soluble nutrients also are released into the steep water, which normally is referred to as light steep water (LSW). The softened grains then are ground gently to break up the kernels. The less dense germs are recovered in a hydroclone system. The recovered germs receive further processing to remove loose starch and gluten and excess water. They are then dried and cooled for storage. Oil can be extracted from the germs on-site. After germ separation, the slurry goes through an intense grinding which further loosens the starch and gluten from the remaining fibre. The slurry is screened to remove the fibre, which then is washed and pressed to about 60% moisture. The LSW from the steeping step is concentrated in evaporators to produce heavy steep water (HSW).

This nutrient rich concentrated product is typically dried together with the fibre to be sold as corn gluten feed (CGF) to the livestock industry. The CGF contains about 21% protein. The starch is washed and processed through a series of up to 14 hydroclones to remove impurities. The final product which is 99.5% pure starch is then used for fermentation in dedicated plants or processed further to produce modified starch, corn syrups (CS) and high fructose corn syrups (HFCS) in integrated plants. All fermentation processes used in wet milling plants today are continuous (Drapcho *et al*., 2008).

2.10.2 Dry milling process

Because wet milling plant are complex and capital intensive, most new and smaller ethanol plant use the dry milling process. The process begins by adding water to the milled corn grains, adjusting the pH to about 6 and adding a thermostable α-amylase. The next step is starch liquefaction (Lewis, 1996). An indirect laboratory method to determine total ethanol production is to measure total carbon dioxide production and calculation to corresponding ethanol production using the stoichiometric equation shown below (Drapcha *et al*., 2008).

$$3Xylose+3ADP+3Pi \longrightarrow 5C_2H_5OH+5CO_2+3ATP+3H_2O \quad .....(2.7)$$

2.10.3 Comparison between wet and dry milling methods

The difference between the two processes is that in the dry milling process. The whole corn is ground and fed into the fermenter for fermentation, whereas in the wet milling process. The corn components are fractionated first and then only the starch fraction is used in fermentation. As a result, the wet milling process requires much higher capital investment and ethanol plants using this process are much larger than those using the dry milling process. Both processes generate a number of ethanol related co-products. These co-products include distillers dried grains with soluble (DDGS) and carbon dioxide in the dry milling process, whereas corn oil, corn gluten meal, corn gluten feed and carbon dioxide are obtained in the wet milling process. All these co-products are of relatively moderate values. However, the wet milling plants can easily be modified to produce other products such as corn syrups and high fructose corn syrups, which can be produced independent of ethanol production. These independent co-products are not just economically beneficial but can also be strategically important, especially during the times of reduced ethanol market demand (Drapcho *et al*., 2008).

2.10.4. Biomass pre-treatment

Pre-treatment affects the structure of the biomass by solubilising hemicelluloses, reducing crystallinity and increasing the available surface area and pore volume of the substrate. Pre-treatment improves the digestibility and access for microbial and enzymatic attack by removing the core and non-core lignin fraction. This results in the enlargement of the inner surface area of the substrate particles due to the partial solubilisation and degradation of hemicelluloses and lignin. This further leads to fractionation and opening of the cellulose structure for enzymatic attack. A study carried out by Adsul *et al*. (2005) showed that high cellulose productivities can be achieved by the use of chemically pre-treated biomass as carbon source for specific micro-organism. Again Lynd (2003) reported that an ideal biomass/ lignocellulosic material should meet with some of the stated requirement after chemical pre-treatment:

(a) High rates of hydrolysis and high yield of fermentable sugars.

(b) No production of compound that are inhibitory to micro-organisms used in the subsequent fermentation step.

(c) Recycle of chemicals to reduce operating costs.

(d) Be effective at low moisture content.

(e) Inexpensive materials of construction.

(f) Minimal degradation of the carbohydrate fractions.

(g) Minimal wastes

(h) Have high degree of simplicity.

An ideal pre-treatment will also reduce the lignin content and crystallinity of the cellulose and increase the surface area. There is currently no single pre-treatment process that meets the entire above requirement. In selection of a pre-treatment process, all of the above requirement should be considered.

2.10.5 Concentrated H_2SO_4 hydrolysis

The use of concentrated acid, especially H_2SO_4 for cellulose hydrolysis has been known for long time. The process developed by Arkenol by far has the best chance of reaching commercialization. In the Arkenol's process Farone and Cuzens (1996) reported that decrystallization of cellulose and hemicelluloses is carried out by adding 70-77% H_2SO_4 to a biomass that has been dried to 10% moisture. The acid is added to achieve a ratio of acid to total cellulose plus hemicellulose of 1:25:1 and the temperature is maintained at about 50^0C. The concentrated acids disrupt the hydrogen bonding between cellulose chains and convert it to an amorphous state, which is extremely susceptible to hydrolysis. Dilution of acid to 20–30% will cause hydrolysis of both cellulose and hemicelluloses to monomeric sugars. The hydrolysate is separated from the residual biomass by pressing. The partially hydrolyzed biomass then undergoes a second hydrolysis, which uses conditions similar to the first one. This second hydrolysis releases the rest of the sugars. The sugars are separated from the acid in a moving chromatography column containing a cross-linked polystyrene cation exchange resin. The product is a liquid containing at least 15% sugar and less than 3% acid. The sugar solution is then neutralized and the acid from the ion-exchange column is reconcentrated in a triple effect evaporator and recycled (Drapcho *et al*., 2008).

2.10.6 Dilute H_2SO_4

Dilute sulfuric acids can also be used for pre-treatment of lignocellulosic biomass. However dilute acids can only partially hydrolyze biomass to monomeric sugars. Following dilute acid treatment, the enzyme cellulose is needed for hydrolysis of the remaining carbohydrates in the treated biomass. H_2SO_4 is the acid that is most widely used. Dilute acid pre-treatment can be a simple-stage process in which biomass is treated with diluted H_2SO_4 at suitable acid concentrations and temperatures for a period of time. Nguyen *et al*. (1998) gave the pre-treatment

conditions for softwood to be 0.4% H_2SO_4 at 200 – 230^{0}C and 1–15min. When Douglas fir chips were heated under these conditions, 90–95% of the hemicelluloses and 20% of the cellulose were solubilised and 90% of the remaining cellulose was hydrolyzed to glucose by cellulose.

The dilute acid hydrolysis process is effective in releasing fermentable sugars from several types of biomass. However, it has a major drawback. In dilute acid treatment of biomass, a number of degradation compounds are formed. These compounds, which include furan derivatives such as furfural and 5-hydroxy-methylfurfural (5 – HMF); organic acids such as acetic, formic and levulinic acid and phenolic compounds are inhibitory to ethanol – producing organisms intended for conversion of biomass – derived sugars (Larsson *et al*., 1999).There are other methods of chemical pre-treatment of lignocellulosic materials for bio-ethanol production and these include the following:

2.10.7 Steam explosion

In this pre-treatment process, biomass is exposed to super heated steam in a reactor. The high – pressure steam penetrates the biomass and initiates an auto hydrolysis reaction. The organic acids, which are formed initially from the acetyle groups in the biomass, catalyze hydrolysis of most of the hemicelluloses fraction to soluble sugars (Lora and Wayman, 1978). After a specific reaction time, a ball valve is rapidly opened to discharge the biomass explosive into a collection tank at much lower pressure. Upon hydrolysis by celluloses, the biomass treated by steam explosion process yields much higher fermentable sugars than the untreated material. Tucker *et al*. (2003) reported that higher pre-treatment temperatures and shorter contact time results to higher enzymatic cellulose digestibility. Again enzymatic hydrolysis of poplar chins treated by the process of steam explosion achieves 90% of theoretical yield compared to 15% obtained with untreated material (Grous *et al*., 1986). Morjanoff and Gray (1987) reported on

optimization of steam explosion as a method for increasing susceptibility of sugar cane bagasse to enzymatic saccharification.

2.10.8 Ammonia treatment

Alkali such as NaOH, KOH and NH3 can be used for biomass pre-treatment. Base solutions cause swelling of biomass which subsequently leads to decrease in the degree of polymerization, decrease in crystallinity, disruption of lignin structure and separation of structural linkages between lignin and carbohydrates (Fan *et al*., 1987). Among the bases investigated ammonia has the highest potential for use in commercial processes since it can be recovered and recycled due to its high volatility, thus reducing chemical and waste treatment cost (Kurakake *et al*., 2001) reported the pre-treatment of bagasse; corn husk and switch grass using ammonia water for enzymatic hydrolysis.

2.10.9 Lime treatment

Biomass can also be pre-treated with lime to improve subsequent enzyme hydrolysis to fermentable sugars. Typical lime loading is 0.1g Ca $(OH)_2$ per gram biomass. A minimum amount of 5g water per gram biomass is needed. Lime treatment can be performed at temperatures below 100^0C to avoid the use of expensive pressure vessels, but at such low temperatures, the required treatment times normally are very long. For instance, Kims and Holtzapple (2005) reported that corn stover treated with excess lime at 0.5g Ca $(OH)^2$ per gram biomass at 55^0C with aeration, needed 4 weeks to accomplish the treatment, after which 87.5% of the lignin was removed and some of the carbohydrate fractions we solubilized. The total yields of glucose and xylose after enzyme hydrolysis were 93.2% and 79.5% respectively.

2.10.10 Alkaline peroxide treatment

This involves the use of alkaline solutions of hydrogen peroxide. In this treatment, large fractions of the hemicelluloses and lignin are solubilised whereas most of the

cellulose remains intact. The cellulose in the residual solid can be hydrolyzed with enzymes at very high rates and near theoretical yields. The optimum pH is 11.5, which is the pH for the dissociation of H_2O_2. When this pre-treatment was applied to corn stover, most of the hemicelluloses and as much as 50% of the lignin were solubilised. The residual solid fraction which still contains most of the original cellulose was hydrolyzed with cellulose to over 90% of theoretical glucose yield (Zhang *et al*., 2007).

2.10.11. Wet oxidation

In the wet oxidation process, biomass is treated with water and air or oxygen at elevated temperatures and pressures (McGinnis *et al*., 1983). Similar to the alkaline peroxide treatment process large fractions of hemicelluloses and lignin are solubilised during wet oxidation leaving a solid residue high in cellulose. The cellulose in the residual solid can be hydrolyzed with enzymes at high rates and yields (Bjerre *et al*., 1996). The main advantage of the wet oxidation over the alkaline peroxide process is replacement of hydrogen peroxide by air, which helps reduce the chemical costs significantly (Drapcho *et al*., 2008).

2.11 Gelatinization and saccharification of starch

It is needful that the starch from their feed stocks be converted first into soluble sugar before it is fermented to ethanol. The soft and tender nature of the starch suggests the use of mild saccharifying agents, since the polysaccharide must be broken down into fermentable sugars which can be utilized by the micro-organisms. The starch degradation process can be achieved in complete term by enzymatic agents or by acid hydrolysis and it involves gelatinization or hydration, liquefaction and saccharification. The practice of cooking involved in the first two steps is undertaken in order to release the starch granules and this facilitates the reaction of the substrate with the saccharifying agent. It has been observed that starch granules gelatinize in water when the temperature is raised to about 60-70^{o}C

range. As the temperature is raised further, the granules swell progressively to form a paste or sol and the shorter linear molecules dissolve. There is thus a disintegration of the granules into molecules and starch granules fragments. The paste then forms a gel upon cooling, depending on the variety and concentration of starch present. The best definition of the gelatinization temperature is that point at which the granule loses birefringence when viewed under a polarizing microscope (Chen, 2003).

When the organization of the starch granule is disrupted by mechanical means such as extensive grinding of the starch in the dry state (dry milling), there is a rupture along certain lines of cleavage and the starch tends to gelatinize even in cold water. In this disrupted state, it is more susceptible to action of enzymes.

The temperature of gelatinization in water could be altered by the addition of certain chemicals such as caustic alkalis, urea and some amines. But if these chemicals are present in proper concentration, they are solvents for starch even at room temperature. Salts such as sodium sulphite suppresses gelatinization by preventing excessive swelling of the starch granules.

Gelatinization is a net endothermic process involving hydration of the starch molecules, separation and rupture of some hydrogen bonds between glucose units. The heat of gelatinization varies from 5700 cal per glucose unit for very small starch granule to 9080 cal. per glucose unit for relatively large starch granule. An important property of starch from an industrial stand point is the case of hydrolysis of bonds between glucose units by acid or enzymes. Acids may be used to hydrolyze starch in either its native from or in gelatinized state, while enzymes (amylases) hydrolyze starch in either its native form or in gelatinized state, while enzymes (amylases) hydrolyze starch efficiently only in the swollen granule state or gelatinized form. Although an individual starch granule gelatinizes quite sharply, not all of the granules in sample gelatinize at the same temperature but

rather between 8-10^{o}C range and each variety of starch have a characteristic gelatinization temperature range as given in Table 2.1below.

Table 2.2: Gelatinization Temperature of Some Starch Sources

Starch Source	Gelatinization Temperature (^{o}C)
Rice	68-78
Sorghum	68-78
Corn	62-72
Cassava	71-44
Cocoyam	65-70
Potato	59-68
Wheat	58-64

Source: Ofoefule (2012)

2.11.1. Enzymatic hydrolysis method.

Enzymes suitable for the hydrolysis of starch can be obtained from many sources such as bacteria, fungi, vegetables and animals. In the United States and in other advanced countries, submerged fermentation process was initiated and successfully used in hydrolysis of tapioca for alcohol production. Barley malt moulds that grow on rice, bread and wheat bran are commonly used in most Asian countries. Recently, the use of rhizopus was introduced in France to supplement the mould bran process. Malt contains the three important enzymes necessary for the complete hydrolysis and degradation of starch. These are **α** -amylase, β -amylase and glucoamylase (glucosidase). The breakdown of gelatinized starch occurs through the hydrolysis of a – 1, - 4 linkages that join the glucose molecules into long chains and through the hydrolysis of the **α** – 1, 6 links that form the branch points of the amylopectin component of starch. **α** - amylase also known as liquefying enzyme causes more or less random cleavage of the starch molecule by hydrolyzing the **α** – D- (1,4) glucosidal bonds. Characteristic of this reaction is the

rapid decrease in the viscosity of the gelatinized starch slurry to produce more chain ends for the action of the other enzymes e.g the saccharrifying enzyme. Microbial **α**-Amylases are used commercially to produce low viscosity size adhesive and syrups from native starch. The presence of this enzyme in saliva and pancreatic juice of animals is necessary for digestion of the starch in their diet. β–amylase also attacks the **α** –1, 4 linkages of dextrin and splits the disaccharide maltose directly from the starch molecules.

The hydrolysis involves the stepwise removal of maltose units from the non-reducing ends of the amylase and amylopectin components of the starch. Amylase molecules are almost quantitatively hydrolyzed to maltose in this manner. Amylopectin molecules are hydrolyzed only to branch points in the molecules because β-amylase does not bypass the 1.6- glucosidic linkages present at these points. The residue which remains (limit dextrin) is of comparatively high molecular weight and contains 1 to 6 bonds for the attack of the third enzyme. Neither **α**–amylase nor β-amylase attacks of the **α** -1, 6 linkages but they rather assist in facilitating the attacks of the glycosidase which is the third enzyme. Glucoamylase acts specifically on **α** -1,6 linkages of the resulting maltose and dextrin thus completing the hydrolysis of starch into fermentable sugar. It involves the splitting of the disaccharides directly to form monosaccharide, D-glucose. The hydrolysis proceeds by the stepwise removal of glucose from non-reducing ends of the molecules chains. The hydrolysis pathway involves the progressive shortening of all the chains beginning at the non-reducing ends of the molecules of amylopectin component. When a-D- (1-6 linkage is encountered at a branch point in this molecule, the hydrolysis occurs at a much slower rate. The hydrolysis of amylase also begins at the non-reducing ends and proceeds with a progressive shortening of all the amylase chains until they are completely hydrolyzed to D-glucose.

2.11.2. Acid-catalyzed hydrolysis method

All starches are hydrolyzed by acid to sugar. In the past, this reaction was the commercial method for the production of dextrose and starch syrups. Sugar yields achieved from acid hydrolysis is usually low; however, high yields can be obtained by hydrolyzing the starch slurry with 0.1M HCl at one atmosphere using starch to acid ratio of 1:3. Acid hydrolysis process which involves the mixing of starch slurry with HCl until the pH approximate 2.0 was investigated by Robinson and Kutianawala (1979). The desired conversion was achieved by heating the mixture. This gave a maximum yield of about 90% as some of the glucose and dextrin were consumed in the side reactions and polymerized under such conditions of the process to form compounds of high molecular weight.

The sugar syrup is then neutralized with N_a2CO_3 and centrifuged to remove impurities. It can be further refined by filtration for certain purposes, but as a substrate for fermentation, the syrup would require a little more than the desired sugar level. Today, enzymatic methods of starch hydrolysis are replacing all or part of the acid processes because enzymatic methods are more economically viable and result in better and purer products. Acid hydrolysis is more expensive and causes extensive corrosion of the equipment. Expensive corrosion-proof equipment are therefore needed resulting in high capital cost.

Again, a number of side reactions have been discovered to accompany the acid hydrolysis of starch glucose which may recombine to give various disaccharides and polysaccharides, or it may decompose into products such as 5-hydroxymethyl furfural and levulinic acid. The compounds produced as a result of partial decomposition or degradation by acid can inhibit microbial growth in enzyme – acid dual process. The starch hydrolysis process is given by a simple equation.

$$(C_6H_{10}O_5)n + H_2O \longrightarrow nC_6H_{12}O_6 \quad \text{..... (2.8)}$$

2.11.3 Enzymatic hydrolysis

The enzymatic hydrolysis reaction is carried out by means of enzymes that act as catalysis to break the glycosidic bonds. This is the degradation of cellulose chains into glucose molecules by trichoderma viride (cellulase) enzymes. There are two types of hydrogen bonds in cellulose molecules: those that form between the C_3OH group and the oxygen in the pyranose ring within the same molecules and those that form between the C_4OH group of one molecule and the oxygen of the glucosidic bond of another molecule. Ordinarily, beta-1, 4 glycosidic bonds themselves are not too difficult to break. However, because of these hydrogen bonds, cellulose can form very tightly packed crystallites. These crystals are sometimes so tight that neither water nor enzyme can penetrate them; only exogluconase, a subgroup of cellulose that attacks the terminal glucosidic bond, is effective in degrading it. The inability of water to penetrate cellulose also explains why the crystalline is insoluble. On the other hand, amorphous cellulose allows the penetration of endogluconase, another subgroup of cellulose that catalyzes the hydrolysis of internal bonds. The natural consequence of this difference in the crystalline structure is that the hydrolysis rate is much faster for amorphous cellulose than crystalline cellulose. The process of breaking the glucosidic bonds that hold the glucose basic units together to form a large cellulose molecule is called hydrolysis because a water molecule must be supplied to render each broken bond inactive. In addition to crystallinity, the chemical compounds surrounding the cellulose in plants, e.g. lignin, also limit the diffusion of the enzyme into the reaction sites and play an important role in determining the rate of hydrolysis. Sometimes, wood chips are pretreated with acid to strip hemicellulose and lignin before they are treated with an enzyme or a mixture of enzymes. In general, 20 to 70 percent yield of glucose can be expected after 24 hours. Hence, it is sometimes referred to as Acid-Enzyme hydrolysis.

Pre-treatment is necessary to break down the crystalline structure of the lignocellulose material isolating the cellulose away from the lignin in the cell walls for hydrolysis. Pre-treatment is carried out to increase the surface area and accessibility of the plant fiber to enzymes and thus, achieve high sugar yield for ethanol fermentation. The face stock is subjected to steam and acid whose temperature, concentration respectively and treatment time are mild so that the cellulose surface area is greatly increased as the fibrous feedstock is converted to a muddy texture with little conversion of the cellulose to glucose. This process is referred to as pre-treatment of the feedstock. The feedstock however becomes acidic and the pH is adjusted by the addition of alkaline often NaOH to 4 to 6, which is the optimal pH range for celluloses. Higher pH is acceptable if alkalophilic cellulose is used. The pre-treated cellulose is then hydrolyzed with cellulose. Cellulose is a generic term denoting a multi-enzyme mixture comparing exocellobiohydrolases (CBH) endoglucanases (EG) and Aspergillus luchunesis that can be produced by a number of plants and microorganisms. CBH and EG catalyze the hydrolysis of insoluble cellulose to cell oligosaccharides (majority cellulobiose) which is then catalyzed to glucose by βG.

2.11.4 Important Factors in Enzymatic Hydrolysis

Substrate concentration and quality, applied pretreatment method, cellulose activity, and hydrolysis condition such as temperature, pH, and mixing are the main factor in enzymatic hydrolysis of starch materials. The optimum temperature and pH is function of raw material, enzyme source and hydrolysis duration. The temperature and pH of different cellulose are usually reported to be in the range of 40 to 50^{o}C and pH 4 to 5. However, the optimum pH and residence time might affect others.

One of the main factors that affect the yield and the initial rate of enzymatic hydrolysis is substrate concentration of slurry solution. High substrate concentrate

can cause substrate inhibition, which substantially lowers the rate of hydrolysis. The extent of inhibition depends on the ratio of total enzyme to total substrate.Problem in mixing and mass transfer also arise in working with high substrate concentration. The ratio of enzyme to substrate used is another factor in enzymatic hydrolysis. Obviously, application of more cellulase, up to a certain level, increases the rate and yield of hydrolysis. However, increase in cellulose level will increase the cost of the process. BH and EG bind to cellulose in the feedstock via carbohydrate-binding modules (CBMs), such as cellulose-binding domain (CBDs), while most βG enzymes, including Trichnoderma and Aspergillus β-glycosidase enzymes, do not contain such binding modules and thus remain in solution.

Advantages:

1. Low utility costs since process can be run at low temperatures.
2. Process allows for very high rate of conversion.

However the problems with enzymatic hydrolysis are:

1. Cellulose accounts for up to 50 percent of the cost of hydrolysis due to the requirement of large amount of cellulose hence increased cost of process.
2. Presence of compounds that reduce rate of cellulose and/or microorganisms in the subsequent fermentation of the sugar. E.g., glucose released during the process inhabits cellulose, particularly

2.12 Chemistry of fermentation

Fermentation is an internally balanced oxidation / reduction of organic compounds that take place in the absence of external acceptors (O_2, NO_3, SO_4^2). In fermentation, the oxidation of an intermediate organic compound is coupled to the reduction of another organic intermediate fermentation resulting in the formation of carbon compound that is more reduced than the original organic electron donor and a carbon that is more oxidized (Drapcho *et al*., 2008). Fermentation also

includes phosphorylation, dehydrogenation and decarboxylation reactions. It produces esters, alcohols, higher alcohols, aldehydes etc as by-products (Skinner, 1947). In many fermentation processes, the reactions involved with oxidizing a simple sugar under anaerobic conditions involve two phases; glucose oxidation and pyruvate metabolism. Glucose metabolism often occurs through glycolysis otherwise known as the Embden-Meyerhof Parnas (EMP) pathway in the same manner as in aerobic or anaerobic respiration. However, because oxygen is unavailable for use as an external electron acceptor or the microorganism does not have the capacity to use alternative inorganic compounds such as nitrate or sulphate, the electron carrier molecule NAD^+ must be regenerated by donating electrons to intermediate organic compounds.

The yeast *Saccharomyces cerevisiae* is the universal organism for fuel ethanol production using starch and sugar fees stocks. The sugars that are metabolisable by this organism include glucose, fructose, mannose, galactose, sucrose, maltose and maltotriose. In the simplest form, production of ethanol from glucose can be expressed by the following equation.

$$C_6H_{12}O_6+2Pi+2ADP \longrightarrow 2C_2H_5OH+2CO_2+ATP+2H_2O\text{----------} \quad (2.9)$$

Glucose $\longrightarrow$ 2 ethanol + 2 carbon dioxide + Energy

From the above equation, it can be calculated that the theoretical yield is 0.522g ethanol produced per gram of glucose consumed this yield can never be realized in practice since not all of the glucose consumed is converted to ethanol but part of it is used for cell mass synthesis, cell maintenance and production of by-products such as glycerol, acetic acid, lactic acid and succinic acid (Thomas *et al*., 1996). The mechanism starts with the conversion of the disaccharide (sucrose) into monosaccharide by the enzyme invertase.

$$C_{12}H_{22}O_{11}+H_2O \xrightleftharpoons{\text{invertase}} C_6H_{12}O_6+C_6H_{12}O_6\text{----------} \quad (2.10)$$

from yeast Glucose Fructose

2.12.1 Distillation of fermented wort

Ethanol is recovered from the fermented wort by distillation. This is achieved by using the distillation apparatus consisting of a round bottomed flask, with a connecting arm, sealed on a thermoregulator. The flask is fitted with a cork carrying a thermometer. The connecting arm is subsequently connected to a condenser through cold water tap to enable the vapourized alcohol to condense. The distillate is then collected in a flask tightly connected to the condenser. Ethanol is obtained by setting a thermo-regulator at 78 – 79^0C and monitoring the temperature with the aid of the thermometer. The volume of ethanol recovered is then measured when there is no more condensate at the set distillation temperature. The distillate is allowed to run through a funnel containing calcium oxide or anhydrous sodium sulphate to absorb any traces of water in the distillate. This latter process however is carried out for industrial ethanol for beverage alcohol, distillation alone is used. The distillation is done over and over again to increase the concentration of ethanol available.

Alcohol yield is expressed as the amount of distillate per volume of fermented wort percentage yield is calculated as;

$$\% \text{ Yield} = \frac{\text{Volume of ethanol obtained by distillation}}{\text{Volume of fermented wort}} \text{-------- (2.11)}$$

2.12.2 By-products of alcoholic fermentation

Alcoholic fermentation is usually carried out by live yeast cells and is associated with the production of a number of substances other than alcohol. Apart from the stillage, carbon dioxide and problems of azetrope, ethanol from the fermented wort contains many by-products such as glycerol, succinic acid, acetaldehyde and fossil oils. These substances usually account for less than one percent of the total volume of ethanol (Baldwin, 1967). They are largely responsible for the characteristic

flavours and aromas observed in most of the distilled alcoholic beverages. The proportions of these by-products are variable and depend mainly on the condition of the yeast and the nature of nutrients. Research findings have shown that succinic acid exists in all fermented liquors. Glycerol is also an inevitable by-product of sugar fermentation many other subsidiary products have now been recognized in addition to succinic acid and glycerol. These are formic acid, acetic acid, propionic acid, butyric acid and several other higher acids which occur as esters. Formaldehyde, acetaldehyde and traces of higher aldehydes, alcohols like n-amyl and iso-amyl alcohols that are main constituents of fossil oil are also among the typical by-products. Stillage, which is the spent mash resulting from the distillation of wine is usually obtained in proportion of 12 parts for each of alcohol. It is a highly polluting waste which when not properly controlled, causes serious environmental problems. However, stillage can be fed to cattle and poultry and also used as a fertilizer though the quantities and procedures for adding it to the soil have not been extensively developed. Stillage is also used in the manufacture of several fermentation products such as enzymes, vitamins and antibiotics. The fossil oil and second grade alcohol (6 litres and 50 litres respectively per 1000 litres anhydrous alcohol), could be used commercially. The carbon dioxide produced during fermentation also has some industrial applications. It is used in the preparation of dry ice and for carbonation of beverages like beer and soft drinks and in the lyophilisation of micro organisms (Prescoth and Duun, 1959).

2.13 Cocoyam as a tropical food crop

The world has focused entirely on a comparatively small number of crops to meet the various needs for food and industrial fibre; the total number of economic crops of significance to global trade hovering just above one hundred. The consequence is that thousands of plant species with a considerably larger number of varieties fall into the category of under-utilised or neglected crops. These crops are

marginalized by agricultural, nutritional and industrial researchers. One of such neglected crops is cocoyam and its species, which over the years has received only minimal attention from researchers and other stakeholders of interest.

Cocoyam (*Colocasia esculenta*) and wild cocoyam (*Xanthosoma sagittifolium*) are the important species of edible aroids grown in tropical and sub tropical countries (FAO, 2006). They contribute significantly to the carbohydrate diet, even though ranked less important after yam, cassava and potato (Obomeghei *et al*., 1998; FAO, 2006). Cocoyam is produced in abundance in eastern part of Nigeria, but less valued in this area as it is regarded as staple food for rural dwellers, the poor and the less privileged in society (IITA, 1992). Enwere (1998) reported that the corms and cormels are cooked and pounded with cassava or yam into fufu and eaten with stew or soup, and that the cormels are exclusively used as a thickener in preparation of soup. Wild cocoyam (*Xanthosoma sagittifolium*) shown in Figure 2.2 is another specie of cocoyam that grows wild in the bushes and is common in most part of the tropics. Though its corm is edible, but it is rarely consumed.

The nutritional and chemical compositions as reported by FAO (2006) shows that cocoyam if fully exploited would enhance the food security of people living in the Tropics. A major problem of cocoyam is that the corms are susceptible to physical damage during harvesting and thus leading to high post harvest losses (Onwueme and Simha, 1991; FAO, 2006). To overcome these losses, Onyeike *et al*. (1995) reported that the corms and cormels may be processed into flour. Kwarteng and Towler (1994) reported that the flours stores much longer than the unprocessed tubers of cocoyam. The need to widen the scope of information on the physical, chemical and engineering characteristics has been stressed by FAO (2006). This will improve cocoyam competitiveness alongside other roots and tuber crops, enhance its application in other food systems and improve its marketing potential.

Figure 2.2. Wild cocoyam (Xanthosoma sagittifolium) growing in the forest

2.14.1 Uses of cocoyam.

Cocoyam (*Xanthosoma sagittifolium)* is grown in tropical region for human nutrition, animal feed, and cash income for both farmers and traders. Cocoyam flour can be used in making biscuits or as composites in bread making (Idowu *et al*., 1996). The corm's flour is a good source of carbohydrate for diabetics and production of weaning food for infants and for those with gastrointestinal disorders (Onwueme, 1978). The taro and tannia flours in precooked forms may find good uses in pie filling, binder in sausage and as emulsifier in food systems (Fagbemi and Olaofe, 1998). In the current study, sorption isotherm, physical and chemical properties of cocoyam flour are reported. This is expected to provide information for those involved in processing of flour and its application in food formulation.

2.14.2 Wild Cocoyam as bio-ethanol feedstocks.

Wild cocoyam (*Xanthosoma sagittifolium)* is one of the potential feedstocks for bio-ethanol production. The crop grows wild in the bushes. The food shortage concern expressed by most individuals over the use of food crop for bio-fuels production would not apply in the case of wild cocoyam. This is because the crop falls into the group of highly underutilised crops. Also, information on the crop is very minimal and highly outdated. A few research works have been reported on the

use of this crop mainly for pharmaceutical purposes with no attention given to its uses for ethanol and biogas production.

2.15 Jackfruit as a tropical tree

Jackfruit (*Artocarpus heterophyllus* lam) is a large fruit of a milky-juice tree, of Moraceae family (Fig. 2.3). The edible, pulpy part represents the parianth. Jackfruit is the largest edible fruit in the world (Naik, 1949; Sturrock, 1959). It was believed to have originated from the forests of the Western Ghats (India), where it still grows in the wild, as well as in the evergreen forests of Assam and Myanmar. It is cultivated throughout Bangladesh, Burma, India, Indonesia, Malaysia, the Philippines, Sri Lanka, and Thailand to some extent in Brazil and Queensland (Australia). It has been reported to contain high levels of protein, starch, calcium, and thiamine (Brukill, 1997; Bobbio *et al*., 1977). The juicy pulp of the ripe fruit is eaten fresh as a dessert. The bulbs (excluding the seeds) are rich in sugar, fairly well in carotene and also contain vitamin C (Bhatia *et al.*, 1955).

Figure 2.3: A jackfruit tree with fruits.

Fig. 2.4: Ripe Jackfruit cut longitudinally showing the seeds

Fig. 2.5: Ripe Jackfruit seed

2.15.1 Uses of jackfruit

Jackfruit is rich in nutrients such as sodium, potassium, iron, vitamin B6, calcium, zinc, and many other nutrients. Jackfruit can lower blood pressure, cure fever and diarrhea. According to Bobbio *et al*. (1977), jackfruit is also known to be beneficial to fighting asthma, ulcers, indigestion, tension, nervousness and constipation. It can slow down aging and cell degeneration. Jams, beverages, candies, conserves and dehydrated forms are other industrial uses for which the jackfruit can be utilized (Naik, 1949).

2.15.2 Jackfruit as bio-ethanol feedstocks

Production of jackfruit as feedstocks will be relatively less expensive for ethanol production and would not compete with human and animal consumption. At present in Nigeria, jackfruit is mainly grown for its ornamental values. The consumption of its seeds is still not popular as it is regarded as waste or as feed for domestic animals.

Thus, jackfruit is a potential feedstock for ethanol and biogas production. The starch of the crop is found to be high in sugar yield which will translate to high ethanol yields. Much attention has not been paid to the crop by some researchers leading to underdevelopment of the crop as a potential feedstock for bio-fuel

production. In all the literatures, no documentation has been cited or found about making fermentation/alcohol beverage or biogas production from crop's seeds.

Bio-ethanol production from jackfruit may increase due to the vast area of land and abundant labour available for growing the crop in Nigeria. More so, the crop is not widely consumed and as such would not compete largely with human or animal food.

2.16 Water Yam as a tropical food crop

Yam is a common name for some plant species in the genus Dioscorea family (*Dioscoreaceae*) that form edible tubers. These are perennial herbaceous vines cultivated for the consumption of their starchy tubers in Africa, Asia, Latin America, the Caribbean's, and the Oceania. There are many cultivars of yam. The true yam is a versatile vegetable. It can be barbecued, roasted, fried, grilled, boiled, baked, smoked, and when grated it is processed into a desert recipe. Yams are a primary agricultural and culturally important commodity in West Africa (Wikipedia, 2012) where over 95% of the World yam crop is harvested.

2.16.1 Water yam (*Dioscorea alata)*

Water yam (Fig.2.6) is one of the six yam species of economic importance in Nigeria. It is less utilised for major food products as a traditional bias which has to recognize the unique quality characteristics and the good agronomic flexibility of the species. The species has high yield, high multiplication ratio and better tuber storability than the preferred indigenous *Dioscorea rotundata*. D. alata has an advantage for sustainable cultivation especially when yam production seems to be on the decline as a result of high cost of production, low yields, and post harvest losses among others. Dioscorea alata is widely cultivated in the tropics and subtropical regions of the world and known for high carbohydrates and medicinal values. Starch is the major carbohydrate reserve accounting up to 85% of dry

matter. D. alata tubers are known to contain alkaloid, tannins, and saponins (Osagie, 1992).

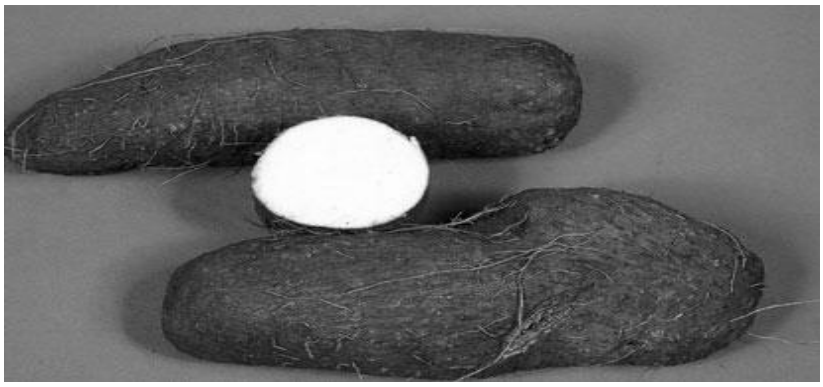

Figure 2.6: Water yam (*Dioscorea alata*)

2.16.2 Uses

Tuber is the main part of the yam plant which has high carbohydrate content (low in fat and protein), and provides a good source of energy. Unpeeled yam has vitamin C (Osagie, 1992). Yam, sweet in flavour, is consumed as boiled yam, as cooked vegetable or fufu or fried in oil and then consumed. It is often pounded into a thick paste after boiling and is consumed with soup. Yam is used as an industrial starch has been established as the quality of some of the species is able to provide as much starch as in cereal.

2.16.3 Water yam as bio-ethanol feedstocks

Food versus fuel has been the major concern with regards to the production of alternative fuels. Most feedstocks are relatively expensive for ethanol production and compete with human and animal food which may lead to higher prices of grain and sugar in future. At present, corn kernel is being utilized for the production of ethanol because it is easier and less expensive to produce ethanol.

Water yam is another potential feedstock for ethanol and biogas production. The starch of the crop is found to be high in sugar yield which will translate to high

ethanol yields. Much attention has not been paid to the crop by some researchers leading to underdevelopment of the crop as potential feedstocks for bio-fuel production.

Bio-ethanol production from water yam may increase due to the vast area of land and abundant labour available for growing the crop in Nigeria. More so, the crop is not widely consumed and as such would not compete largely with human or animal food.

2.17 Yellow yam as a tropical food crop.

Yellow yam (*Dioscorea dumentarum*) Shown in Fig. 2.7, is one of the six yam species of economic importance in Nigeria. It is less utilised for major food products as a traditional bias which has to recognize the unique quality characteristics and the good agronomic flexibility of the species (Wireko-Manu *et al*., 2011). The species has high yield, high multiplication ratio and better tuber storability than the preferred indigenous *Dioscorea rotundata*. Dioscorea dumentarum has an advantage for sustainable cultivation especially when yam production seems to be on the decline as a result of high cost of production, low yields, and post harvest losses among others.

D. dumentarum is widely cultivated in the tropics and subtropical regions of the world and is known for its high carbohydrates and medicinal values (Siddaraju *et al*., 2008). Starch is the major carbohydrate reserve accounting up to 85% of dry matter. D. dumentarum tubers are known to contain alkaloid, tannins, and saponins (Osagie, 1992; Addy, 2011; Policarp *et al*., 2012).

Figure 2.7: Yellow yam (*Dioscorea dumentarum*)

2.17.1 Uses of yellow yam

Yellow yam is cultivated for its carbohydrate content (low in fat and protein), and provides a good source of energy. Unpeeled yam has Vitamin C (Policarp *et al*., 2012). Yellow yam is sweet in flavour, rich in medicinal value, and consumed when boiled (Addy, 2011).

2.17.2 Yellow yam as bio-ethanol feedstocks

Food versus fuel has been the major concern with regards to the production of alternative fuels. Most feedstocks are relatively expensive for ethanol production and compete with human and animal consumption which may lead to higher grain and sugar prices in future. At present, corn kernel is being utilized for the production of ethanol because it is easier and less expensive to produce ethanol (Taherzabeh *et al*., 2007).

Yellow yam is another potential feedstock for ethanol and biogas production. The starch of the crop is found to be high in sugar yield which will translate to high ethanol yields. However, much attention has not been paid to the crop by researchers leading to underdevelopment of the crop as potential feedstocks for bio-fuel production. Reported research works on the use of water yam have been mainly for food and pharmaceutical purposes (Osagie, 1992). Policarp *et al*. (2012) reported on characterization of chemical composition andante-nutritional factors in seven species of yam germplasm. Addy (2012) worked on comparative

study of yam varieties in Ghana as case study in Asante Mampong. In all the literatures, no documentation has been cited or found about making fermentation/alcohol beverage or biogas production from yellow yam.

Bio-ethanol production from yellow yam may increase due to the vast area of land and abundant labour available for growing this crop in Nigeria. More so, the crop is not widely consumed and as such would not compete largely with human or animal food.

2.18 Food versus bio- energy

The impact of bio-energy on food security and prices is complex; assessing the impact requires careful analysis of many variables. It is true that bio-energy production can change the availability of food and price of food by competing for land with food crops or livestock. This will depend however on the type of crop and region. Crops currently being used specifically for bio-fuels utilize about 0.025 billion hectares (2%) of the 1.5 billion hectares used to produce arable crops. A number of recent studies show that the impact of bio-fuels is causing rise in price of foodstuff (FAO, 2007). World Bank has also attributed this share at 80% increased demand for bio-fuels to recent rise in grain prices. Also, food prices have been affected by higher oil and fertilizer prices, bad harvests due to weather events. But higher food prices have its positive and negative effects. For instance, higher food prices will encourage farmers to produce more and make more money thereby raising their standard of living and checking youth migration from rural to urban areas. This will check crime too. Though in the short term, access to food may decrease for poorer urban dwellers that must spend either more of their limited incomes on food or can afford only insufficient quantity of food. However, much of the food versus fuel debate could be eliminated if bio-energy feedstocks were produced on land that is not suitable for food or from wastes and residues which is the focus of second generation bio-energy technologies. Utilizing marginal and

degraded lands unsuitable for food production such as lands affected by rising salinity level, may be able to restore environmental values.

The world is still at dilemma regarding dependence on food crops as energy sources, however, Zegada-Lizarazu *et al*. (2010) has observed that the recent policies enacted by the EU foresee an increased interest in the cultivation of energy crops. Hence, systematized information on new energy crops and cropping strategies is necessary to optimize their production quantitatively and qualitatively and to integrate them into traditional production systems. This kind of information will offer farmers new perspectives and options to diversify their farming activities. Some of these crops, however, may compete for land and resources with existing food crops, while others could be grown on marginal or degraded lands with consequent beneficial effects on the environment. Therefore, choosing the appropriate management components and species should be site specific and oriented to minimize inputs and maximize yields.

The paper further noted that in some cases, traditional food crops are used as dedicated energy crops with the advantage that their management practices are well known. On the other hand, the management of new dedicated energy crops, such as perennial herbaceous crops, often demands a range of structural features and tactical management approaches that are different to those commonly used for traditional food crops. Most of these crops are largely undomesticated and are at their early stages of development and improvement. The foregoing points strongly at one fact notably that it is important to research into more and more energy crops, wherever they may be found so as to tap their potential for the benefit of global progress.

2.19 Biogas production

2.19.1 Bases for biogas technology

Biogas typically refers to a gas produced by the biological breakdown of organic matter in the absence of oxygen. Biogas originates from biogenic material and is a type of bio-fuel. One type of biogas is produced by anaerobic digestion or fermentation of biodegradable materials such as biomass, manure or sewage, municipal waste, green waste and energy crops. This type of biogas comprises primarily methane and carbon dioxide.

The other principal type of biogas is wood gas which is created by gasification of wood or other biomass. This type of biogas is comprised primarily of nitrogen, hydrogen, and carbon monoxide, with trace amounts of methane

Biogas generators or digesters yield two products: The biogas itself and a semi-solid by-product called effluent or sludge. Biogas systems are most popular for their ability to produce fuel from products that might otherwise be wasted crop residues or manures. The fuel is a flammable gas suitable for cooking, lighting, and fuelling combustion engines.

The digested waste – sludge is a high quality fertilizer. The digestion process converts the nitrogen in the organic materials to ammonium, a form that becomes more stable when ploughed into the soil. Ammonium is readily fixed or bonded in the soil so that it can be absorbed by plants (Mattocks, 1984) Moreover, biogas systems offer a need to sanitize wastes. Thus, the systems are capable of destroying most bacteria and parasitic eggs in human and animal wastes, enabling the digested sludge to be applied safely to crops.

2.20 History of biogas

Anecdotal evidence indicates that biogas was used for heating bath water in Assyria during the 10th century BC and in Persia during the 16th century. Jan Baptista Van Helmont first determined in 17th century that flammable gas could evolve from decaying organic matter. Count Alessandro Volta concluded in 1776 that there was a direct co-relation between the amount of decaying organic matter

and the amount of flammable gas produced. In 1808, Humphrey Davy determined that methane was present in the gas produced during the anaerobic digestion of cattle manure. Also scientific interest in the manufacturing of gas produced by the natural decompositions of organic matter was first reported by Robert Boyle, and Stephen Hale in the 17th Century. In their report, they noted that flammable gas was released by disturbing the sediment of streams and talus. Also in 1808, Sir. Humphry Davy reported that methane was present in the gases produced by cattle manure (Oderinde and Tahir, 1988). In 1859, the first anaerobic digester was built by a leper colony in Bombay, India while the technology was developed in Exeter, England in 1895 using a septic tank to generate gas for the server gas destructor lamp, a type of gas lighting.

In 1904, the first dual purpose tank for both sedimentation and sludge treatment was installed in Hamptom England. 1n 1907, Germany was granted patent right for Inhof tank, an early form of digester. Anaerobic digestion gained academic recognition in the 1930's through scientific research. These researches led to the discovery of anaerobic bacteria, the micro organisms that facilitate the process. More research have been conducted in this field and during World War II, both France and Germany increased the application of anaerobic digestion for the treatment of manure. However, further research was carried out to investigate the conditions under which methanogenic bacteria are able to grow and reproduce. These researches led to the discovery of anaerobic bacteria, the micro organisms that facilitate the process.

2.21 Definitions of biogas

Biogas is a colourless, flammable gas produced through anaerobic digestion of animal, plant, human, industrial and municipal waste amongst others. It is composed of mainly methane (50-70%), carbon dioxide (20-40%), water vapour (2-7%), and traces of other gases such as ammonia, nitrogen, hydrogen, hydrogen

sulphide as shown in Table 2.3 (Maishanu *et al*., 1990; Umeghalu *et al*., 2012). Also, Sagagi *et al*. (2009) defined biogas as a flammable gas produced when organic materials are fermented under anaerobic condition. Biogas originates from biogenic material and is a type of bio-fuel. To produce biogas, water is added to animal/plant waste in a certain ratio to form slurry and digestion takes place in the process of anaerobic digestion (Ravita, 2012).

Biogas has globally remained a renewable energy source derived from plants that use solar energy during the process of photosynthesis. Being a source of renewable natural gas, it has been adopted as one of the best alternative for fossil fuels after 1970's world energy crisis (Al Iman *et al*., 2013). Biogas is a product of the metabolism of methane bacteria and is created when the bacteria decomposes a mass of organic materials. It is smokeless, hygienic and more convenient to use than other solid fuels. Gas gotten from anaerobic digestion is called several other names such as; drug gas, marsh gas, goober gas and swamp gas (Sagagi *et al.*, 2009).

Biogas technology is a biochemical conversion technology of bio-energy conversion where decomposition or degradation of organic matter occurs in the absence of oxygen by microorganisms (Harka *et al*., 2010).Biogas technology is based on the phenomenon that when organic matter containing cellulose is fermented in the absence of air (anaerobically), combustible gases (chiefly methane) are emitted (Umeghalu *et al.*, 2012). Biogas technologies commonly apply consortia of microbes. These communities form an intricate microbiological food chain (Kovacs *et al.*, 2013).

Table 2.3: Composition of biogas

Component	Concentration by volume (%)
Methane (CH_4)	50-70

Carbon dioxide (CO_2)	20-40
Water (H_2O)	2-7
Hydrogen sulphide (H_2S)	2
Ammonia (NH_3)	0-0.55
Nitrogen (N)	0-2
Oxygen (O_2)	0-2
Hydrogen (H)	0-1

Source: Mattocks (1980)

2.22 Anaerobic digestion (AD)

Anaerobic digestion (AD) is a microbial process in which micro-organisms breakdown biodegradable material in the absence of oxygen (Nwabanne *et al*., 2012). It is widely used to treat wastewater, sludge and organic waste because it provides volume and mass reduction of the input material. Anaerobic digestion is also a biological process in which organic material is decomposed in the absence of oxygen to produce biogas which is a mixture of methane (55-75%), carbon dioxide (25-45%), Hydrogen (0-3%), nitrogen (1-5%), carbon monoxide (0-0.3%), Hydrogen sulphide (0.1-0.5%), oxygen, water vapour and other trace of gases. The organic matter can be degraded by the sequential action of hydrolytic, acetogenic and methanogenic bacterial to produce biogas (Gupta *et al.*, 2011).

According to Nwabanne *et al*. 2012) anaerobic treatment comprises decomposition of organic material in the absence of free oxygen and production of methane, carbon dioxide, ammonia and traces of other gases and organic acids of low molecular weight. Anaerobic digestion has been considered as waste-to-energy technology and is widely used in treatment of different organic waste for example; organic fraction of municipal solid waste, sewage sludge, food waste, animal manure, etc. (Chen *et al.,* 2010). Anaerobic digestion (AD) is a controlled biological degradation process which allows efficient capturing and utilization of

biogas for energy generation. A well detailed definition of AD was given by WRAP, (2010) and it says: Anaerobic digestion is a process of controlled decomposition of biodegradable materials under managed conditions where free oxygen is absent at temperature suitable for naturally occurring mesophilic or thermophilic anaerobic and facilitative bacteria and archae species, that convert the inputs to biogas and whole digestate. Anaerobic digestion consists of several interdependent, complex sequential and parallel biological actions in the absence of oxygen, during which the products from one group of transformation of micro-organisms serve as the substrate for the next , resulting in transformation of organic matter (biomass) mainly into a mixture of methane and carbon dioxide (Chukwuma, 2013; Aworanti *et al*., 2011). Key products of anaerobic digestion include digested solids and liquids, which may be used as soil amendments or liquid fertilizers (Buendia *et al*., 2009; John, 2010; Chen *et al*., 2010).

The anaerobic digestion produces biogas for heat and electricity generation, the by-product is bio-fertilizer for plant use. Thus sustainable cycle is established.

2.23 Biogas model of European countries

The development of biogas plant that co-digest agricultural waste with other organic wastes or energy crops or industrial wastes has been aggressive over the past two decades, this has resulted because of economic, social and environmental pressure. The Kyoto Protocol, which requires countries to meet 1990 levels of GHGs, is a very significant driver. The efforts in co-digestion in Europe have been reviewed in the following.

2.23.1. United Kingdom (UK)

United Kingdom has led government initiatives driving the anaerobic digestion and renewable energy industry. Notably the "climate change levy" and the

“Renewable obligation” are UK energy initiatives that are helping the development of anaerobic digestion. Regulations are driving the use of renewable energy and environmental beneficial technology like anaerobic digestion.

2.23.2. Denmark

Denmark has a relatively large livestock population and has the need for the development of biogas plants. Many of these plants have been subsidized by their national government in order to make them economically viable. In Europe, Denmark has been the world leader in anaerobic digestion development and implementation, especially for generating manure to electricity systems. One of the driving forces in Denmark is their goal of having 33% of their total energy produced derived from renewable energy sources by the year 2030. It is believed that the biogas production in Denmark will be increasing by a factor of 10 by the year 2020, with most of them using co-substrates (Braun and Wellinger, 2002). Germany along with other countries in Europe has been using anaerobic digestion plants since World War II (LUST, 1998).

2.23.3. Germany

In total, Germany processes eight million metric tonnes of bio-waste but 85% of these wastes are composted rather than treated through anaerobic digestion. Most of the larger biogas plants in Germany use co-digestion of animal waste, human waste sewage, and processing wastes. Typically, a low solids waste stream is used for these types of plants. This is in construct to Austria which has numerous biogas plants, but there is very little if any co-digestion taking place (Holm-Nielsen and Al Seadi, 2001).

2.23.4. Sweden

Sweden has increased greatly in the use of other European countries; regulation is the impetus behind the adoption of this technology. The inability to use animal by-products in feed in Sweden has made slaughterhouse and associated wastes available for anaerobic digestion. And also, the ban on lad filing of organic wastes, and the regulations on phosphorus reduction in Sweden has given anaerobic digestion on outlet for these wastes to be handled and processed for biogas production. Manure is the primary feedstocks for most of the biogas plants, so other waste streams co-digested are secondary substrates. A good example of a Swedish anaerobic digestion plant is the kristainstad plant that processes a number of wastes.

2.24 Biogas technology in some Asian countries

Biogas generators or digesters operate throughout Asia, for example, with more than 100,000 reported in India, about 30,000 in Korea, and several millions in China. Ancient Chinese experimented with burning the gas given off when vegetables and manures were left to rot in a closed vessel.

Presently, China has successfully promoted the use of biogas as a source of household energy since the 1980s, especially in the rural areas where wood for fuel was in short supply and rural electricity was not available. Each household builds its own plant to channel waste from the domestic toilet and nearby shelters for animals, usually pigs, into a sealed tank. The waste ferments and is naturally converted into gas and compost. In addition the project has resulted in better sanitary conditions in the home.

2.25 Biogas technology in Africa

Biogas technology can serve as a means to overcome energy poverty, which poses a constant barrier to economic development in Africa. Biogas production from energy crops, agricultural wastes, industrial wastes, municipal water, crop residues etc., does not compete for land, water and fertilizers with food crops like is the

case with bio-ethanol and biodiesel production. In Africa, biogas technology has been further stimulated by the promotion efforts of various foreign aid agencies and international organization through their meetings, visits and publications. Some digesters have been installed in several Africa countries utilizing a variety of organic wastes from slaughterhouses, industrial waste, municipal wastes, human excreta and animal dung. Biogas plants (small scale) are located all over the continent but very few of them are operational (Mschandete and Parawira, 2009).

In developing countries like Nigeria, Uganda, Tanzania etc, biogas is currently used mainly for cooking and lighting and in some cases also for heating. For this reason, the gas is stored in sample plastic bags or directly in the digester above the substrates. The low efficiency of gas utilization and the lack of storage capacity can be counteracted by the installation of simple compressors, the compression of the gas and filling it into gas bottles. This also opens the opportunity to provide the neighbourhood with biogas as an energy source for cooking and lighting.

2.26 Biogas technology in Nigeria

Anaerobic digestion has been deemed one of the most useful decentralized sources of energy supply by the United Nations Development Programme (Wauton and Gumus, 2012). In the past decades, the consumption of poultry in Nigeria and in many other countries has been on the increase. As a result of this growing poultry demand, there has been a corresponding increase in the poultry industry and consequently increasing amount of organic solids by-products and wastes. Umeghalu *et al.* (2012) reported that about 724.8 tons of poultry droppings and 184,128 cows are slaughtered in major abattoirs in Anambra State of Nigeria. They also reported that only a small proportion of poultry droppings generated in major farms in the state is utilized for manure application (majorly during planting season) and fish farming. Poultry droppings can be considered as a sustainable

biomass. A broiler produces approximately 11gDM/bird/day of poultry droppings while a layer generates 32.9gDM/bird/day (Wauton and Gumus, 2012).

Akinbami *et al.* (1996) reported that Nigeria produces about 227, 500tons of fresh animal waste daily. The following are a review of the various biogas technology researches conducted in Nigeria.

Usman *et al*. (2011) carried out a research on biogas generation in University of Lagos, Lagos State from two batch digesters containing domestic solid wastes and poultry dropping for a period of 20days at mesophilic situation (40^0C). Water was added in the ratio 1:1 (substrate; water). Digester A was fed with 300g of the feedstocks while Digester B contained 200g of the feedstocks blended with 100g of poultry droppings. It was observed that biogas production was optimized in digester B when the feedstocks was blended with poultry dropping.

Sagagi *et al*. (2009), at Kano University of Science and Technology carried out a research on biogas production from fruits and regrettable waste blended with cow dung from his research, it was observed that the highest production rate of biogas was recorded for cow dung slurry with average production of 1554cm^3.

Dahunsi and Oranusi (2013), at Covenant University, Ota, Nigeria investigated on co-digestion of food waste and human excreta for biogas production. The mixture used was a combination of 12kg of food wastes and 3Kg of excreta as seed material with water in the ratio of 1:1 for a period of 60days. They concluded that food waste and human excreta when used in combination are good substrates for biogas production.

Yusuf *et al*. (2011) investigated on biogas production in Port Harcourt, River State, Nigeria from five batch digesters containing varying ratios of mix of horse and cow dung for a period of 30 days at ambient temperature. It was observed that biogas production was optimized when horse and cow dung was mixed in a ratio of 3:1.

According to Momoh and Nwaogazie (2008) from the University of Port Harcourt, they studied the effect of waste paper on biogas production from co-digestion of fixed amount of cow dung and water hyacinth at room temperature in five batch reactor for a period of 60days. It was reported that an optimum waste paper amount of 17.5g needs to combine with 5g of cow dung and 5g of water Hyacinth in 250ml of water for maximum biogas production. They further advised that similar equivalent in kilograms and tones can be utilized in large-scale production of biogas which can provide centralized source of fuel for university laboratories and also local supply of energy for electricity production. Anaerobic digestion of cow dung for biogas production was investigated by Abubakar and Ismail (2012) at the University of Maiduguri, Borno State. They used a 10litre jacketed fermented (bio stat B) equipped with pH probe stirrer, sampling ports and temperature controller. The results showed that cow dung might be one of feedstocks for efficient biogas production and waste treatment.

Chukwuma and Bassey (2012) investigated on the evaluation of mixture effect on biogas production of paunch manure and cow dung under tropical condition at Nnamdi Azikiwe University, Awka for 60days. It was reported that the optimum biogas yield obtained from the research was when the substrates was in the ration of 1:2

Lortyer *et al*. (2012) at University of Agriculture, Makurdi, investigated on the effect of mixing ratio of cattle and piggery dung on biogas production for a 40day retention period in batch digesters. The substrates was used in the ratio of 4:1 (cattle: piggery dung), 3:2, 1:1 and so on. It was observed that the highest biogas yield was in the ratio of 4:1. It was concluded that mixing cattle and piggery dung can produce reasonable quantities of biogas for meeting the energy requirement of people that have access to both dung types and may even give higher yields than obtainable when using only one of the two types of dung especially at the mixing

ratio 4:1. Iyagba *et al*. (2009) subject of investigation was co-digestion of cow dung with rice husk for biogas production at laboratory scale. Sample with 50wt% cow dung and 50wt% rice husk was not significant, while there was no production from sample 0wt% cow dung and 100wt% rice husk.

Chukwuma *et al.* (2013) carried out a research on determination of optimum mixing ratio of cow dung and poultry droppings in biogas production under tropical condition at Nnamdi Azikiwe University, Awka. The mixing ratio used were 100:0, 75:25, 50:50, 25:75, and 0:100 for cow dung (CD) and poultry droppings (PD) respectively. The biogas yields obtained in the work were in the order of 25% CD + 75% PD > 100% CD + 0% PD > 50% CD + 50% PD > 0% CD + 100% PD> 75% CD + 25% PD. It was observed that the optimum mixing ratio for cow dung and poultry droppings is 25% CD + 75% PD which gave 16.35 L/total mass of slurry (TMS) within the period of study.

Ojolo *et al*. (2007) at University of Lagos carried out a research on utilization of poultry, cow and kitchen wastes for biogas production, comparative analysis for a period of 40 days. The average biogas production from poultry droppings, cow dung and kitchen waste was 0.0318dm^3/day, 0.0230dm^3/day and 0.0143dm^3day, respectively. It was concluded that the wastes can be managed through conversion into biogas, which is a source of income generation for the society.

Ofoefule *et al*. (2010) at University of Nigeria, Nsukka, researched on biogas production from paper wastes and its blend with cow dung. Result obtained showed that paper waste had a cumulative gas yield of 6.23 ± 0.07dm^3/kg of slurry. Blending increased the cumulative gas yield to 9.34 ± 0.11dm^3/kg slurry representing more than 50% increase. The study shows that paper waste which abounds everywhere and is either burnt off or thrown away constituting nuisance to the environment would be a very good feedstocks for biogas production.

Adeleke and Bamgboye (2009) carried out a research on comparison of biogas productivity of cassava peels mixed in selected ratios with major livestock waste types at Olabisi Onabanjo University, Ogun State for a retention period of 30 days and within the mesophilic temperature range. Biogas yield was significantly influenced by the different mixing ratios of livestock waste with cassava peels. The cumulative average biogas yield from digested cassava peels was 0.61/kg-TS. The average cumulative biogas 4.0l/kg and 9.01/kg respectively for 1:1, 2:1, 3:1, and 4:1 mixing ratios when cassava peels was mixed with poultry waste. On mixing with piggery waste the average cumulative biogas yield increased to 35.0, 26.5, 17.1 and 9.31/kg-TS respectively for 1;1, 2:1 and 3:1, and 4:1 mixing ratios. In the case of mixing with cattle waste, the average cumulative biogas yield increased to 21.3, 19.5, 15.8 and 11.21/kg-TS respectively for 1:1, 2:1, 3:1 and 4:1 mixing ratios. Results showed that for all livestock waste types, mixing with peels in the ratio 1:1 by mass produced the highest biogas volumes and highest in piggery waste.

Ezeoha and Idike (2007) from the Department of Agricultural and Bio-resources Engineering at University of Nigeria, Nsukka investigated on biogas production potential of cattle paunch manure. The research was carried out for 93days under mesophilic temperature of 39^0C, pH of 8, volatile acid concentration (VAC) of 278mg/L_1 NH_3-N of 18.3 and alkalinity of 2800mg/L. it was concluded from the research that cattle paunch manure has biogas production potential.

The effectiveness of using fresh maize leaves for biogas production was investigated under varying dilution ratios by Imasuen *et al*. (2011) in Auchi, Edo State. The experimental result showed that the digester with 1:5 content (maize to water) had a significant higher performance compared to the rest.

At National Centre for Energy Research and Development UNN, Ofoefule *et al*. (2010) researched on the comparative study of biogas production from cassava

peels treated with different chemicals namely; potassium hydroxide (KOH, 50% w/v) and locally available potash. They reported that biogas yield from cassava peels can be enhanced by chemical treatment and that locally available potash is a better treatment to be employed in the biogas production of cassava peel.

Olugbemide *et al*. (2012) on their work on anaerobic co-digestion of fresh maize leaves with elephant grass at Auchi Polytechnic, Edo State, Nigeria investigated the synergistic and antagonistic effect of co-digestion of maize leaves with elephant grass and the optimal ratio of the substrates. The research was carried out using batch digestion once daily. A set of five batch reactors were used as digester. The compositions of the reactors are 100% maize leaves, 90% maize leaves and 10% elephant grass, 80% maize leaves and 20% elephant grass, 70% maize leaves and 30% elephant grass and finally 60% maize leaves and 40% elephant grass. The results showed that co-digestion of maize leaves with elephant grass have both synergistic and antagonistic effects on biogas production depending on the mixture proportion. It was reported that the proportion with 60% maize leaves and 40% elephant grass gave the optimum biogas yield of 67.3% higher than the control. They however, recommended that further research work should be extended into the investigation of biogas yield by co-digesting the substrates with other organic wastes and the antagonistic effect.

Chukwuma and Chukwuma (2014) in Nnamdi Azikiwe University, Awka investigated on batch anaerobic kinetics of bio-production process of cattle paunch blended with poultry waste. The fermentation was for a period of 45 days in a batch type bio-digesters and the mixing ratios of cattle paunch (CP) and poultry droppings (PD) assessed were: 0 : 100, 25 : 75, 35 : 65, 50 : 50, 75 : 25, and 100 : 0. The synergistic effect of co-digestion was observed on biogas production potential and maximum biogas rate. The result showed that 50% CP + 50% PD had the highest biogas production potential.

Also, Chukwuma (2012) in the same institution carried out a research on co-digestion of selected animal wastes for biogas optimization in a 30 litres batch tank reactors incubated at ambient temperature for a 30 day retention period. The mixing ratio of the substrates were as follows: 100 : 0, 75 : 25, 50 : 50, 25 : 75, and 0 : 100. The kinetics of anaerobic digestion process of the various digestion mixtures were also evaluated. The result obtained showed that poultry droppings had the highest short term biodegradability index of -2.4 while the 50% CD = 50% PYD digester had the highest removal rate of the biodegradable fractions (k) of – 0.199 among cow dung and poultry droppings mixtures. The kinetic parameter used in the assessment of the substrates performance agreed with the experimental result and showed that the 50% CD + 50% PYD digester had the overall best performance.

2.27 Methane and biogas

Biogas is a mixture of methane (CH_4), carbon dioxide (CO_2) and small quantities of hydrogen, nitrogen, carbon monoxide and other compounds. This mixture of gases is combustible if the methane content is greater than 50%. In anaerobic digestion of organic matter first the complex organic materials are broken down into simple organic acids, alcohols and CO_2;**.**next the simple organic acids and CO_2 are either oxidized or reduced to methane. Optimal conditions for this process to occur include:

• Oxygen:

This is an anaerobic process so there should be no oxygen/air.

• Temperature:

Optimum temperature is 15-35C (65-95F). With suitable temperatures micro-organisms are more active and biogas is produced at higher rate.

• Moisture:

Water should comprise around 90% of the slurry weight. Too much water will reduce the rate of biogas production per unit of volume; too little water causes accumulation of acetic acids which inhibit fermentation and biogas production.

• Acidity:

Micro-organisms require neutral or mildly alkaline conditions; optimum pH is between 7.0-8.5

• C:N Ratio:

Carbon and Nitrogen are the main nutrients required by micro-organisms. The optimum C:N ratio is 25:1 - 30:1. Too much carbon quickly digests the nitrogen and the process slows; too little carbon is used up quickly, the process stops and the excess nitrogen escapes as ammonia.

2.27.1 Properties of methane

• Methane is odorless, colorless, and tasteless.

• The weight of methane is roughly half that of air (so it will tend to rise).

• The solubility of methane in water is very low (this allows it to be cleaned by passing through a simple water scrubber).

• The combustion of methane produces a blue flame and a great amount of heat.

The nutrients rich digestate also produced can be used as fertilizer.

2.27.2 Process stages

Anaerobic digestion is a multistep process taking place in a closed digester. Anaerobic digestion kinetics is influenced by many factors such as: temperature – usually kept in mesophilic domain, mixing – in order to keep the solids in the digester form forming deposits in the bottom side and to realize a good homogenization of the tank's content, hydraulic retention time which is decisive in percent of stabilized organic matter; solids concentration in the influent; inhibitors. The sludge stabilization is the result of multiple group of microorganism's activity. The first group of microorganisms turns the macromolecular organic matter in

more simple compounds, like sugars, long chain fatty acids and amino acids. The solubilised compounds are converted by acidogenesis to hydrogen and carbon dioxide. In the acidogenesis phase, long chain fatty acids and alchols are turned into acetic acid and hydrogen. In the last stage of the anaerobic digestion methanogenic bacteria methane is produced from acetic acid, carbon dioxide and hydrogen. The process efficiency can be influenced by the different kinetics of the different groups of bacteria involved. Hydrolysis develops slowly, depending on the particulate matter introduced in the anaerobic digester, thus slowing down the whole process kinetics. Acidogenesis kinetics on the other hand is one order of magnitude higher than methanogenesis, so any disturbance that changes the methanogenic bacteria activity may have a negative effect on the process efficiency. *There are four key bi*ological and chemical stages of anaerobic digestion which are as follows: (i). hydrolysis, (ii). acidogenesis (iii). actogenesis. (iv). Mthanogenesis

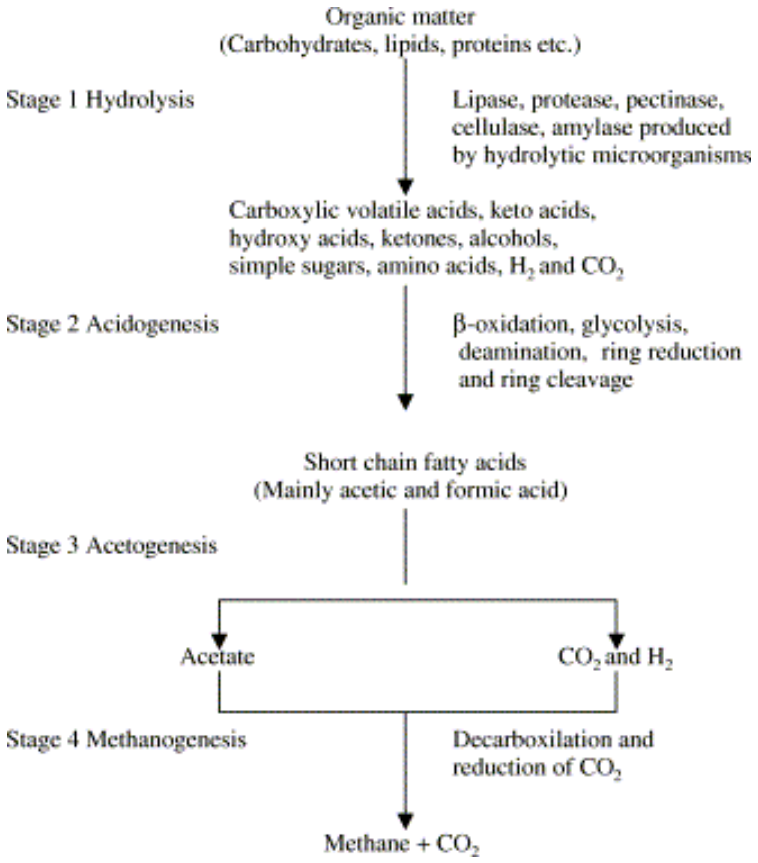

Fig. 2.8: Process stages of anaerobic digestion

2.28 Pathways in anaerobic degradation

The degradation of organic matter to produce methane relies on the complex interaction of several other different groups of bacteria. Stable digester operation requires that these bacterial groups be in dynamic and harmonious equilibrium. Changes in environmental conditions can affect this equilibrium. It is of paramount importance to understand the basic microbiological and biochemical pathways, in order to master the biogas digestion system. A diversity of micro-organisms is involved in the many steps of anaerobic degradation of complex substrates. Effective digestion of organic matter requires the combined and coordinated metabolism of different kinds of carbon catabolizing anaerobic bacteria. Carbohydrates are rapidly converted via hydrolysis to simple sugars and alcohols and subsequently fermented to volatile fatty acids. Proteins are hydrolyzed to

polypeptides and amino acids and glycerol. During acidogenesis, glycerol is converted to acetate and long chain fatty acids are converted to acetate and hydrogen. Fermentation bacterial is responsible for converting the intermediates to volatile fatty acids, carbon dioxide and hydrogen. And finally during methanogenesis, methane and carbon dioxide are produced which are the main components of biogas. Figure 2.9 illustrates the anaerobic degradation pathways.

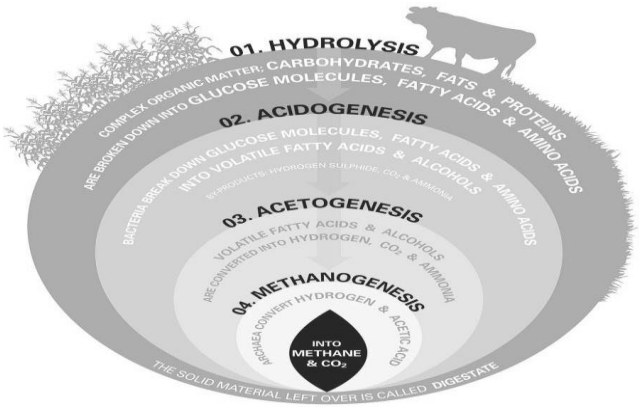

Figure 2.9: Pathways in anaerobic degradation

Source; (www.biogas.co.uk).

2.28.1 Hydrolysis

In most cases biomass is composed of large organic polymers, for the bacteria in anaerobic digesters to access the energy potential of the material, these chains must be broken down into their smaller constituent parts. These constituent parts or monomers such as sugars are readily available to other bacteria. The powers of breaking these chains and dissolving the smaller molecules into solutions are called hydrolysis. Thus, hydrolysis is the first step in anaerobic digestion (Mattocks, 1984). In this process, the complex organic molecules are broken down into simple sugar, amino acids, and fatty acids.

2.28.2 Acidogenesis

The biological process of acidogenesis results in further breakdown of the remaining components by acidogenic or fermentative bacteria. Here the volatile fatty acids (VFAs) are created, along with ammonia, carbon dioxide and hydrogen sulfide as well as other by-products.

2.28.3 Acetogenesis

In this stage, the simple molecules created through the acidogenesis phase are further digested by acetogens produce largely acetic acid, as well as carbon dioxide and hydrogen.

2.28.4 Methanogenesis

This is the terminal stage of anaerobic digestion. In this biological process of methanogenesis, the methanogens use the intermediate products of the proceeding stages and converts them into methane, carbon dioxide, and water. These components make up the majority of the biogas emitted from the system. A simplified generic chemical equation for the overall processes is as follows.

$$C_6H_{12}O_6 \rightarrow 3CO_2 + 3CH_4 \text{---.....} \quad (2.12)$$

2.29 Substrates for anaerobic digestion

A wide range biomass types can be used as substrates (feedstocks) for the production of biogas from anaerobic digestion. Figure 2.10 shows substrates for anaerobic digestion. The most common biomass categories used in biogas production as outlined by Al Seadi *et al*. (2008) are as follows:

- Animal manure and slurry
- Agricultural residue and by-products
- Digestible organic wastes from food and agro-industries (vegetable and animal origin)
- Organic fraction of municipal waste and from catering (vegetable and animal origin)

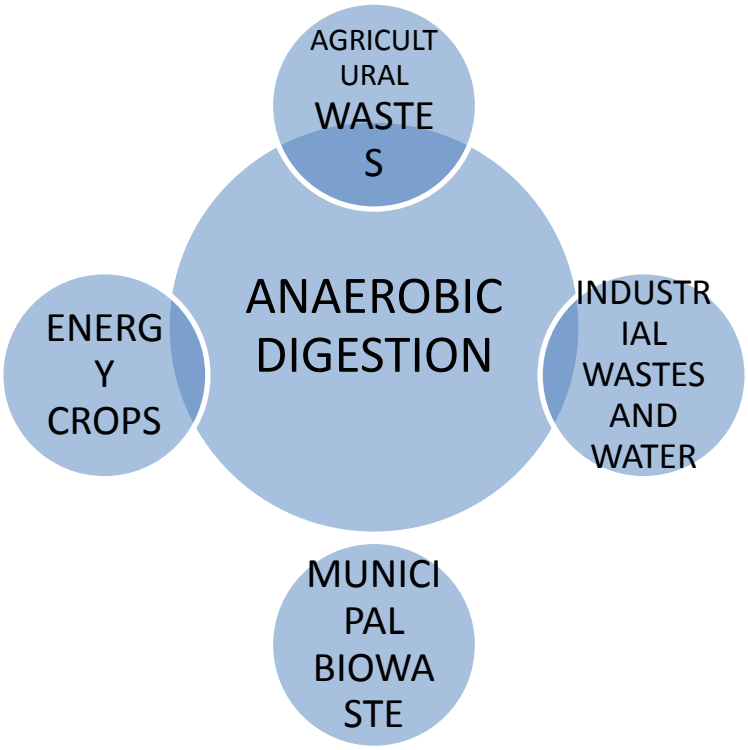

Figure 2.10: Substrates for anaerobic digestion

Table 2.4: **Gas yields and methane contents for various substrates, at the end of a 10-20 day retention time, at a process temperature of about 30^0C.**

Substrates	Gas Yields (1/kg vs)	Methane content (%)
Pig manure	340-550	65-70
Cow manure	90-310	65
Poultry droppings	310-620	60
Horse manure	200-300	
Sheep manure	90-310	
Grass	280-550	70
Elephant grass	430-560	60
Vegetable residue	330-380	
Sunflower leaves	300	59
Agricultural waste	310-430	60-70
Fallen leaves	210-290	58
Water hyacinth	315	

Source: Information and Advisory Service on Appropriate Technology (ISAT).

2.30 Benefits of biogas technology

The production and utilization of biogas from AD provides environmental and socio-economic benefits for the society as a whole as well as for the involved farmers. Utilization of the internal value chain of biogas production enhances local economic capabilities, safeguards jobs in rural areas and increases regional purchasing power. It improves living standard and contributes to economic and social development. In United States, there has been considerable interest in the process of anaerobic digestion as an approach to generating a safe clear fuel as well as source of fertilizer (Usman *et al*., 2012).

2.30.1 Benefits to the society The current global energy supply is highly dependent on fossil sources (crude oil, lignite, hard coal, natural gas). These are fossilized remains of dead plants and animals, which have been exposed to heat and pressure in the Earth's crust over hundreds of millions of years. For this reason fossil fuels are non-renewable resources which reserves are being depleted much faster than new ones are being formed. Unlike fossil fuels, biogas is permanently renewable (Al Seadi *et al*., 2008). Nearly two thirds of renewable energy sources in the European Union stem from biogas inducing wastes (Vindis *et al*., 2008). More so, government like the Thailand government has a clear policy to promote alternate energy in a significant scale for encouragement in increasing the proportions of alternate energy resources.

2.30.2 Reduced greenhouse gas (GHGs) emissions and mitigation of global warming:

The production of green house energy resources in the world is the major cause of global warming and climate change (Harka *et al*., 2010). Ravita (2012) opined that biogas is a clean fuel and is lighter in terms of carbon chain length and hence less

amount of carbon dioxide is released into the atmosphere during combustion. Al Seadi *et al*. (2008) opined that the main difference when compared to fossil fuels is that the carbon in biogas is readily taken up from the atmosphere by photosynthetic activities of the plants. The carbon cycle of biogas is this closed within a very short time (between one and several years). Biogas production by anaerobic digestion process reduces also emissions of methane and nitrous oxide (N_20) from storage. The greenhouse gas potential of methane is higher than that of carbon dioxide by 23 folds and of nitrous oxide by 296 folds (Al Seadi *et al*., 2008). When Biogas displaces fossil fuels from energy production and transport, a reduction of emissions of CO_2, CH_4 and N_20 will occur contributing to mitigate global warming. The European Union made a commitment to reduce its emissions by 8% in 2010 compared to 1990 (Vindis *et al.,* 2008).

2.30.3 Waste reduction

Human engagements both at the domestic front and in industrial operations are inevitablely accompanied by waste generation (Usman *et al.*, 2012). Utilization of such waste material plays a vital role to improve the environmental conditions. Anaerobic digestion has been considered as waste-to-energy technology and is widely used in the treatment of municipal solid waste, sewage sludge food waste, animal manure etc. (Chens *et al.,* 2010). Anaerobic digestion also contributes to reducing cost of waste disposal.

2.30.4 Job creation:

Production of biogas from anaerobic digestion requires work power for production, collection and transport of anaerobic digestion feedstocks, manufacture of technical equipment, construction, operation and maintenance of biogas plant. This means that the development of a national biogas sector contributes to the establishment of new enterprises some with significant economic potential increases the income in rural areas and creates new jobs.

2.30.5 Low water inputs

Even when compared to other bio-fuels, biogas has some advantages. One of them is that the anaerobic digestion needs the lowest amount of process water. This is an important aspect related to the expected future water shortages in many regions of the world.

2.31 Benefits to the farmer

2.31.1 Additional income to farmer

Production of feedstocks as raw material for operation of a biogas makes biogas technologies economically attractive for farmers and provides them with additional income. The farmer gets also a new and important social function as energy provider and water treatment operators.

2.31.2 Closed nutrient cycle:

From the production of feedstocks, the application of digestate as fertilizers, the biogas from AD provides a closed nutrient and carbon cycle. The methane is used for energy production and the carbon dioxide is released to the atmosphere and re-taken by vegetation during photosynthesis. Some carbon compounds remain in the digestate, improving the carbon content of the soil when digestate is applied as fertilizer. Biogas production can be perfectly integrated into conventional and organic farming where digestate replaces chemical fertilizers produced with consumption of large amount of fossil energy.

2.31.3 Digestate as a good fertilizer

The digestate from anaerobic digesters contains many nutrients and can thus be used as plant fertilizer and soil amendment . Compared to raw animal manure, digestate has improved fertilizer efficiency due to higher homogeneity and nutrient availability, better carbon-nitrogen ration (Al Seadi *et al*., 2008).

2.32 Anaerobic co-digestion

Co-digestion is the term used to describe the combined treatment of several wastes with complementary characteristics, being one of the main advantages of anaerobic technology. The most common situation is when a major amount of a main basic substrate is mixed and digested together with minor amount of a single or a variety of additional substrates (Olugbemide *et al*., 2012).Co-digestion is the simultaneous digestion of a homogeneous mixture of two or more substrate with complementary characteristics so as to enhance biogas production (Campos *et al*., 1999; Chukwuma *et al*., 2012; Braun and Wellinger, 2002; Larzor *et al*., 2010; Mata-Alvarez *et al*., 2002). The use of co-substrates usually improves the biogas yields from anaerobic digester due to positive synergism established in the digestion medium and the supply of missing nutrients by the co-substrates (Chukwuma *et al*., 2012; Olugbemide *et al*., 2012). The mixing of several waste types has also positive effects both on the anaerobic digestion itself and on the treatment economy (Chukwuma *et al*., 2012; Mata-Alvarez *et al*., 2000; Campos *et al*., 1999). Co-digestion strategic is widely applied in order to enhance the methane production in agricultural biogas plant (Campos *et al*., 1999; Larzor *et al*., 2010). The expression co-digestion is applied independently to the ratio of the respective substrates used simultaneously with the aim of improving the efficiency of the anaerobic digestion process; it is very important to establish the best blend in order to maximize methane production, avoid inhibition processes and made profitable biogas plant (Mata-Alvarez *et al*., 2000; Chukwuma *et al*., 2012). Manure was digested to produce energy, sewage sludge was anaerobically stabilized and industrial waste water was pretreated before final treatment in a wastewater treatment plant. Numerous studies show that the sensitivity of the anaerobic digestion process may be improved by combining several waste streams. Co-digestion is therefore a hot tropical issue in energy production. The rate and

efficiency of the anaerobic digestion process is controlled by the following factors (Burke, 2001).

- The presence of toxic materials
- The type of waste being digested
- Its temperature
- Its concentration
- The pH and alkalinity
- The hydraulic retention time (HRT)
- The solids retention time
- The ratio of food to microorganisms
- The rate of digestion loading and
- The rate of which toxic end products of digestion are removed.

2.32.1 Advantage of co-digestion

Co-digestion offers many ecological, economical and technological advantages. According to Braun and Wellinger (2002) the following list of advantages of co-digestion of wastes in the farm was developed.

i) Improved nutrient balance:

The digestion of a variety of substrates instead of a single waste type improves the nutrient ratio of C: N: P which optimally should be around 300:5:1. It also maintains a reasonable mix of minerals (Na, K, Mg, Mn, etc.) as well as a balanced composition of trace metals. Co-digestion therefore helps to maintain a stable and reliable digestion performance and a good fertilizer quality of the digester.

ii) Optimization of rheological qualities

Waste with poor fluid dynamics aggregating wastes, particulate or bulking materials and floating wastes can be much easier digested after homogenization with dilute substrate such as sewage sludge or liquid manure. The mixing of

different substrates allows some flexibility to be able to compensate for seasonal mass fluctuations of wastes. Under loading and overloading of digesters can be avoided and the digestion process can be maintained at a constant rate.

iii) Gate fees and biogas recovery

In agricultural digesters, the application of co-substrates can considerably improve the overall economics (payback time) of the plant. Gate fees create a win-win situation. The ponder pays significantly lower prices at a farm-scale AD-plant than that of an incineration or composting facility (usually factor of 3 to 4). The farmer takes credit of the increased biogas production and the income from the gate fee.

iv) Energy crops as co-substrate

In a limiting situation of industrial wastes, energy crops might have become an interesting alternative, especially when the plants are grown on fallow or set aside land which attracts subsidies. A number of crops demonstrate good biogas potentials.

Other advantages identified are:

- Increased savings for farmers
- Improved fertilizer efficiency
- Reduced greenhouse gas emissions
- Cost effective and environmentally sound waste recycling
- Reduction of odours
- Reduction of flies
- Potential pathogen reductions
- Renewable energy production

2.32.1 Disadvantages of co-digestion

Chukwuma (2012) outlined the following disadvantages observed on co-digestion of substrates. They are as follows:

- Increased digester effluent COD
- Additional pre-treatment requirements
- Increased mixing requirements
- Wastewater treatment requirement
- High utilization degree required
- Increasing availability and rates
- Hygienisation requirements
- Restrictions of land use for digestate
- Economically critical dependent on crop costs and yield.

2.33 Biogas plant

In many countries worldwide biogas plants are in operation, producing biogas from the digestion of manure or other biomass. In addition, with success small scale biogas plants are utilized to displace woody fuels and dung in many developing countries. For example, the Dutch Development Organization, SNV, implemented with success in Nepal and Vietnam over 220,000 household on site biogas plants (FAO, 2007). Moreover, in China and Indian millions of plants are in operation. Biogas plants have proven to be an effective and attractive technology for many households in developing countries. Under the right conditions a biogas plant will yield several benefits for the end-users, the main benefits are (GTZ, 2007):

1. Production of energy for lighting, heat, electricity

2. Improved sanitation (reduction of pathogens, worm eggs and flies)

3. Reduction of workload (less firewood collecting) and a biogas stoves has a bettercooking performance

4. Environmental benefits (fertilizers substitution, less greenhouse gas emission)

5. Improved indoor air quality (less smoke and harmful particle emission of a biogas stove compared to wood or dung fuels) (Milich, 1999)
6. Economical benefits (substitution of spending on expensive fuels and fertilizer)

Consequently, biogas plants are of great benefit to the end-users and the environment.

2.34 Bioreactor types

The reactor is the place where any substrate is digested. The classification of reactors is basedon the mixing of fluid (substrate and sludge) in the reactor. There are various types of systems. Concerning the feed method, mainly two different forms can be distinguished:

i. Batch plants

ii. Continuous plants

iii. Plug flow

iv. Fed batch (accumulation)

v. Completely Stirred Reactor (CSTR)

2.34.1 Batch plants

These plants are filled and then emptied completely after a fixed retention time.Each design and each fermentation material is suitable for batch filling, but batch plants require high labor input. As a major disadvantage, their gas-output is not steady in time.

2.34.2 Continuous plants

These are fed continuously. The CSTR and plug flow systems are characterized by automatic overflow when new material is filled in. Therefore, the substrate must be fluid and homogeneous. Continuous plants are suitable for rural households as the necessary work fits well into the daily routine. Gas production is constant.

A CSTR system is characterized by a continuous feeding rate and a complete mixture and substrate and at constant loading rate, a constant gas production rate is achieved in time.The plug flow system is continuously fed and the feed passes through the reactor in a horizontal direction and concentration reduces from left to right.

2.34.3 Completely Stirred Reactor (CSTR) system plug flow

Active stirring is applied to mix the contents of the digester with the added substrate, Completely Stirred Reactor (CSTR). The concentration of the degraded substrate is the same in the reactor as at the outlet of digested substrate as a result of stirring. These types of reactors have different implication for the hydraulic and sludge retention time and subsequently the loading rate of substrate and the volume of the reactor. The hydraulic retention time (HRT) refers to the time water and bacteria remain in the reactor and the sludge retention time (SRT) refers to the time the substrate is in the reactor. The volume of the reactor necessary to produce enough gas for cooking depends on the HRT, SRT and the loading rate of substrate. CSTR system, plug flow systems and system based on a similar set up always need an additional storage for the digestate, to overcome periods that digested manure cannot be applied on the field on low temperature countries this can be as long as 5-6 months.

2.34.4 Biogas plants in developing countries

In developing countries there are several digesters in operation, the most familiar is the fixed dome digester, in addition the floating dome digester and bag digester are found in many developing countries.

2.34.5 Fixed dome digester

The fixed dome digester (Fig. 2.11) is the most popular digester. its archetype was developed in China. The digester comes in various types, notably the Chinese fixed dome, Janata model and Janata II model (Arafa, 1986).

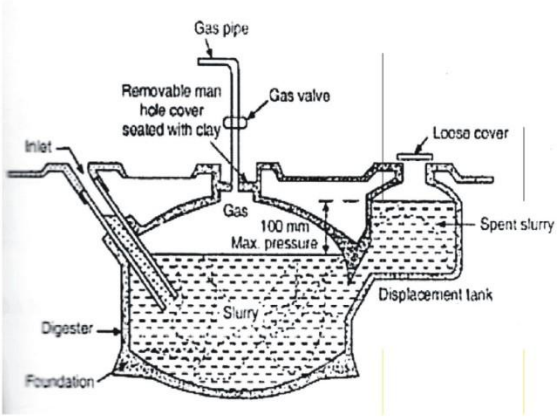

Fig. 2.11: Fixed dome plant (Source: Mattocks, 1980)

2.34.6 Characteristics and function of fixed dome plant

A fixed dome digester is a closed dome shaped digester. The waste (manure, dung, human excrement) is fed to the digester. After that the methanogenic bacteria 'digest' the waste and produce biogas and slurry (digested waste). The gas is captured in the gasholder and the slurry is displaced in the compensating tank. The more gas is produced, the higher the level at the slurry outlet will be. The level of slurry in the digester depends on the loading rate, gas production and consumption. During gas production slurry is pushed back sideways, displaced to the compensation tank. When gas is consumed slurry enters back into the digester from the compensation tank. As a result of these movements, a certain degree of mixing is obtained of slurry of different ages; therefore this design approaches a mixed digester reactor. In such a reactor the HRT is the same as the SRT. The volume of the sludge filled part of the digester is therefore equal to the retention time of the sludge times the flow rate.

The Gas holder is adapted to fit the gas requirement of the end-user or family. Important to consider is the daily pattern of cooking. The longer period between

the meals, the more gas is produced in between meals and subsequently the larger the gasholder should become.

2.34.7 Floating drum digester

The floating drum digester was originally developed in Bombay, by J.J. Patel, who named it Gramalaxmi (Sathianathan, 1975). It is comprised of an underground cylindrical masonry digester having an inlet pipe for feeding animal slurry and an outlet pipe for sludge. There is a steel dome for gas collection which floats over the slurry. It moves up and down depending on accumulation and discharge of gas guided by the dume guide shaft. Fig. 2.12 shows the floating drum biogas plant (KVIC model).

In this model a partition wall is provided in the digester to improve circulation, required for fermentation. The pressure in the digester built by the floating gas holder forces out the spent slurry through a sludge pipe.

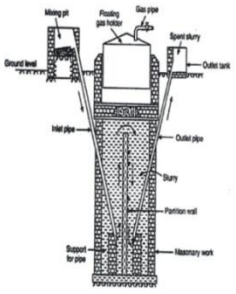

Figure 2.12: Cross- section of a floating drum digester

Source:www.ganesha.co.uk/Articles/Biogas%20Technology%20in%20India.htm

2.34.8 Flexible bag digester/ balloon plants

A balloon plant also referred to as a bag digester is a plastic or rubber bag combining the gas holder and digester. The flexible bag digester was developed in Taiwan in the 1960's and is now common in China. This is a plug-flow type reactor. Gas is collected in the upper part and manure in the lower part; the inlet and outlet are attached to the skin of the bag. The pressure of the gas is adjustable by laying stones on the bag. As the biogas is used , the bag collapses like a ballon being emptied of air. Figure 2.13 shows the example of a flexible bag digester.

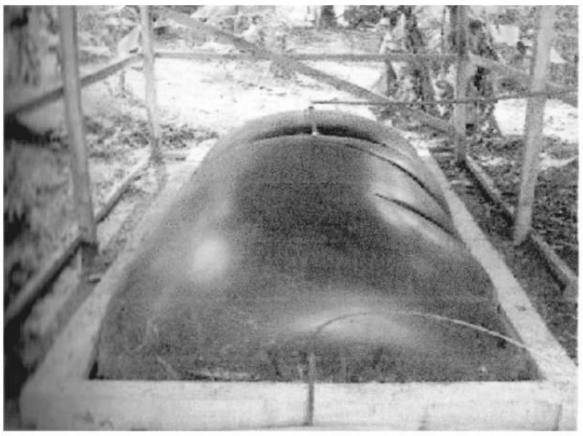

Fig. 2.13: Flexible bag digester. (Source: www.tecnologiadesarrollo.tk)

2.34.9 Deenbandhu biogas plant (DBP)

This biogas plant was developed by Action for Food Production (AFPRO). It was designed to cut cost and to be built mainly with cheaper material like bamboo where bricks may not be handy. The main feature of this type of biodigester is the fixed underground digester chamber constructed with a layer of bricks and an additional layer of cement mortar forming the roof above. Connected to the underground chamber is an inlet tank through which manure is fed into the plant. The manure then ferments separating the slurry from the methane gas which rises and collects at the top of the digester tank, and is released through the gas outlet pipe. The slurry passes into the outlet tank where it is ejected from the pit and can be used as fertilizer. Figure 2.14 shows the Deenbandhu biogas plant.

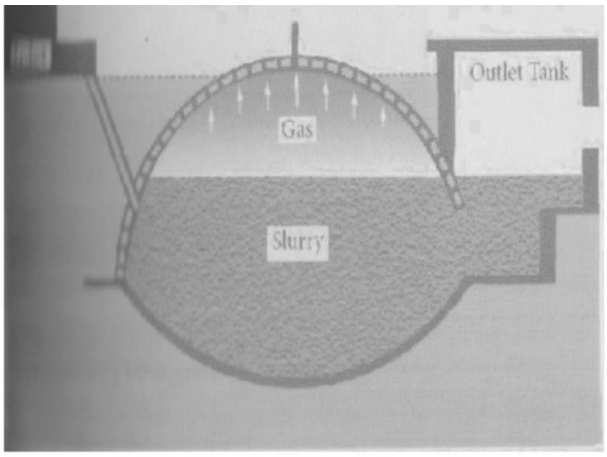

Fig.2.14: Deenbandhu biogas plant

Source: www.tecnologiadesarrollo.tk

2.34.10 Flow-thru digester

A flow-thru digester (Fig. 2.15) can also be made from any size container. Three 200 liter (55 gal) metal drums welded end to end make a good size. The drums should be clean and in good condition. A drum with 2 threaded holes in the lid works well for the top drum. The bottom is cut from the top drum; the top and bottom are cut from the middle drum; and the top is cut from the base drum. The drums are then welded together end to end. The three drums can be placed on a stand that holds them at a slight angle (most micro-organism activity takes place near the surface of the slurry so the angle should be very low). A 5cm (2in) slurry inlet is installed in the lid of the top drum, it should curve upward with the opening above the top of the barrel unit. A fitting for the gas exit is also installed in the lid of the top drum, at the highest point. A 5cm (2in) slurry outlet with a valve is installed in the bottom of the base drum, at the lowest point. Once the barrel unit, gas lines, gas collector and cookstove are prepared and connected, the unit can be filled with slurry.

When the unit begins producing biogas, the gas should be released for about the first week before trying to ignite the stove to assure that there is no air left in the

system. Once the system is purged of oxygen and ready to use, new slurry can be added and expended slurry released on a regular basis. To maintain production, 20lt (5gal) of slurry should be processed through the unit each week. To maximize production, 20litres (5gal) of slurry can be processed daily. This amount can be adjusted according to local conditions of weather, slurry, usage, etc. Never release too much slurry so that air enters the unit (if air does enter, the unit must be purged again). Occasionally some of the expended slurry can be added back in to boost micro-organism levels. Once this unit is established, it provides a continual supply of biogas and slurry fertilizer. It is estimated that this 3-drum unit could produce about 27 cubic feet of biogas per day and that 2 cows or 10 pigs could supply sufficient manure.

Fig. 2.15: A flow-thru digester (Source: Doerr and Lehmkuhl, 2008)

2.35 Waste

Waste (also called rubbish, trash, refuse, garbage, litter, and wort) is unwanted or useless materials. In biology, waste is any of the many unwanted substances or toxins that are expelled from living organisms, metabolic waste; such as urea,

sweat, and feces fountain large quantity of fresh and soft textured waste products. Waste is directly linked to human development, technologically and socially.

2.35.1 Composition of wastes

The compositions of different wastes have varied over time and location, with industrial development and innovation being directly linked to waste materials. Some components of waste have economical value and can be recycled once correctly recovered. It is widely recognized that waste materials are a valuable resource. There are many waste types defined by modern systems of waste management, which include:

(i) Municipal waste - This include household waste, commercial waste, demolition waste.

(ii) Hazardous waste - includes industrial waste

(iii) Bio - *medical*- include chemical waste

(iv) Special hazardous waste- radioactive waste, explosive waste

2.36 The effects of physical parameters on anaerobic digestion

2.36.1 Temperature

The anaerobic digestion process is carried out by a delicately balanced population of various bacteria. These bacteria can be very sensitive to changes in their environment. Temperature is a prime example as the temperature falls, bacteria activity decreases and biogas production decreases. As the temperature increases some bacteria begin to die, once again biogas production decreases. Insulation, heat exchangers, heating elements water baths, and steam injection are all means which have been used to control digester temperature. Temperature control is an important consideration when designing digesters. Anaerobic digestion will occur even at room temperature; however, any method of maintaining digester temperature constant near 35^0C will improve digester performance.

In technical applications three different temperature ranges are distinguished:

(1) Psychophilic (or Cryophilic) temperature from 10 to 20^0C;

(2) Mesophilic temperature from 20 to 40^0C;

(3) Thermophilic temperature from 40 to 60^0C. Temperature affects the activity and the growth of micro-organisms.

The methanogenic bacteria are more sensitive to changes in temperature than other micro-organisms in anaerobic digesters (Ofuefule and Onukwili, 2010). The mesospheric and thermophilic ranges mainly provide optimum treatment conditions for an effective COD removal and methane production in anaerobic treatment. Most of the experiments carried out so far were conducted at 30^0C, but it is well known that the optimal temperature for mesosphilic growth is situated near 40^0C. On the other hand, there is a less difference between mesophilic and thermophilic digestion. The main difference is higher volumetric methane yield per day, which can be reached with thermophilic digestion, thus allowing higher specific methane yields from a given volume of biogas reactor (Burton and Turner, 2003).

2.36.2 Hydraulic retention time

Hydraulic retention time (HRT) is the amount of time the digester liquid remains in the digester. HRT is crucial because if the feed does not stay in the reactor long enough for the entire treatment process to take place, biogas will not be produced (Garcelon and Clark, 2000). The required retention time for completion of the anaerobic treatment reactions varies with different technologies, process temperature, and waste composition (Verma, 2002). For instance, Burton and Turner (2003) reported that the digestion of pig manure with its high fat content requires lower HRTs than cattle manure, which contains comparably high cellulose and hemicellulose concentrations. In mesosphilic conditions, they proposed average HRTs for cattle manure of 12 to 25 days, for cattle manure with straw

bedding 15 to 38 days, and the pig manure 10 to 20 days. Furthermore, Wellinger (1999) and Rchling (2001) proposed a 20 – 40 day range of HRT in the treatment of liquid hen manure. They emphasized that the optimum value of retention time actually depends on many factors, such as feed stock, environment temperatures and type of gas plant.

2.36.3 Organic loading rate

Organic loading rate (OLR) is a measure of the biological conversion capacity of the anaerobic treatment system. It is an important parameter that significantly affects the microbial ecology and characteristics of a given systems. As such, this parameter integrates reactor characteristics, operational characteristics, and bacterial mass and activity into the volume of media.

2.36.4 Volatile fatty acids

In anaerobic treatment, the control of the process is usually affected by volatile fatty acids (VFA). Generally, change in VFA concentration is the most sensitive parameter, the reason being that the primary cause of digester failure hinges around an imbalance between acidogenic, acetogenic and metabolism regulation. The fraction of undisssociation of VFA increases when the pH decreases due to VFA production by acetogens. When the concentration of undissociated VFA remains high for prolonged periods, methanogens are slowly wiped out and acetogenns predominate in the bioreactors. If sufficient buffering capacity is present, the eventual production of VFA during occasional overloading will not decrease the pH and, consequently, the undissociated VFA fraction will be too small to significantly disturb the methanogens (Florencio *et al*., 1997). With the production of VFA, little COD removal is achieved. Hence the controlling of VFA

accumulation is inevitable in order to obtain an effective COD removal and methane production.

2.36.5 Ammonia

Ammonia can act as a potent inhibitor of methanogens in a digester by the break down of proteins present in the manure. The pH and temperature have a strong effect on the inhibitory concentration of NH_3, by influencing the equilibrium. Hence, NH_3, toxicity thresholds are very sensitive to pH above 7. A portion of the nitrogen in poultry manure is in the NH_4^+ form. NH_4^+ and NH_3 can interchange rapidly depending on the pH. NH_4^+ will convert to ammonia at a pH that is greater than 6.5. Increasing the pH (more alkaline or less acid) increases the amount of ammonia and decreases the amount of ammonium. Burton and Turner (2003) suggested that the free NH_3, levels should be maintained below 80 mg L^{-1}. However, ammonium iron can generally be tolerated up to 1500 mg L^{-1} as NH_4^{+}.

2.36.6 Sulphides and heavy metals

In anaerobic reactors, sulphides are produced by the reduction of sulphates present in the influent and also by the degradation of proteins present in the manure. If a sulphide exceeds 200 ppm, then the metabolic activity of methanogenic bacteria will be strongly inhibited, leading to the failure of the process. They also pointed out that heavy metals form highly insoluble precipitates with sulphide. Hence, the addition of a metal, such as iron, provides a simple means of reducing the soluble sulphide concentrations. Heavy metals are toxic to both major anaerobic populations even at very low concentrations. Furthermore, it was concluded that other noxious substances such as antibiotics from feed or veterinary treatments or farm disinfectants can slow down the anaerobic process.

2.37 Nutrient content of feed stock

Anaerobic digestion (AD) contain plant nutrients (macro and micro- nutrients, though occasionally, it can also contain heavy metals and persistent organic components as shown in Table 2.5.

Macro-nutrients are essential for all forms of plants, animal, and bacterial life. However, animal, do not use these nutrients efficiently and high proportions are excreted. Resent research result indicate that 55–99 percent of the nitrogen (N) in animal diets is excreted through feces and urine. High proportions of phosphorus (P) and potassium (K) in animal diets are also excreted.

Animal manures and slurries are therefore rich in nutrients (Lukehurst *et al*., 2008). This is also the case for many other types of anaerobic feedstocks, making digestate a valuable biofertilizer. By making the best possible use of digestate as a biofertilizer, nutrients are returned to the land through natural cycles to replace the input of inorganic fertilizer. Recycle in this way closes a loop to create more sustainable agricultural production systems. Lukehurst *et al*. (2008) reported that the composition of animal manure depends mainly on the digestive system of the animal (ruminant, omnivore, etc) and on its diet. Other factors that effect the composition of manure include the species sex and age of the animals as well as geographical and climatic conditions

However, manures alone as substrate (feedstocks) for anaerobic digestion gives relatively low biogas yields per unit of fresh weight; as a result, it is frequently mixed and co-digested with other feed stock types which have higher biogas yields (Braun and Wellingen, 2003).

Table 2.5: Nutritional contents of substrates

Nutrient	Of metals composition
Macro –nutrients	Nitrogen (N), Phosphorus (p) potassium (K), calcium (Ca), magnesium (mg), Sulphur (s)
Micro/trace	Baron (B), Cobalt (Co), Copper (Cu), Chlorine (Cl), Iron (Fe),

elements	Manganese (Mn), Molybdenum (Mo), Nickel (N), Selenium (Se), Zinc
Heavy metals	Lead (Pb), Chromium (Cr), Cadmium (Cd), Mercury (Hg)

Source: Mattocks (1984)

2.38 Unit process of secondary treatment

The basic ingredient needed for conventional aerobic secondary treatment are the availability of many microorganisms, good contact between these organisms and the organic material, the availability of oxygen, and the maintenance of other favourable environmental conditions. A variety of approaches have been used in the past to meet these basic needs. The most common approaches are,

(1) Activated sludge,

(2) Trickling filters and

(3) Oxidation ponds (or lagoons).

A process that does not fit precisely into either the trickling filter or the activated sludge category but does employ principles common to both is *rotating biological contactor* (RBC).

2.38.1 Role of microorganisms

The stabilization of organic matter is accomplished biologically using a variety of microorganisms. The microorganisms convert the colloidal and dissolved carbonaceous organic matter into various gases and into protoplasm. Because protoplasm has a specific gravity slightly greater than that of water, it can be removed from the treated liquid by gravity setting.

It is important to note that unless the protoplasm produced from the organic matter is removed from the solution, complete treatment will not be accomplished because the protoplasm, which itself is organic, will be measured as BOD in the effluent. If the protoplasm is not removed, the only treatment that will be achieved is that associated with the bacterial conversion of a portion of the organic matter originally present to various gaseous end products.

2.38.2 Aerobic decomposition

It is important however to note that molecular oxygen (O_2) must be present as the terminal electron acceptor for decomposition to proceed by aerobic oxidation. As in natural water bodies, the oxygen is measured as DO. When oxygen is present, it is the only terminal electron acceptor used. Hence, the chemical end products of decomposition are primarily carbon dioxide, water, and new cell material. Odiferous gaseous end products are kept to a minimum. In healthy natural water systems, aerobic decomposition is the principal means of self-purification. Based on the large amount of energy released in aerobic oxidation, most aerobic organisms are capable of high growth rates. Consequently, there is a relatively large production of new cells in comparison with the other oxidation systems. This means that more biological sludges are generated in aerobic oxidation than in the other oxidation systems.

2.38.3 Anoxic decomposition.

Some microorganisms can use nitrate (NO_3) as the terminal electron acceptor in the absence of molecular oxygen. Oxidation by this route is called denitrification. The end products from denitrification are nitrogen gas, carbon dioxide, water, and new cell material. The amount of energy made available to the cell during denitrification is about the same as that made available during aerobic decomposition. As a consequence, the rate of production of new cells, although not as high as in aerobic decomposition, is relatively high. One other important aspect of denitrification is in relation to final clarification of treated wastewater. If the environment of the final clarifier becomes anoxic, the formation of nitrogen gas will cause large globs of sludge to float to the surface and escape from the treatment plant into the receiving water. Thus, it is necessary to ensure that anoxic conditions do not develop in the final clarifier.

2.38.4 Anaerobic decomposition.

In order to achieve anaerobic decomposition, molecular oxygen and nitrate must not be present as terminal electron acceptors. Sulphate (SO^2_4), carbon dioxide, and organic compounds that can be reduced serve as terminal electron acceptors. The reduction of sulphate results in the production of hydrogen sulphide (H_2S) and a group of equally odiferous organic sulphur compounds called *mercaptans*.

The anaerobic decomposition (fermentation) of organic matter generally is considered to be a three-step process. In the first step, waste compounds are hydrolysed. In the second step, complex organic compounds are fermented to low-molecular-weight fatty acids (volatile acids). In the third step, the organic acids are converted to methane. Carbon dioxide serves as the electron acceptor. Anaerobic decomposition yields carbon dioxide, methane, and water as the major end products. Additional end products include ammonia, hydrogen sulphide, and mercaptans. As a consequence of these last three compounds, anaerobic decomposition is characterized by an unbelievably horrid stench. Direct anaerobic decomposition of wastewater generally is not feasible for dilute waste. The optimum growth temperature for the anaerobic bacteria is at the upper end of the mesophilic range. Thus, to get reasonable biodegradation, we must elevate the temperature of the culture. For dilute wastewater, this is not practical.

If the oxidation of an organic compound is carried out by microorganism using the organic matter as a food source, the oxygen consumed is known as biochemical oxygen demand (BOD). Because the organisms also use oxygen in the pours of consumption of the waste, the process is called aerobic decomposition. During the early stage (the first few days) the rate of oxygen depletion is rapid because of the high concentration of organic matter present. As the concentration of organic matter diseases, so does the rate of oxygen consumption. Oxygen concentration is mostly associated with the delay of the bacteria that grew during the early days.

The rate of oxygen consumption is directly proportional to the concentration of degradable organic matter remaining at any time. Acidogenic bacteria convert the sugars and amino acids into carbon dioxide, hydrogen, ammonia, and organic acids. This is followed by the actions of the acetagonic bacteria that convert these resultant organic acids into acetic acid, along with additional ammonia, hydrogen, and carbon dioxide. Finally, methanogenic bacteria convert these products to methane and carbon dioxide. Anaerobic digestion can widely be used as source of renewable energy (Nwabanne *et al*., 2012).

2.38.5 Population dynamics

Population dynamics is the term used to describe the time-varying success of the various species in competition. It is expressed quantitatively in terms of relative mass of microorganisms. In the discussion of the behaviour of bacterial culture which follows, there is the inherent assumption that all the requirements for growth are initially present. Since these requirements are fairly extensive and stringent, it is worth taking a moment to recapitulate them. The following list summarizes the major requirements that must be satisfied.

1. A terminal electron acceptor
2. Macronutrients
    a. Carbon to build cells
    b. Nitrogen to build cells
    c. Phosphorus for ATP (energy carrier) and DNA
3. Micronutrients
    a. Trace metals
    b. Vitamins are required by some bacteria
4. Appropriate environment
    a. Moisture
    b. Temperature

c. pH

Thus, for growth in pure cultures, when bacteria of single species are introduced into a synthetic liquid medium, initially nothing appears to happen. The bacteria must adjust to their new environment and begin to synthesize new protoplasm. This phase of growth is called the *lag phase*. At the end of the lag phase the bacteria begin to divide. Since all of the organisms do not divide at the same time, there is gradual increase in population. This phase is labelled *accelerated growth* on the growth plot. At the end of the accelerated growth phase, the population of organism are large enough and the difference in generation time is small enough that the cells appear to divide at a regular rate. Since reproduction is by binary fission (each cell divides producing two new cells), the increase in population follows in geometric progression: 1, 2, 4, 8, 16, 32 and so forth. The population of bacteria (P) after the nth generation is given by the following expression:

$$P = P_0 (2)^n \qquad (2.13)$$

Where

P_o is the initial population at the end of the accelerated growth phase. If we take the log of both sides of Equation 2.13, we obtain the following:

$$\log P = \log P_o + n\log 2 \qquad (2.14)$$

This means that if we plot bacterial population on a logarithmic scale, this phase of growth would plot as a straight line of slope n and intercept Po at t_0 equal to the end of the accelerated growth phase. Thus, this phase is called the *log growth or exponential growth phase*.

The log growth phase tapers off as the substrate becomes exhausted or as toxic by-products build up. Thus, at some point the population becomes constant either as a result of cessation of fission or a balance in death and reproduction rates. This is depicted by the *stationary phase* on the growth curve. Following the stationary phase, the bacteria begin to die faster than they reproduce. This death phase is due

to a variety of causes that are basically an extension of those which lead to the stationary phase. In wastewater treatment, as in nature, pure cultures of microorganisms do not exist. Rather, mixtures of species compete and survive within the limits set by the environment.

The prime factor for governing dynamics of the various microbial populations is the competition for food. The second most important factor is the predator-prey relationship. The relative success of a pair of species competing for the same substrate is a function of the ability of the species to metabolize the substrate. The more successful species will be the one that metabolizes the substrate more completely. In so doing, it will obtain more energy for synthesis and consequently will achieve a greater mass.

Because of their relatively smaller size, bacteria will predominate over fungi. For the same reason, the fungi predominate over the protozoa. When the supply of soluble organic substrate becomes exhausted, the bacterial population is less successful in reproduction and the predator population increase. In a closed system with an initial inoculum of mixed microorganisms and substrate, the populations will cycle as the bacteria give way to higher level organisms which in turn die for lack of food and are then decomposed by a different set of bacteria.

2.38.6 Micro-organisms and their classification

1. By kingdoms:

Microorganisms are organized into five broad groups bases on their structural and functional differences. The groups are called kingdoms. The five kingdoms are as follows

i. Animals (eg) rotifeas, crustaceans

ii. Plants (eg) mosses, ferns, some algae

iii. Protista (eg) mushrooms, yeasts

iv Fungi (eg) amoebas, some algae

v. Bacteria (eg) salmonella, escherichia

2. By energy and carbon source

The relationship between the source of carbon and the source of energy for the microorganism is vital. Carbon is the basic building block for cell synthesis. A source of energy must be obtainable from outside the cell to enable synthesis to proceed. If the microorganism uses organic material as a supply of carbon, it is called heteratrophic. Autotrophic requires only CO_2 to supply their carbon needs. Organisms that rely only on the sum for their energy are called phototropic. Chemotropis exerts energy from organic or inorganic oxidation/reduction reaction. While organotropus use organic materials, and lithotropus oxidize inorganic compounds.

2.38.7 By their relationship to oxygen

Bacteria also are classified by their ability or inability to utilize oxygen as a terminal electron acceptor, in oxidation /reduction reactions. Obligate aerobes are microorganisms that must have oxygen as the terminal electron acceptor. If waste water contains oxygen and can support obligate aerobes, it is called aerobic.

By their preferred temperature regime each species of bacteria reproduces best within a limited range of temperature - four temperature ranges are used to classify bacteria:

i. *Psychrophiles*- These are bacteria that grow best at temperatures below 20^0C

ii. *Mesophiles* - grow best at temperatures between 25 - 40^0C

iii. *Themophiles*- grow best between 45 - 60^0C

iv. *Stenothermophiles* grow best above 60^0C

Bacteria will grow over a range of temperatures and will survive at a very large range of temperatures. If frozen, rapidly, they may be stored for years with no significant death rate. (G) Escherichia coli.

2.38.8 Bacterial biochemistry

(i) Metabolism:

This is the term used to describe all the chemical activities performed by a cell. It is further divided into two - anabolism and catabolism.

(ii) Anabolism includes all the biochemical process by which the bacterium synthesizes new chemical compounds needed by the cells to live and reproduce. The synthesis process is driven by the energy that was stored in the energy carrier while *catabolism* includes all the biochemical processes by which a substrate is degraded to end products with the release of energy. A substrate is food. Food in turn is the organic material from the human digestive tract and other biodegradable wastes. Substrate one oxidized and the oxidation process releases energy that is transferred to an energy carrier which stores it for future use.

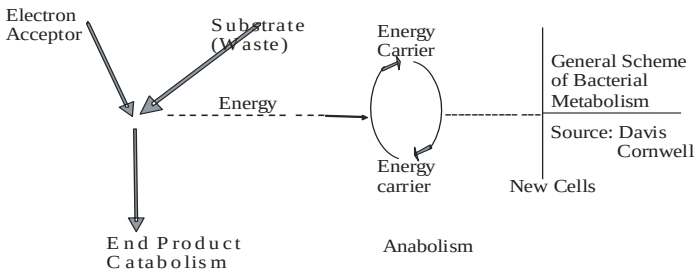

Figure 2.16: Schematic representation of decomposition of waste

The type of electron acceptor available for catabolism determines the type of decomposition (i.e aerobic, anoxic, or anaerobic) used by a mixed culture of microorganism. Each type of decomposition however, has peculiar characteristics which affects its use in waste treatment.

2.39 Biochemistry of anaerobic digestion pH and alkalinity

Anaerobic bacteria, especially the methanogens, are sensitive to the acid concentration within the digester and their growth can be inhibited by acidic conditions. It has been determined that an optimum pH value for anaerobic treatment lies between 5.5 and 8.5 (Verma, 2002). Methane bacteria are very sensitive to pH value. They need a pH range between 6.5 and 7.8 whereas the acid-producing bacteria have optimum pH value between 5 and 6. as the methane step is the rate limiting step pH should be kept near to 7. the optimal pH for bacterial growth of anaerobic organisms is in the range of 6.5 to 7.5, and consequently for a rapid sludge granulation the reactor pH should be maintained at this range (Amatya, 1996).

Alkalinity refers to the ability of a solution to resist changes in pH. Alkalinity is important because as acid is added to solution, carbonates will contribute hydroxide ions, which tend to neutralize the acid. This is known as buffering effect of alkalinity. The pH in anaerobic system is controlled by the interaction of the carbon dioxide/bicarbonate buffer system and a net strong base, which is the summation of all strong acids and bases including volatile fatty acids and ammonia. In cases of pH decrease, it can be maintained in the optimum range by the addition of sodium hydroxide, sodium carbonate or calcium hydroxide (Amatya, 1996).

2.40 Kinetic Study on Anaerobic Digestion

Anaerobic digestion is a complex process that is performed by a variety of microorganisms. Both Archaea and Bacteria are involved in anaerobic digestion. At least five different groups of microorganisms are involved in methane production. They are fermentative bacteria, hydrogen-producing acetogenic bacteria, hydrogen-consuming acetogenic bacteria, hydrogenotrophic methanogens and acetotrophic methanogens.

Due to the complexity of anaerobic digestion, studies on all sub-processes and the essential microbial groups involved in each step of anaerobic digestion are needed. A detailed understanding of the overall process and microbial activity is useful to improve and optimize anaerobic digestion process through new engineering and biotechnological approaches. Kinetic modeling of anaerobic digestion is a useful tool to design and optimize anaerobic digestion process. It helps describe biological treatment processes in a series of quantitative equations (Pavlostathis and Giraldo-Gomez 1991b), and help simplify or optimize the further study based on simulation results. The kinetic constants were often used in default values. Although the process of anaerobic digestion involves multiple biochemical pathways and different microorganisms.

Kinetic models are normally divided into two classes: structured and unstructured . Structured models take metabolic pathways into consideration and are much simpler than the unstructured ones. In the unstructured kinetic models microorganisms are usually considered to be component or reactant in the system. The unstructured kinetic models are the most frequently employed for modeling microbial systems because they are simple but good enough for technical purposes. Various kinetic models include Monod, Grau second order, modified Stover-Kincannon and first order model.

Nwabanne *et al.* (2009) proposed a kinetic model of anaerobic digestion of municipap solid waste (MSW) which considered both Monod's kinetics of biomass growth and substrate utilization. It was applied to estimate the hydraulic retention time (HRT) required for a batch digestion process. Moreover, Also on Kinetic study of Nwabanne et al (2009), proposed a continuation model for a continuous mode homogeneous reactor under steady state condition. The model was simulated to study the influence of different variables like substrate and biomass concentration (at various food to microorganism (F/M) ratio) on the efficiency of

the anaerobic digestion and to predict the HRT (hydraulic retention time) and solid retention time (SRT) required for the reaction for any specific. Process kinetic studies of anaerobic degradation of organic substrates can contribute to basic process analysis, optimal design and operation, and maximum substrate utilization rate in anaerobic digestion (Pavlostathis and Giraldo-Gomez 1991b). Modelling of biological processes in anaerobic digestion is a good tool to describe and convert complex biological problems and extensive experimental data into simple formula (McCarty and Mosey 1991).

2.41 Design of Experiment (DOE)

An experiment is a test or series of tests where the purposeful changes are made to the input variables of a process or system so that the output variables are identified. An experiment may require testing so many factors to know their effects, which can be done using One Factor At a Time (OFAT) method. In OFAT, the first factor is fixed as a "good" value, the next factor is examined, and on and on to the last factor. Because each experimental run considers only one factor, many runs are needed to get sufficient information about the set of conditions contributing to the problem. This consumes a hefty amount of time and money, along with running a high risk of error. Another limitation is that when factors change, they generally change together, so it is impossible to understand the best solution by pointing to a single, isolated factor. Traditional OFAT experimentation frequently reduces itself to no methodology whatsoever—just trial and error and a reliance on common sense. This was the method that Edison used in developing the light bulb (Anthony, 1995). But, that has now changed with the development of a new, faster and more effective method called Design of Experiment (DOE).

Design of Experiment is a computer-enhanced systematic approach to experimentation that considers all factors involved simultaneously (Anthony, 1995). DOE is concerned with the planning and conduct of experiments to analyze

the resulting data so that a valid and objective conclusion is obtained. DOE fits response data to mathematical equations. Collectively, these equations serve as models to predict what will happen for any given combination of values. DOE techniques enable designers to determine simultaneously the individual and interactive effects of many factors that could affect the output results in any design. DOE also provides a full insight of interaction between design elements; therefore, it helps turn any standard design into a robust one. Simply put, DOE helps to pin point the sensitive parts and sensitive areas in designs that cause problems in Yield. Designers are then able to fix these problems and produce robust and higher yield designs prior going into production.

2.41.1 Advantages of Design Of Experiment

1. It saves a lot of time. It can also be used to test many factors in just a short time rather than the months or even years that will be used if the traditional method of testing one factor at a time is used
2. It shows how interconnected factors respond over a wide range of values without requiring the testing of all possible values directly.
3. It determines where to set the inputs so that the influence of uncontrollable factors is minimized (robust design)
4. It saves cost, that is, it is cost effective
5. It determines which variables are the most influential in a processor system.
6. It determines where to set the inputs so that the output variability is minimized.
7. It determines the interactive effects of many factors that could affect the output results in any design.
8. It determines where to set the inputs so that the output is always near the desired state

Consider the experience of the John Deere Engine Works (Waterloo, Michigan) and its search for a way to improve paint adhesion to aluminum components while

eliminating the increasingly expensive chromate conversion coating process as a pretreatment to painting. It was difficult to find a basis for improving the paint's adhesion because John Deere's data did not clearly indicate the cause of the paint's limited performance, says supply management engineer Wayne Mills.

John Deere first ran a screening design to identify the important variables among the 12 that its experimental team had established which included the casting method, pretreatment process, paint type, and the concentration of adhesive ingredients. The results surprised everyone. Chromate conversion had very little effect on paint adhesion, but paint type proved a very significant factor. No one at John Deere had considered paint type as a major player. More tests narrowed the variables to three: chromate conversion, paint type, and surface treatment. A three-dimensional-cube plot generated by the DOE software clearly showed how the three variables interacted. The results again identified paint type as the major problem. To confirm the software's results, Mills and his colleagues performed a traditional one-variable test with several data points. This final test convinced all the members of the experimental team. As a result, John Deere solved its paint adhesion problem and eliminated the chromate-conversion pretreatment process from aluminum parts—a change that has saved the company nearly $500,000 annually, according to Mills.

2.41.2 Surface Response Methodology (RSM)

Response surface methodology (RSM) is an empirical statistical technique employed for multiple regression analysis by using quantitative data obtained from properly designed experiments to solve multivariate equations simultaneously. A full factorial design, which includes all possible factor combinations in each of the factors, is a powerful tool for understanding complex processes for describing factor interactions in multifactor systems. The relationships which link inputs with outputs are complex and difficult to describe with elemental mathematical models.

Therefore, the need arises for tools that are capable of more complex modeling and that achieve maximum refinement of the role of each variable in the system as well as the synergetic and/or antagonistic interrelationships between the same variables. The Response Surface Methodology (RSM) emerged in the 1950s (Montgomery, 2001) within the context of Chemical Engineering in an attempt to construct empirical models able to find useful statistical relationships between all the variables making up an industrial system. This methodology is based on experimental design with the final goal of evaluating optimal functioning of industrial facilities, using minimum experimental effort. Here, the inputs are called factors or variables and the outputs represent the response that generates the system under the causal action of the factors. Afterwards, the use of the RSM was shown in the design of new processes and products. In recent years it is being applied successfully in other scientific fields such as biology, medicine, and economy.

Montgomery (2001) has exhaustively reviewed the literature in the sense, describing the developments and applications of this methodology. Very recently, RSM has been used even to validate new experimental methods.

Response Surface Methodology is a collection of mathematical and statistical techniques used for the modeling and analysis of problems in which a response of interest is influenced by several variables and the objective is to optimize the response (output variable) which is influenced by several independent variables (input variables or factors). Different levels or values of the operating conditions comprise the factors in each experiment (Montgomery, 2001). Some may be categorical (e.g., the supplier of raw material) and others may be quantitative (feed rates, temperatures, time, etc). In practice, categorical variables must be handled separately by comparing our best operating conditions with respect to the quantitative variables across different combinations of the categorical ones. The

fundamental methods for quantitative variables involve fitting first-order (linear) or second-order (quadratic) functions of the predictors to one or more response variables, and then examining the characteristics of the fitted surface to decide what action is appropriate.

The designs of the response surface methodology (RSM) are those in which problems are modeled and analyzed; in these problems the response of interest is influenced by different variables. The RSM is widely used as an optimization, development, and improvement technique for processes based on the use of factorial designs—that is, those in which the response variable is measured for all the possible combinations of the levels chosen of the factors. The main effect of a factor is defined as the variation in response caused by a change in the level of the factor considered, when the other ones are kept constant. There is an interaction (dependence) between the variables when the effect of one factor depends on the behavior of another. The application of the RSM becomes indispensable when, after the significant factors affecting the response have been identified, it is considered necessary to explore the relationship between the factor and dependent variable within the experimental region and not only at the borders. Response surfaces are recommended for these types of factorial designs for their effectiveness and quick execution. This consists of correlating the *k* variables put into action through a second-degree polynomial expression of the following form: Here is a general overview of RSM. First, it provides functions and data types that provide for the coding and decoding of factor levels, since appropriate coding is an important element of response-surface analysis (Russell, 2009). Secondly, it provides functions for generating standard designs (currently, central-composite design and Box-Behnken design);

Thirdly, it extends R's function to simplify the specification of standard response surface models, and provide appropriate summaries. Fourthly, it provides means of

visualizing a fitted response surface. Finally, it provides guidance for further experimentation, e.g., along the path of steepest ascent. Most RSM functions take advantage of R's formula capabilities to provide intuitive and transparent ways of obtaining the needed results (Russell, 2009).

For example, the growth of a plant is affected by a certain amount of water x_1 and sunshine x_2. The plant can grow under any combination of treatment x_1 and x_2. Therefore, water and sunshine can vary continuously. When treatments are from a continuous range of values, then a Response Surface Methodology is useful for developing, improving, and optimizing the response variable. In this case, the plant growth y is the response variable, and it is a function of water and sunshine. It can be expressed as

$$y = f(x_1, x_2) + e \qquad (2.15)$$

The variables x_1 and x_2 are independent variables where the response y depends on them. The dependent variable y is a function of x_1, x_2 and the experimental error term, denoted as e. The error term e represents any measurement error on the response, as well as other type of variations not counted in f. It is a statistical error that is assumed to distribute normally with zero mean and variance s2. In most RSM problems, the true response function f is unknown. In order to develop a proper approximation for f, the experimenter usually starts with a low-order polynomial in some small region. If the response can be defined by a linear function of independent variables, then the approximating function is a first-order model. A first-order model with 2 independent variables can be expressed as

$$y = \beta_o + \beta_1 X_1 + \beta_2 X_2 + \varepsilon \qquad (2.16)$$

If there is a curvature in the response surface, then a higher degree polynomial should be used. The approximating function with 2 variables is called a second-order model, it is given by

$$Y = \beta_o + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X^2_{11} + \beta_{22} X^2_{22} + \beta_{12} X_1 X_2 + \varepsilon \qquad (2.17)$$

In general all RSM problems use either one or the mixture of the both of these models. In each model, the levels of each factor are independent of the levels of other factors. In order to get the most efficient result in the approximation of polynomials the proper experimental design must be used to collect data. Once the data are collected, the Method of Least Square is used to estimate the parameters in the polynomials. The response surface analysis is performed by using the fitted surface. The response surface designs are types of designs for fitting response surface. Therefore, the objective of studying RSM can be accomplish by

1. Understanding the topography of the response surface (local maximum, local minimum, ridge lines), and
2. Finding the region where the optimal response occurs. The goal is to move rapidly and efficiently along a path to get to a maximum or a minimum response so that the response is optimized.

2.41.2.1 Central Composite Design (CCD)

Two msajor Response Surface optimization designs are the Central Composite Design (CCD) and Box-Behnken Design (BBD). They are available to generate standard response-surface designs. The most popular response-surface design is the central-composite design (CCD), due to Box and Wilson. A simple example is the chemical-reaction experiment presented in the preceding section. These designs allow for sequential augmentation, so that we may first experiment with just one block suitable for fitting a first-order model, and then add more block(s) if a second-order fit is needed. Typically, we generate the whole design at once, but only actually run the parts that are needed.

The blocks in a CCD are of two types the "cube" block which contains design points from a two-level factorial or fractional factorial design, plus center points;

and the “star" block type which contains axis points plus center points. In the following discussion, the term “design points" refers to the non-center points in a block. The levels of the factors are coded, so that the cube blocks contain design points with coordinate values all equal to ±1, and center points at (0, 0, ... , 0). The design points in the star blocks are at positions of ±α along each coordinate axis. The value of α, and choices of replications of design points and center points, are often selected based on considerations of rotatability (i.e., the variance of the prediction depends only on the distance from the center) and orthogonality of blocks (so that the coefficients of the fitted response-surface equation are not correlated with block effects).

2.41.3 Coding of data

An important aspect of response-surface analysis is using an appropriate coding transformation of the data. The way the data are coded affects the results of canonical analysis and steepest-ascent analysis; for example, unless the scaling factors are all equal, the path of steepest ascent obtained by fitting a model to the raw predictor values will differ from the path obtained in the coded units, decoded to the original scale. Using a coding method that makes all coded variables in the experiment vary over the same range is a way of giving each predictor an equal share in potentially determining the steepest-ascent path. Thus, coding is an important step in response-surface analysis (Montgomery, 2001), directly linked to human development, technologically and socially.

CHAPTER 3

MATERIALS AND METHODS

3.1 Bioethanol Production

3.1.1 Materials and equipment

The wild cocoyam used for this research was obtained from the bushes in Ojoto where they were growing wild, while the water yam, yellow yam tubers, and jackfruit were purchased from Eke Ojoto Market in Idemili South Local Government Area of Anambra State of Nigeria. The cow paunch was obtained from Umeba Slaughter House at Umuoji in Anambra State of Nigeria. The malted barley utilized as the source of enzyme was obtained from Intafact Breweries Limited, Niger Bridge Industrial Layout Onitsha, Anambra State of Nigeria. The chemicals utilized for the reducing sugar measurements were procured from a chemical dealer at Head Bridge Market in Onitsha. The chemicals were used without further purification.

In collecting the cow paunch, care was taken to avoid contamination by spreading a clean water proof on the ground upon which the bowel of the slaughtered cow was slid open and the content was collected and put in a 50 liter plastic container and covered with the lid. The materials were taken to Springboard Laboratory at Udoka Housing Estate Awka, Anambra State for analysis.

Equipments utilized include Thomas Willey laboratory mill model ‘4’ equipped with 0.25mm sieve, Heating mantle (1 liter capacity, Sunbim, India), hot plate stoves, digital weighing balance (Ohaus, Adventurer, model- AR 3130), digital pH meter (Jenway 3510), hydrometer, specific gravity bottles, Abbe refractometer, Autoclave (Equitron–Medical Instruments Mfg. Co, Model – 740657.18 153), Centrifuge (80 - 2B), Oven (Memmert, UK, Model – HBTOV 14-23), Solar dryer, Oxygen bomb calorimeter (XRY–1A: Shanghai Chagi, China), Heizung, heating chauffage / coolant (HAAKE, Type F4391, No: 761060), digital

spectrophotometer (Jenway, 6305), 1 litre plastic bottles, 250ml graduated cylinders, plastic bowels, retort stands, corks, water troughs, thermometer, water hose.

3.2 Extraction of starches

The starches from the four feedstocks (cocoyam, jackfruit seeds, water yam, and yellow yam) were extracted using wet milling method according to the method of Kunle *et al.* (2003).

3.2.1 Extraction of starch from tubers.

Wild cocoyam, water yam and yellow yam are tubers, thus, their starch was extracted as follows: the tubers (10 kg) each were washed and peeled to remove the epiderm. The peeled bulks were thoroughly washed with clean water, cut and sliced into small pieces. They were milled with mechanical grinder to break down the plant cells, thereby releasing the starch granules. The resultant paste was sieved with 0.25mm mesh to extract the starch using some quantity of water. The water from the resulting starch suspension was removed by allowing the starch to sediment by gravity and decanting of the water.

The sedimented starch was squeezed in a clean muslin bag to remove the water. It was then allowed to sun dry for a period of 7 days. This ensured thorough drying of the starch. The starch which was in cakes forms was dry milled with an electronic blender, which reduced it to a very fine powdery starch. The resulting starch flour was sieved through 150 µm vibration screens, weighed and stored dry for subsequent use.

3.2.2 Extraction of starch from jackfruit seeds.

Ripe fruits were thoroughly washed and cut into halves lengthwise. The seeds were manually separated from the mucilage, and the aril and spermoderm were peeled off remaining the cotyledom. The peeled seeds (7.8kg) were slurried using mechanical grinder to break down the plant cells, thereby releasing the starch

granules. The resultant paste was sieved with 0.25mm mesh to extract the starch using some quantity of water. The water from the resulting starch suspension was removed by allowing the starch to sediment by gravity and decanting of the water. The sedimented starch was squeezed in a muslin clear bag to remove the water. It was then allowed to sun dry for a period of 7 days. This ensured thorough drying of the starch. The starch which was in cake form was dry milled with an electronic blender, which reduced it to a very fine powdery starch. The resulting starch flour was sieved through 150 μm vibration screens, weighed and stored dry for subsequent use. A yield of 51.65% was obtained.

3.3 Proximate analysis of starch feedstocks

3.3.1 Determination of moisture content

The hot oven air method of Association of Official Analytical Chemists (AOAC, 2010) was adopted for this analysis. Porcelain crucibles were washed and dried in an oven at 100^0C for 30 minutes. These were allowed to cool in the desiccators. Different starch feedstock (2 g) were placed into weighed crucibles and placed in an oven at 105^0C for 4 hours, the samples were removed and all the crucibles with the samples were re-weighed until a constant weight was obtained. The percentage moisture was calculated from the loss of weight of the sample using the following formula:

$$\frac{W_1 - W_2}{W_1} \times \frac{100}{1} \quad \text{..... (3.1)}$$

Where W_1 = wt of the original sample

W_2 = wt of final dried sample

3.3.2 Determination of ash content

This was carried out using the standard method by (AOAC, 2010). One silica dish was heated to 600^0C and weighed. The starch sample (2g) each was transferred into the dish and weighed. The dish was placed in a muffle furnace and heated

(ashed) at a temperature of 600^0C in a furnace for 3 h, and allowed to cool. Percentage ash content was calculated using the formula below:

$$\% \text{ Ash} = \frac{\text{Weight of ash}}{\text{Weight of fresh sample}} \times \frac{100}{1} \text{ ---------- (3.2)}$$

3.3.3 Determination of crude fibre

This was also carried out according to the method outlined by AOAC (2010). Pre-heated sulphuric acid (0.225M, 150ml) was added to 1.5 g of each of the starch samples (W_1) and then heated to boiling for 30 min and then filtered. Some drops of antifoaming agent were added before being boiled slowly for another 30 min and then filtered. The residue was washed three times with hot water and another three times with acetone. It was dried at 103^0 for 1 h weighed (W_2) and heated at 500^0C and then weighed again (W_3). Percentage fibre was calculated using the formula as shown below:

$$\% \text{ Fibre} = \frac{W_2}{W_1} - \frac{W_3}{1} \times 100 \quad \text{.....(3.3)}$$

Where:

W_1 = weight of the starch sample

W_2 = weight of residue after drying at $103\ ^0$C

W_3 = weight of ash

3.3.4 Determination of crude fat content

This was determined using soxhlet exraction method described by Pearson (1976). Soxhlet fat extraction method involves continuous extraction of oil/fat in a sample with non-polar organic solvent such as petroleum ether for about 1 h or more depending on the volume of the sample. An extraction flask was thoroughly washed and dried in hot oven for 30 min. It was placed in a dessicator to cool. Starch samples (2 g) were weighed and transferred into a rolled ashless filter paper

and then placed inside the extractor thimble, which was placed inside the soxhlet extractor. Some petroleum ether, about ¾ volume of the flask was added. The apparatus was set up and then heated and allowed to run for 4 h. The ether was recovered at the end of the extraction before the thimble was removed. The oil collected in the flask was dried at 100 ^{0}C in an oven and then weighed. The percentage (%) fat content was calculated using the following:

$$\% \text{ Fat} = \frac{C - B}{B} \times \frac{100}{1} \quad \text{----------} \quad \text{(3.4)}$$

Where:

B = Weight of sample; C = Weight of flask + oil after drying.

3.3.5 Determination of crude protein/nitrogen content

This was carried out using the micro-Kjeldahl method described by Person (1976). The method involves estimation of the total nitrogen in the sample and the subsequent conversion of the nitrogen to protein with the assumption that all in the samples are present as nitrogen. Using a conversion factor of 6.25, the actual percentage of protein in the sample was calculated as:

$$\% \text{ crude protein} = \% \text{ nitrogen} \times F \quad \text{----------} \quad \text{(3.5)}$$

Where

F = conversion factor (6.25)

Micro-Kjeldahl digestion/distillation apparatus and 50 ml Kjeldahl flasks were utilized in carrying out the analysis.

3.3.6 Digestion:

Samples (2g) were weighed in Kjeldahl flasks. Catalysts such as sodium sulphate and copper sulphate were added in the flask in the ration of 3:1. Oxiding agent (cone, H_2SO_4 15 ml) was added, glass beads were added to prevent bumping during heating. Heating was carried out continuously on a digestion rack under fume cupboard until a greenish clear solution appeared. The digest was allowed to

clear for about 30 min, before it was heated for another 30 min and allowed to cool. 10 ml of distilled water was added to avoid caking after which the digest was transferred with several washings into 25 ml volumetric flask and made up to the mark with distilled water.

3.3.7 Distillation of the protein:

A 50 ml receiver flask containing 5 ml boric acid (methyl red and blue indicator) were placed under the condenser of the distillation apparatus so that the tip was 2 cm inside the indicator. A 10 ml of 40% NaOH solution was added to the digested sample in the apparatus through the funnel stop cork. Closing the steam by-pass and opening the inlet stop cork on the steam jet arm of the distillation apparatus started off the distillation. The distillate was collected in the conical flask (35 ml) with its indicator–methyl red and blue). Titration was then carried out using 0.01M HC1 to first pink colouration. The percentage of nitrogen and protein was calculated using the formula below:

$$\% \text{ nitrogen (N)} = \frac{\text{Titre} \times 0.0014 \times 250}{\text{Weight of original sample}} \times \frac{100}{1} \quad \text{---------- (3.6)}$$

Where % crude protein = % N x 6.25

3.3.8 Determination of calorific value (energy content)

This was carried out using the AOAC (2010) bomb calorimetric method. It involves igniting the waste sample in adiabatic oxygen bomb calorimeter (under a high pressure of oxygen gas, usually 25 atmospheres). This helps to bring about oxidation of organic constituent of the waste sample to water and carbon dioxide while oxidizing some elements such as S, N and P with resultant release of heat energy. The heat energy released is absorbed by the water surrounding the bomb calorimeter and gives rise to increase in temperature of the water which is then

used to estimate the energy/calorific value of the sample. Known quantities of the samples were pelleted and burnt in adiabatic oxygen bomb calorimeter. The heat of combustion were calculated as the gross energy of the sample:

$$\text{Calorific value (W)} = \frac{£\Lambda T - Ø}{M} \text{---------- (3.7)}$$

Where;

ΛT = Net corrected temperature; Ø = Correction for heat of combustion of fining wire; V = Volume of standard alkali solution; M = Mass of the sample being analyzed/evaluated.

3.3.9 Determination of fructose

This was carried out according to Pearson (1976) method. Starch samples (1 g) each were weighed out, macerated with 50 ml distilled water and filtered. 1 ml of the filtrate was pipetted into a flask. 1 ml of reagent B (50 ml cone HCI + 10 ml distilled water) and 1 ml of reagent A (50 mg resorcinol dissolved in 50 ml ethanol) were added to the flask containing 1 ml of the filtrate. It was heated in a water bath at 80^0C for 6 min, cooled to room temperature and the absorbance measured at 530 nm using spectrophotometer.

3.3.10 Determination of sucrose

This was carried out according to Pearson (1976) method. Starch samples (1 g) each were weighed out, macerated with 50 ml distilled water and filtered. 1 ml of the filtrate was pipetted into a flask. 5 ml of 2% resorcinol was added and boiled for 12 min. It was cooled and made up to 25 ml with distilled water. The absorbance was measured at 620 nm using spectrophotometer.

3.4 Preliminary phytochemical analysis (qualitative)

The tests carried out were based on procedures outlined by Harbourne and modified by Trease and Evans (1996).

3.4.1 Test for carbohydrate

Molisch test

The starch samples (0.1g) were boiled with 2 ml of distilled water and filtered. To the filtrate, were added, a few drops of naphthol solution in ethanol (Molisch's reagent); concentrated sulphuric acid in a Pasteur pipette was then gently poured down the side of the test tube to form a lower layer. A purple interfacial ring indicated the presence of carbohydrate.

3.4.2 Test for glycosides

Dilute sulphuric acid (0.5M, 5ml) was added to 0.1 g of the extract in a test tube and boiled for 15 min in a water bath, cooled and neutralized with 20% potassium hydroxide solution. Equal parts of Fehling's solution A and B (10ml) each were added and boiled for 5 min. a dense brick red precipitate indicated the presence of glycosides.

3.4.3 Test for saponins

Distilled water (20ml) was added to 0.25 g of each of the starch samples and boiled in a water bath for 2 min. the mixtures were filtered while hot and allowed to cool. The filtrates were used for the following tests:

i) Frothing test

The filtrate (5 ml) was diluted with 15 ml of distilled water and shaken vigorously. A stable froth (foam) upon standing indicated the presence of saponins.

ii) Emulsion test

Two drops of olive oil were added to the frothing solution and the contents shaken vigorously. The formation of emulsion indicated the presence of saponins.

iii) Fehling's test

To 5 ml of the filtrate was added 5 ml of Fehling's solution. Equal parts of A and B were added and the mixture heated on a water bath. Reddish precipitate which

turned brick red on further heating with sulphuric acid indicated the presence of saponins.

3.4.4 Test for tannins

Starch samples (1 g) each were boiled with 20 ml of water, filtered and used for the following tests:

i) Ferric chloride test

Few drops of ferric chloride were added to 3 ml of the filtrate. A greenich black precipitate indicated the presence of tannins.

ii) Lead acetate test

Lead acetate solution was added to a little quantity of the filtrate. Reddish colour indicated the presence of tannins.

3.4.5 Test for flavonoids

Ten milliliters (10ml) of ethyl acetate were added to 0.2 g of each of the starch samples and heated on a water bath for 3 min. the mixture was cooled, filtered and the filtrate was used for the following tests:

i) Ammonium test

ii) Four milliliters (4 ml) of the filtrate was shaken with 1 ml of dilute ammonia solution. The layers were allowed to separate and the yellow colour in the ammoniacal layer indicated the presence of flavonoids.

iii) 1% Aluminum chloride solution test

Another 4 ml portion of the filtrate was shaken with 1 ml of 1% aluminum chloride solution. The layers were allowed to separate. A yellow colour in the aluminum chloride layer indicated the presence of flavonoids.

3.4.6 Test for steroids

Ethanol (9 ml) was added to 1 g of the extract and refluxed for a few minutes and filtered. The filtrate was concentrated to 2.5 ml in a boiling water bath. Hot distilled water (5 ml) was added to the concentrate solution, the mixture was

allowed to stand for 1 h and the waxy matter was filtered off. The residue was extracted with 2.5 ml of chloroform using separate funnel. To 0.5 ml of the chloroform extracted in a text tube was carefully added 1 ml of concentrated sulphuric acid to form a lower layer. A reddish brown interface showed the presence of steroids.

3.4.7 Test for reducing sugar

Equal parts of Fehling's solution A and B were added to 5 ml of aqueous starch solution and then heated in a water bath for 5 min. brick red precipitate showed the presence of reducing sugar.

3.4.8 Test for cyanogenic glycoside

Distilled water (20 ml) was added to 0.1 g of the starch solution. A piece of sodium picrate paper was suspended over the solution and placed on a water bath for 1 h. a change of colour from yellow to orange of the sodium picrate paper indicated the presence of cyanogenic glycoside.

3.5 Quantitative determination of phytochemical contents of the starch feedstock

All the quantitative determination of the phytochemistry of the starch were carried out using Pearson (1976) method.

3.5.1 Determination of flavonoids

The samples (1 g) each were weighed out. It was macerated with 20 ml of ethyl acetate for 5 min and filtered. 5 ml of the filtrate was pipetted and added to 5 ml of the dilute ammonia. It was shaken for 5 min. the supplement was decanted and the absorbance was measured at 490 nm.

3.5.2 Determination of reducing sugar

The samples (1 g) each were weighed and macerated with 20 ml of distilled water and then filtered. 1 ml of the filtrate was pipetted and added to 1 ml of alkaline

copper reagent. This was boiled for 5 min and cooled. 1 ml of phosphomolybdic acid was added to the cooled mixture followed by the addition of 7 ml of distilled water. The absorbance was measured at 420 nm.

3.5.3 Determination of glycoside

The starch samples (1 g) each were weighed and macerated with 20 ml of distilled water. 2.5 ml of 15% lead acetate was added and the mixture filtered. 2.5 ml of chloroform was added and the mixture was shaken vigorously. The residue was collected and evaporated to dryness. It was dissolved in 3 ml glacial acetic acid. 0.1 ml of 15% ferric chloride and 0.25 ml of cone sulphuric acid were added, shaken and put in the dark for 2 h. the absorbance was measured at 530 nm.

3.5.4 Determination of tannins

The starch samples (1 g) each were weighed, measured with 50 ml of distilled water and filtered. 5 ml of the filtrate was pipetted into a flask. 0.3 ml of 0.1N ferric chloride in 0.1N HCI and 0.3 ml of 0.008M potassium ferricyanide were added. The absorbance was measured at 720 nm.

3.5.5 Determination of cyanide

Starch samples (1 g) each were weighed and measured and macerated with 50 ml of distilled water. They were left to stand for 24 h and filtered. 1 ml of the filtrate was pipetted into a flask, 4 ml of alkaline picrate solution was added and the mixture was boiled for 5 min and cooled. The absorbance was measured at 400 nm.

3.5.6 Determination of soluble carbohydrate

Starch samples (1 g) each were weighed, macerated with 50 ml distilled water and cooled. 1 ml of the filtrate was pipetted into a flask and 2 ml of saturated picric acid was added. The absorbance was measured at 530 nm.

3.5.7 Determination of steroid

The starch samples (1 g) each were weighed, macerated with 20 ml of ethanol and filtered. 2 ml of the filtrate was pipe.

3.5.8 Determination of saponin

The starch samples (1 g) each were weighed and macerated with 10 ml of petroleum ether. The supernatants were decanted into a beaker. Another set of petroleum ether (10 ml) was used to macerate the samples and the supernatants decanted again. The two decanted supernatants were combined and evaporated to dryness. The residue was dissolved with 6 ml ethanol. 2 ml from it were transferred to a test tube and 2 ml of a colour reagent was added. It was allowed to stand for 30 min at noon temperature. The absorbance was measured at 550 nm.

3.5.10 Amylose and amylopection ratio determination

a). Preparation of standard amylase for calibration

About 100 mg amylase was weighed into 100 ml volumetric flask, dissolved with 10 ml of 1% (w/v) NaOH and made up to mark with water. From this stock solution, different concentrations ranging from 10 µg to 100 µg were prepared. Colour development was achieved by adding 5 ml of 1solution and 1 ml of 6N HCI to each solution. The absorbance of each concentration was determined at 640 nm in a digital spectrophotometer. A plot of the absorbance against concentration in µg/ml gave a standard curve (Lin and Xu, 2005).

b). Amylase/amylopectin ratio determination for the feedstocks

The four starches were treated with n-hexane to remove any residual lipids present as reported in Adiukwu (1998) was used. About 0.1 g of each starch material was weighed into a 100 ml volumetric flask and moistened with 2 ml absolute ethanol. The mixtures were heated on a hot plate until a clear solution was obtained. The mixtures were then made up to 100 ml with distilled water and used for amylase

determination. A 5 ml aliquot of the above stock solution was mixed with 3 drops of 1N HCI and 5 ml of I_2 solution and made up to 100 ml. The mixture was allowed to stand for 20 minutes for maximum colour development after which the absorbance were determined. For each absorbance obtained, the amylase content was estimated from the standard curve earlier derived for pure amylase. The percentage amylase in 100 mg of starch was estimated and the amylopectin content was determined mathematically by subtracting the amylase content from 100%.

3.6 Gelatinization process for starch feedstocks

All the gelatinization processes were carried out according to the method of Novellie and Shutte (1961).

3.6.1 Gelatinization process for cocoyam starch

Four sets of 100 g of cocoyam starch were weighed out accurately using a digital weighing balance. To each of these four sets of 100 g of the starch was added 200 ml, 250 ml, 300 ml and 350 ml of distilled water contained in a 1000 ml breaker (representing slurry concentrations of 2.0, 2.5, 3.0 and 3.5 ml/g) respectively. The starch content of the feedstock determined the starting quantity of water used. The mixtures were thoroughly stirred and put into a water bath. The temperature of the water bath was maintained at a constant boiling range. The starch water mixture was stirred very well for the first ten minutes of heating, while monitoring the temperature. After this first ten minutes, the mixtures were occasionally stirred until gels of moderate viscosities were formed. The gel formation ended the gelatinization process, the temperature range noted. This process was replicated and the mixtures were set to cool down for further conversion.

3.6.2 Gelatinization process for jackfruit seed starch.

Four sets of 100 g of jackfruit seed starch were weighed out and to each of them were added 200 ml, 250ml, 300 ml, and 350ml of distilled water (representing slurry concentrations of 2.0, 2.5, 3.0 and 3.5, ml/g) respectively. Each of these

starch water mixture was contained in 1000 ml beaker. The mixtures were each stirred thoroughly to form a homogeneous mixture and then put in a hot water bath on an electric heating stove. The temperature of the water bath was maintained at a constant boiling temperature. While monitoring the temperature of the starch-water mixture they were stirred very well for the first ten minutes of heating. After that, they were occasionally stirred until gels of moderate viscosity were formed for each of them. The gel formation ended the gelatinization. The temperature ranges of the gel formations were noted. The process was replicated and the mixtures were set to cool down for further conversion.

3.6.3 Gelatinization process for water yam starch.

Four sets of 100 g of water yam starch were weighed out accurately and added to 100 ml, 200 ml, 300 ml and 400 ml of distilled water representing slurry concentrations of 1.0, 2.0, 3.0 and 4.0 ml/g respectively. They were placed in 1000 ml beaker each. The starch content of the water yam determined the starting water quantity and the others were in increments of 1.0 ml/g water content. The temperature of the water bath was maintained at a constant boiling range. While monitoring the temperature of the starch-water mixtures, they were stirred very well for the first ten minutes of heating after which they were occasionally stirred until a gel of moderate viscosity was formed. The temperature range of the gelatinization process was noted. The gel formation ended the gelatinization process. The process was replicated and the mixtures set to cool down for further conversion.

3.6.4 Gelatinization process for yellow yam starch.

Four sets of 100 g of yellow yam starch were weighed out accurately and added to 100 ml, 200 ml, 300 ml and 400 ml of distilled water (representing slurry concentrations of 1.0, 2.0, 3.0 and 4.0 ml/g) respectively. Each of the mixtures was contained in a 1000 ml beaker. The mixtures were thoroughly stirred to form a

homogenous mixture and then put in a water bath on an electric hot plate. The temperature of the water bath was maintained at a constant boiling range. The starch-water mixtures were stirred continuously while monitoring the temperature for the first ten minutes. After that, they were occasionally stirred until a gel of moderate viscosity was formed the temperature ranges of the gel formation were noted. The formation of the gel ended the gelatinization process. The process was replicated and the mixtures set to cool down for further conversion.

3.7 Saccharification process for the starches

3.7.1 Hydrolysis of the starches using Design of Experiment (DOE)

Hydrolysis of the starches for optimization of percentage reducing sugar yield was carried out using Response Surface Methodology (RSM). It uses qualitative data from appropriate experimental designs to determine and simultaneously solve multivariable equations graphically represented as response surfaces which can be used in three ways:

1). To describe how the test variables affect the response.

2).To determine the inter-relationships among the test variables on the response.

3). To describe the combined effects of all the test variables on the response.

3.7.2 Central Composite Design (CCD)

The Central Composite Design (CCD) was used to study the effects of the variables towards their responses and subsequently in the optimization studies. This method is suitable for fitting a quadratic surface and it helps to optimize the effective parameters with a minimum number of experiments, as well as to analyze the interaction between the parameters. In order to describe the effects of temperature, water quantity, enzyme concentration and time on the percentage reducing sugar yield were conducted based on the CCD. The coded values of the process parameters were determined by the following Equation 3.8: (Rajeshkannan *et al,* 2012)

$$x_i = \frac{X_i - X_o}{\Delta X} \quad (3.8)$$

Where:

x_i – coded value of the ith variable,

X_i – uncoded value of the ith test variable and

X_o – uncoded value of the ith test variable at center point.

The Central Composite Design of experiment in terms of coded values of the experimental design and range and levels of individual variables are given in Tables 3.1. and 3.2.

A statistical program package, Design Expert 8.0.7.1 was used for regression analysis of the data obtained and to estimate the coefficient of the regression equation. The equations were validated by the statistical tests called the ANOVA analysis. The significance of each term in the equation is to estimate the goodness of fit in each case. Response surfaces were drawn to determine the individual and interactive effects of the test variable on the percentage reducing sugar yield.

The optimal values of the test variables were first obtained in coded units and then converted to the uncoded units. Using four factor variable and six centre points will give the CCD design in Table 3.1 below.

Table 3.1 Central Composite Design of experiment in terms of coded values

Run	Time	Enzyme conc.	Water qty.	Temperature
1	-	-	-	-
2	+	-	-	-
3	-	+	-	-
4	+	+	-	-
5	-	-	+	-
6	+	-	+	-
7	-	+	+	-
8	+	+	+	-
9	-	-	-	+

10	+	-	-	+
11	-	+	-	+
12	+	+	-	+
13	-	-	+	+
14	+	-	+	+
15	-	+	+	+
16	+	+	+	+
17	-α	0	0	0
18	+α	0	0	0
19	0	-α	0	0
20	0	+α	0	0
21	0	0	-α	0
22	0	0	+α	0
23	0	0	0	-α
24	0	0	0	+α
25	0	0	0	0
26	0	0	0	0
27	0	0	0	0
28	0	0	0	0
29	0	0	0	0
30	0	0	0	0

The high (+) and low (-) values for optimal percentage reducing sugar yield were time (2 and 4hours), enzyme concentration (0.1 and 0.3g) and water quantity (2.0 and 4.0gr) temperature (40 and 70^{o}C). The alpha (α) value used was 1.5. The factor levels of the variables are given in Table 3.2 while the Central Composite Design for reducing sugar yield terms of real values for wild cocoyam and jackfruit seed is shown in Table 3.3. Also, Table 3.4 shows the Central Composite Design for reducing sugar yield terms of real values for water yam and yellow yam.

Table 3.2: Factor levels of independent variables for reducing sugar yield

Independent Factors	Low level	Medium	High level

	(-)	level (0)	(+)
Time (hrs)	2	3	4
Enzyme conc. (g/g).	0.1	0.2	0.3
Water quantity (ml/g)	2	2.5	3
Temp, ^{o}C	40	55	70

Table 3.3 Central Composite Design for reducing sugar yield terms of real values for wild cocoyam and jackfruit seed wastes.

Std	Factor 1 A: time (hrs)	Factor 2 B: enzyme conc. (g/g)	Factor 3 C: Water (ml/g)	Factor 4 D; Temp. (^{0}C)
1	2.50	0.20	2.50	55.00
2	2.50	0.20	3.25	55.00
3	2.50	0.20	1.75	55.00
4	2.00	0.30	2.00	40.00
5	2.00	0.10	4.00	40.00
6	3.00	0.10	4.00	40.00
7	2.00	0.30	4.00	40.00
8	3.00	0.30	4.00	40.00
9	2.00	0.10	2.00	70.00
10	3.00	0.10	2.00	70.00
11	2.00	0.30	2.00	70.00
12	3.00	0.30	2.00	70.00
13	2.00	0.10	4.00	70.00
14	3.00	0.10	4.00	70.00
15	2.00	0.30	4.00	70.00
16	3.00	0.30	4.00	70.00
17	1.75	0.20	3.00	55.00
18	3.25	0.20	3.00	55.00
19	2.50	0.05	3.00	55.00
20	2.50	0.35	3.00	55.00
21	2.50	0.20	1.50	55.00
22	2.50	0.20	4.50	55.00
23	2.50	0.20	3.00	32.50
24	2.50	0.20	3.00	77.50
25	2.50	0.20	3.00	55.00
26	2.50	0.20	3.00	55.00
27	2.50	0.20	3.00	55.00
28	2.50	0.20	3.00	55.00

29	2.50	0.20	3.00	55.00
30	2.50	0.20	3.00	55.00

Table 3.4 Central Composite Design for reducing sugar yield for water yam and yellow yam wastes.

Std	Factor 1 A: time hrs	Factor 2 B: enzyme conc. g/g	Factor 3 C:water qty. ml/g	Factor 4 D: temperature ^{0}C
1	2.50	0.20	2.50	55.00
2	2.50	0.20	3.25	55.00
3	2.50	0.20	1.75	55.00
4	2.00	0.30	2.00	40.00
5	2.00	0.10	4.00	40.00
6	3.00	0.10	4.00	40.00
7	2.00	0.30	4.00	40.00
8	3.00	0.30	4.00	40.00
9	2.00	0.10	2.00	70.00
10	3.00	0.10	2.00	70.00
11	2.00	0.30	2.00	70.00
12	3.00	0.30	2.00	70.00
13	2.00	0.10	4.00	70.00
14	3.00	0.10	4.00	70.00
15	2.00	0.30	4.00	70.00
16	3.00	0.30	4.00	70.00
17	1.75	0.20	3.00	55.00
18	3.25	0.20	3.00	55.00
19	2.50	0.05	3.00	55.00
20	2.50	0.35	3.00	55.00
21	2.50	0.20	1.50	55.00
22	2.50	0.20	4.50	55.00
23	2.50	0.20	3.00	32.50
24	2.50	0.20	3.00	77.50
25	2.50	0.20	3.00	55.00
26	2.50	0.20	3.00	55.00

27	2.50	0.20	3.00	55.00
28	2.50	0.20	3.00	55.00
29	2.50	0.20	3.00	55.00
30	2.50	0.20	3.00	55.00

3.7.5 Quantitative test for reducing sugar

A sample (20 ml) of the saccharified material was withdrawn and added to NaOH (20 ml) in a 250 ml volumetric flask. 60 ml of distilledwater was added to bring the final volume to 100 ml bringing the final factor to 10. The sugar content was determined by the Plumer method described below. 50 ml of the material obtained was poured into a 50 ml burette. 25 ml of the Benedict reagent was pipetted into a 100 ml conical flask. 3–5 g of Na_2CO_3 was added and a few pieces of porcelain were added to prevent bumping. The Benedict reagent was boiled on a bursen burner during which the withdrawn material was slowly run from the burette until the last trace of blue colour of Benedict's solution disappeared and white precipitate formed. The titration was stopped at this stage and the volume of the saccharified or withdrawn material used was noted. The weight in grammes of reducing sugar per 100 ml of the solution was calculated using the formula:

$$\text{Weight of reducing sugar/100 ml} = \frac{K \times 100 \times N}{V} \quad (3.9)$$

Where;

K = Reducing sugar constant which for glucose is K =50

N = Extent of dilution i.e. number of times solution was diluted = 10

V = Volume of saccharified solution used for the titration

3.8 Preparation of yeast inoculums.

The yeast used for this experiment was obtained from yeast extract and was carefully prepared according to the method of Pfaller *et al*. (1985). Glucose 10 g was weighed out and introduced into 100 ml of distilled water. The mixture was

placed at a temperature of 121^0C for 15 min. this was to ensure sterility of the solution. The glucose solution was left overnight to cool. Yeast extract (10 g) was introduced into the cooled sugar solution and left for 24 h. after that, the mixture was centrifuged to separate the sugar solution from the yeast cells. The supernatant being sugar solution was decanted and the yeast cells kept ready for the fermentation process under the temperature of $2 – 6^0$C.

3.9 Preparation of fermentation medium

These were carried out according to the method of Zhang *et al*. (2003). The enzyme – hydrolyzed starch slurry for the variants (cocoyam, jackfruit, water yam, and yellow yam) were boiled for one hour each to precipitate any available protein. For this experiment, the variants with the highest reducing sugar quantity were used for the ethanol precipitate. The supernatant, containing the soluble sugar were then transferred into 1000 ml conical flask. Nitrogen and phosphorus sources were added in the form of ammonium sulphate (0.85%) and ammonium hydrogen phosphate (0.12%) respectively.

The pH was adjusted to 4.5 – 5.0 using dilute HCI. The flasks were properly plugged with cotton wool and autoclaved at 121^0Cand a pressure of 15 psi for a period of 15 min. the flask was allowed to cool and left overnight to ensure sterility before inoculation. The medium was inoculated with 5 g of the yeast cells. The flask was properly corked with a sterile cork connected to a fermentation tube containing cone H_2SO_4. This was to enable carbon dioxide leave the flask but trap vapour or any volatile alcohol. The flask was then left at room temperature for fermentation. The specific gravity of the broth was measured at 12 h intervals using a hydrometer. The pH of the fermentation broth was also measured and adjusted with ammonia solution at the same interval of 12 h the extent of fermentation was checked by evolution of carbon dioxide using lime water test. Fermentation was stopped when no more carbon dioxide was coming out of the

fermentation medium. The fermentation lasted for a total of 72 h for all the variants.

3.10 Determination of fermentation efficiencies

Fermentation efficiencies were calculated as a ratio of the actual ethanol yield to the theoretical ethanol yield. The total starch contents in the samples were used to calculate the theoretical ethanol yields, assuming 1 g of starch converts to 1.11 g of glucose and that 1 g of glucose may generate 0.511 g of ethanol[50]. Fermentation efficiency was calculated using the formula;

Fermentation efficiency (%) = $\frac{\text{Actual ethanol yield}}{\text{Theoretical ethanol yield}}$ X 100---------- (3.10)

3.11 Distillation and rectification of ethanol from fermented worth

During product recovery, 100 ml of the fermentation wort was dispensed into the distillation-round bottom flask, the distillation apparatus were set by fixing the round bottom flask into the condenser, and a thermometer was fixed on the mouth of the flask to monitor the temperature. Heat was applied to the flask through the Bunsen burner. Before heating, a circulation of cold water was ensured to cool the distillate; heat was applied at a temperature of 78-80^{0}C which is the same temperature at which ethanol boils, ethanol was distilled and the distillate was collected.

The volume of ethanol recovered was then measured when there was no more condensate at the set distillation temperature. The distillates which were mixtures of ethanol, water and other contaminants were collected in a receiver. Theoretically, ethanol obtained by simple distillation scarcely exceeds 95%. The ethanol obtained above was therefore subjected to further rectification by repeated distillation using Heizung, heating chauffage / coolant. This was to ensure that the water content was reduced to a minimum. Further dehydration of the ethanol was carried out using calcium oxide.

3.12 Confirmation of ethanol produced

3.12.1 Experimental procedure

(1). Aqueous solution of sodium monooxoiodate (I) (NaOH) was prepared by dissolving (2 drops) iodine in sodium hydroxide solution. 5ml of the distilled ethanol was warmed gently with sodium monooxoiodate 1 and 3 drops of potassium iodide was added with stirring in progress. The mixture was allowed to stand for 10minutes and pale yellow crystals of triodo-methane (iodoform) appeared with its characteristics smell to confirm the presence of ethanol. The pale yellow crystals of triodo-methane that appeared confirmed the presence of ethanol.

(2). Also, a small quantity of potassium iodide solution was added to a small amount of the distilled ethanol followed by sodium chlorate solution and the mixture warmed gently, a very pale yellow precipitate of triodo-methane or iodoform confirmed the presence of alcohol. Ethanol is the only primary alcohol to give the triodo-methane (iodoform) reaction

3.12.2 Determination of strength and yield of ethanol

3.12.2.1 Experimental procedure

The ethanol was poured into a 100ml cylinder and a hydrometer was immersed into the liquid. The hydrometer was allowed to float at a constant position without leaning to the walls of the cylinder. The readings were taken from the calibration of the hydrometer. These readings gave the specific gravity of the ethanol. This was done for all the samples produced from the different varieties of cassava. During this experiment, the ethanol was maintained in an ice bath at 20^0C and the specific gravity obtained in each case was used to determine the strength and yield of ethanol from a standard of the properties of ethanol.

3.13 Calculation of percentage alcohol produced

The specific gravity of the distillate was determined using a specific gravity bottle. The weight of the empty specific gravity (S.G) bottle was first measured using an

electric weigh balance, then the weight of the bottle and water was determined, the weight of the water was noted by subtracting the weight of the empty bottle from the summation of the weight of the bottle and water. Then the weight of the bottle and ethanol was measured and ethanol weight noted by subtracting the weight of the bottle from the weight of ethanol and bottle.

The specific gravity was calculated by dividing the weight of ethanol over the weight of distilled water, and the value obtained was used to calculate the alcoholic content by comparing the specific gravity of distillate by volume from a prepared reference standard table.

3.14 Measurement of specific gravity

The specific gravity of the fermenting broth was measured at 24 hourly internal (0hr, 24hrs, 48hrs, 72hrs...) using a hydrometer. This was done by dipping the hydrometer into a 50 ml cylindrical flask containing the fermenting broth and taking the readings at eye level as the hydrometer floats on the broth surface.

3.15 Waste analysis

3.15.1 Proximate analysis

Determination of moisture content, ash content, crude fibre, crude fat, crude nitrogen content and calorific value were carried out as described in section 3.2.

3.15.2 Determination of carbon content

This was determined using method of Walkey and Black (1934). One (1) gram of the ground waste sample was weighed into 500 ml conical flask. Potassium dichromate (M, 10 ml) was poured inside the flask and the mixture was swirled. Cone H_2SO_4 (20 ml) was added and the flask was swirled again for 1 min in a fume cupboard. This mixture was allowed to cool for 30 min after which 200 ml of distilled water, 1 g of NaF and 1 ml of phenylalanine indicator were added. The mixture was shaken and titrated with ferrous ammonium sulphate in a burette. The

blank was also treated similarly. The percentage carbon content was calculated using the formula below;

$$\% \text{ Carbon} = \frac{B - T \times C \times 133 \times 0.003 \times 100}{W} \text{---------- (3.11)}$$

Where;

B = Blank titre value

T = Sample titre value

C = Concentration of Fe solution

W = Weight of waste sample

3.15.3 Determination of total solids

Determination of total solids is an effective way of knowing the amount of nutrient that will be available for bacterial action during digestion. It is made up of digestible and non-digestible materials. This was carried out using Bhatia (2009) method. The raw waste sample (5 g) was dried in an oven at 105^0C for 5 h. The dried sample was cooled in a desiccators and then weighed. The weight obtained after all moisture loss is the total solids. It was calculated using the formula below;

$$\% \text{ TS} = \frac{B}{A} \times \frac{100}{1} \text{---------- (3.12)}$$

Where;

TS = total solids

A = Initial weight of sample

B = final weight of dried material

3.15.4 Determination of volatile solids

Volatile solids are the biodegradable constituent of any waste sample. Its content is indication of how much biogas can be obtained from a waste sample assuming every condition is okay. This was carried out using Bhatia (2009) method. The

waste sample residue (1 g) from the total solids determination was heated in a muffle furnace at 600^0C.

After this, the heated residue (ash) was cooled in a dessicator and weighed. Thevolatile solids were calculated using the formula below;

$$\text{Volatile solids (Vs)} = \frac{B - C}{Ws} \times \frac{100}{1} \text{---------- (3.13)}$$

Where;

B = Weight of dried residue from Ts determination

C = Weight of sample residue after heating at 600^0C

Ws = Weight of original sample

3.15.5 Determination of microbial total viable count (TVC)

This is done to determine the number of living microorganisms especially pathogens in the waste. This load determines also how most of the nutrients in the waste are converted to biogas. Surface viable count method of Miles and Misra described by Okore (2004) was used for this determination. The suspension obtained from the isolation of bacteria was diluted with sterilized distilled water using sterile pipette. The aim was to obtain condition that contained approximately 30 cells per 0.015 ml or 0.075 volumes per drop. Agar plates were prepared and the undersides of the plates were divided into eight segments with an indelible marker. A drop of the suspension was inoculated on each segment. These were then incubated for 245 h at 37^0C. Developed colonies were counted from equation below:

Mean count =Number of colonies in each segment (cfu)-------- (3.14)

$$\text{TVC} = \frac{Mc \times D}{Vd} \quad (3.15)$$

Where;

Mc = Mean count (cfu); Cfu = Colony forming unit; D = Dilution factor

Vd = Volume per drop

The higher the dilution factor, the less turbid the solution would be.

3.15.6 Determination of Chemical Oxygen Demand

This was done according to the method outlined by AOAC (2010). 10cm^3 of the digestates was introduced into 100cm^3 round-bottomed flask. 2cm^3 potassium dichromate, 2.5cm^3 mercuric sulphate solution, 10-15 ml concentrated sulphuric acid containing silver sulphate and an anti-bumping rod were added to it. The solution was heated to gentle but steady boiling over an electric hot plate or heating mantle and under a reflux condenser. After exactly 45 minutes of boiling, it was allowed to cool briefly, washed through the condenser into the flask with 2cm^3 of distilled water and cooled completely in cold water. 2 drops of ferroin solution was added as indicator and the excess potassium dichromate with standardized ammonium iron (II) sulphate was titrated until the colour changed from bluish-green to reddish-brown.

Where 0.05 = normality of titrant

$$COD\left(mg/l\right)=\frac{ml\ of\ titrant\ for\ sample-ml\ of\ titrant for blank\times 0.05+8000}{Volume of\ Sample} \qquad (3.16)$$

3.15.7 Determination of Biochemical Oxygen Demand

The Biological Oxygen Demand (BOD) was determined using the procedure outlined by APHA (2005). The general equation for the determination of a BOD_5 value is:

$$BOD_5\left(mg/l\right)=\frac{D_1-D_2}{P} \qquad (3.17)$$

Where D_1 = initial DO 15 minutes after preparation of the sample, D_2 = final DO of the sample after 5 days, and P = decimal volumetric fraction of sample used. If 100 ml of sample were diluted to 300 ml, then P = 0.33. If no dilution was necessary, P = 1.0 and the BOD_5 was determined by D_1-D_2.

3.15.8 Determination of pH of the slurry.

This was carried out using the procedure indicated by APHA (2005). Sufficient amount of the sample was poured into a small beaker and the tips of the electrodes rinsed with portions of the sample before being immersed to a depth of about 2cm with the electrodes being at least 1cm away from the sides and bottom of the beaker. The pH meter was turned on, temperature adjustment dial adjusted and the pH of sample recorded.

3.15.9 Determination of Total Suspended Solids (TSS)

Total suspended solid was determined by subtracting the result of total dissolved solid from total solid. This was determined using the guideline indicated by APHA (2005).

Total solids (TS) – Total dissolved solids (TDS) = Total suspended solids (TSS) (3.18)

3.16 Optimization of biogas production

3.16.1 Design of experiment.

Response Surface Methodology (RSM) was used in the design of the experiment. Cornell (2005) reported that RSM uses qualitative data from appropriate experimental designs to determine and simultaneously solve multivariable equations graphically represented as response surfaces which can be used in three ways:

1). To describe how the test variables affect the response.

2). To determine the inter-relationships among the test variables on the response.

3). To describe the combined effects of all the test variables on the response.

3.16.2 Central Composite Design (CCD)

The Central Composite Design (CCD) was used to study the effects of the variables towards their responses and subsequently in the optimization studies.

This method is suitable for fitting a quadratic surface and it helps to optimize the effective parameters with a minimum number of experiments, as well as to analyze the interaction between the parameters. In order to describe the effects of temperature, water quantity, enzyme concentration and time on the percentage reducing sugar yield were conducted based on the CCD. The coded values of the process parameters were determined by the following Equation 3.19: (Rajeshkannan et al, 2012)

$$x_i = \frac{X_i - X_o}{\Delta X} \quad (3.19)$$

Where: x_i – coded value of the ith variable, X_i – uncoded value of the ith test variable and X_o – uncoded value of the ith test variable at center point.

The Central Composite Design of experiment in terms of coded values for biogas production is given in Table 3.5 along with experimental data and predicted responses. The range and levels of individual variables or factor levels are given in Table 3.6.

A statistical program package, Design Expert 8.0.7.1 trial version was used for regression analysis of the data obtained and to estimate the coefficient of the regression equation. The equations were validated by the statistical tests called the ANOVA analysis. The significance of each term in the equation is to estimate the goodness of fit in each case. Response surfaces were drawn to determine the individual and interactive effects of the test variable on the percentage reducing sugar yield.

The optimal values of the test variables were first obtained in coded units and then converted to the uncoded units. Using four factor variable and six centre points will give the CCD design in Table 3.5 while factor levels of independent variables for biogas production is shown in Table 3.6 bellow.

Table 3.5 Central Composite Design of experiment in terms of coded values for biogas production

Run	Substrate	Water qty	Inoculums	Time
1	-	-	-	-
2	+	-	-	-
3	-	+	-	-
4	+	+	-	-
5	-	-	+	-
6	+	-	+	-
7	-	+	+	-
8	+	+	+	-
9	-	-	-	+
10	+	-	-	+
11	-	+	-	+
12	+	+	-	+
13	-	-	+	+
14	+	-	+	+
15	-	+	+	+
16	+	+	+	+
17	-α	0	0	0
18	+α	0	0	0
19	0	-α	0	0
20	0	+α	0	0
21	0	0	-α	0
22	0	0	+α	0
23	0	0	0	-α
24	0	0	0	+α
25	0	0	0	0
26	0	0	0	0
27	0	0	0	0
28	0	0	0	0
29	0	0	0	0
30	0	0	0	0

The high (+) and low (-) values for optimal percentage reducing sugar yield were substrate (25 and 75gr), water quantity (50 and 150gr), and inoculums (10 and 30g) and hydraulic retention time (10 and 35days).

Table 3.6: Factor levels of independent variables for biogas production

Independent Factors	Low level (-)	Med. level (0)	High level (+)
Substrate conc. (gr)	25	50	75
Water (ml)	50	100	150
Inoculums	10	15	20
Time (days)	10	20	40

Table 3.7: Central Composite Design for biogas production.

Std	COY+CP (gr)	Water (ml)	Inoculums (ml)	Time (days)
1	75	50	30	40
2	50	100	20	25
3	25	50	10	10
4	75	150	30	10
5	25	50	30	10
6	0	100	20	25
7	50	100	40	25
8	75	150	10	10
9	75	50	10	10
10	50	100	20	25
11	50	100	20	55
12	75	150	30	40
13	75	50	10	40
14	50	0	20	25
15	50	100	20	25
16	75	150	10	40
17	75	50	30	10
18	100	100	20	25
19	50	100	20	25
20	25	150	10	10
21	25	50	30	40
22	25	150	10	40
23	50	100	20	25
24	50	100	20	25
25	25	150	30	10
26	25	50	10	40
27	50	100	20	-5
28	50	200	20	25
29	25	150	30	40
30	50	100	30	40

3.16.3 Experimental procedure

The samples were taken according to the experimental run from the Central Composite Design and poured into the micro-digesters, stirred thoroughly to ensure homogenous mixture and subjected to anaerobic digestion. Samples were collected every 5 days for analysis of such parameters as Total suspended solids (TSS), Total viable count (TVC), Biological oxygen demand (BOD), Chemical oxygen demand (COD), pH values according to the approved American Public Health Association (APHA, 2005). The biogas produced was measured once a day by means of downwards displacement of water by the biogas in an inverted measuring cylinder. The study was conducted in duplicate sample at ambient temperature of between 26-32^{o}C for a period of 35 days hydraulic retention time (HRT). The sketch for the experimental setup is as shown in Fig. 3.1.

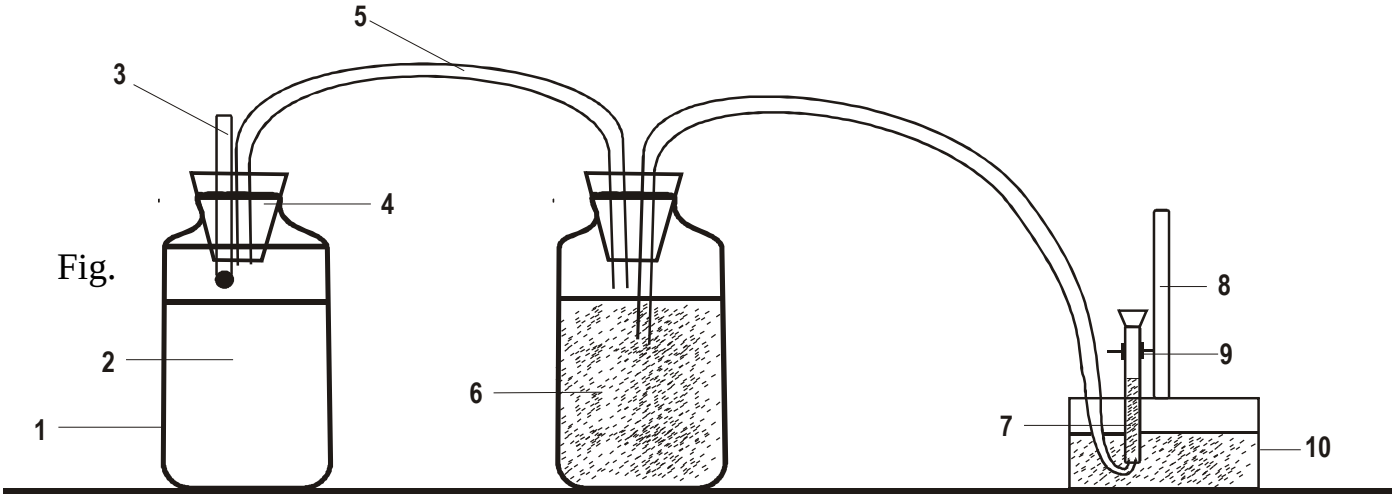

Fig. 3.1: Schematic Diagram of the Experimental Set up for Biogas Production.

Key:

1 - Micro-digester; 2 - Slurry; 3 - Thermometer; 4 - Cork; 5 - Hose; 6 - Plastic Bottle filled with water; 7 - Graduated measuring cylinder.; 8 - Retort Stand with clamp; 9 - Biogas produced; 10 - Trough with water

3.16.4 Characterization of biogas.

Biogas is characterized based on its chemical composition and physical characteristics which results from it. It is primarily a mixture of methane (CH_4) and inert carbonic gas (CO_2). However, the time biogas gathers a large variety of gases resulting from specific treatment processes starting from various organic wastes.

Gas Data GFM406 Biogas Analyser is the instrument used for measuring the four main gas present in bogas which are: Methane (CH_4), carbon dioxide (CO_2), Hydrogen Sulphide (H_2S), and water (H_2O).

3.16.4 Chemical Oxygen Demand

This was done according to the method outlined by AOAC (2010). 10cm^3 of the digestates was introduced into 100cm^3 round-bottomed flask. 2cm^3 potassium dichromate, 2.5cm^3 mercuric sulphate solution, 10-15 ml concentrated sulphuric acid containing silver sulphate and an anti-bumping rod were added to it. The solution was heated to gentle but steady boiling over an electric hot plate or heating mantle and under a reflux condenser. After exactly 45 minutes of boiling, it was allowed to cool briefly, washed through the condenser into the flask with 2cm^3 of distilled water and cooled completely in cold water. 2 drops of ferroin solution was added as indicator and the excess potassium dichromate with standardized ammonium iron (II) sulphate was titrated until the colour changed from bluish-green to reddish-brown.

Where 0.05 = normality of titrant

$$COD\left(mg/l\right)=\frac{ml\ of\ titrant\ for\ sample-ml\ of\ titrant for blank\times 0.05+8000}{Volume of\ Sample} \qquad (3.19)$$

3.16.5 Biochemical Oxygen Demand

The Biological Oxygen Demand (BOD) was determined using the procedure outlined by AOAC (2010). The general equation for the determination of a BOD_5 value is:

$$BOD_5(mg/l) = \frac{D_1 - D_2}{P} \quad (3.20)$$

Where D_1 = initial DO 15 minutes after preparation of the sample, D_2 = final DO of the sample after 5 days, and P = decimal volumetric fraction of sample used. If 100 ml of sample were diluted to 300 ml, then P = 0.33. If no dilution was necessary, P = 1.0 and the BOD_5 was determined by D_1-D_2.

3.16.6 pH of the slurry

This was carried out using the procedure indicated by AOAC (2010). Sufficient amount of the sample was poured into a small beaker and the tips of the electrodes rinsed with portions of the sample before being immersed to a depth of about 2cm with the electrodes being at least 1cm away from the sides and bottom of the beaker. The pH meter was turned on, temperature adjustment dial adjusted and the pH of sample recorded.

3.16.7 Total Suspended Solids

Total suspended solid was determined by subtracting the result of total dissolved solid from total solid. This was determined using the guideline indicated by AOAC (2010).

Total solids (TS) – Total dissolved solids (TDS) = Total suspended solids (TSS)

316.8. Total Viable Count.

Biological analysis was done on the sample using serial dilution method (APHA, 2005). The prepared media was carefully poured into petri dishes with a temperature of 47^{o}C. The petri dishes are rocked to and fro gently, allowed to cool while it was covered. Ten sterile test tubes are arranged in a test tube rack. A 9ml of normal saline/distilled water was pipette into each test tube forming 10^{-10} concentration. From the raw sample 1ml was pipette into the 1st tube and subsequently the last tube. A Pasteur pipette was used to transfer 0.25ml of the last three dilutions onto a sterile plate of prepared nutrient agar and eosins methlene

blue agar. The plate was agitated for an even spreading of the inoculums and incubated at 37^{o}C for 24hrs. The colonies that appeared at the end of incubation were counted and the unit expressed in terms of colony forming unit per millilitre (cfu/ml).

3.17. Kinetics of batch process anaerobic digestion

During the digestion period of the waste samples, the following parameters were tested and monitored for five (5) day interval from the beginning of the digestion to completion they include, chemical oxygen demand COD, total suspended solids TSS, total viable count TVC, Temperature and pH values .

As described by Nwabanne et al. (2009), limited substrate consumption is a first order reaction which can be expressed as:

$$\frac{-ds}{dt} = K^{'}S \qquad (3.21)$$

Where K' is the rate constant, this can be characterized as exponential growth and the substrate concentration profile with respect to hydraulic retention time (HRT) as follows:

$$S_e = S_o\, esp(-K_s t) \qquad (3.22)$$

Where S_0 = the influent substrate concentration (mg/l),

S_e = the effluent substrate concentration (mg/l),

t = is the hydraulic retention time (days).

Equation (3.22) shows the exponential growth of the organism as the substrate is utilized. Rearranging and taking natural logarithm of both sides of Equation 3.22 gives Equation 3.23 (Nwabanne et al., 2009):

$$\ln\left(\frac{S_e}{S_o}\right) = -Kt \qquad (3.23)$$

where K is the first order inactivation rate coefficient (l/day).

A plot of linear $-\ln(S_e/S_0)$ against t is obtained with the regression coefficient showing the first order of reaction.

Also a linear plot of $\frac{1}{U}$ against $\frac{1}{S_e}$ with the slope and intercept were obtained from the equation below;

$$\frac{1}{U}=\frac{K_s}{K}+\frac{1}{K} \qquad (3.24)$$

Where K_s = half-velocity constant/ saturation constant (mg/l)

K = maximum rate of substrate utilization (day^{-1})

$U = \frac{ds/dt}{x}$ = rate of substrate utilization (mg COD/L/day)

x = total suspended solid (biomass concentration) (mg/l)

The biomass yield (Y) and endogenous decay coefficient (K_d) were obtained from a straight line graph generated from Equation 3.24,

where $\frac{1}{\theta}$ was plotted against U

$$\frac{1}{\theta}=YU-K_d \qquad (3.25)$$

Where $\theta=\frac{x}{dX/dt}$ = the mean cell residence time (day)

SRT = sludge retention time, where:

U = the specific rate of substrate utilization

Y = the biomass yield/microbial growth yield (mg/mg)

K_d = endogenous decay coefficient (day^{-1})

The value of Y (biomass yield) obtained was used in Equation 3.25 to obtain the maximum specific growth rate of microorganism, μ_{max}.

$$K=\frac{\mu_{max}}{Y} \qquad (3.26)$$

Where K = maximum rate of substrate utilization (day^{-1})

μ_{max} = maximum specific growth rate of microorganisms (day^{-1})

According to Igoni et al. (2008), from the Monod equation,

$$\mu_{net} = \mu_{max} \frac{S}{K_s + s}[X] - K_d[X] \quad (3.27)$$

Dividing through by X ,

$$\mu_{net} = \mu_{max} \frac{S}{K_s + s} - K_d \quad (3.28)$$

μ_{net} = net specific growth rate of microorganisms (day^{-1})

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Starch yields of the feedstocks

The starch yields of wild cocoyam (*Xanthosoma sagittifolium*) and jackfruit seeds *(Artacapus heterophillus lam*) were 65.42% and 51.65% respectively. These were quite higher than the yields of 41.65% and 26.17% obtained from water yam (*Dioscorea alata)* and yellow yam (*Dioscorea dumentarum*) respectively. This indicates that the starches of cocoyam and jackfruit seeds would be very good for bioethanol production. But despite the fact that from these results, the starch yield of water yam and yellow yam were comparatively lower, they can still be considered as good supplementary feedstocks to the existing ones for bioethanol production. The starch yield of wild cocoyam (65.42%) obtained in this study can compare with 64.19% yield reported by Owuamamam et al. (2010), though these are quite lower than the yield of 80.44% obtained by Awokoya et al. (2012) and higher than the 21-32% range obtained by Tattiya et al. (2009). However, variations in composition of physicochemical properties are as the different in soil types, nutrient level of the soil and agro-climatic condition of the area where the

feedstocks were cultivated (Ofoefule, 2012). The starch yields of the feedstocks studied (wild cocoyam, jackfruit, water yam, and yellow yam) are shown in Table 4.1

Table 4.1: Starch yield of the feedstocks

Feedstock	Yield %
Wild cocoyam (*Xanthosoma sagittifolum*)	65.42
Water yam (*Dioscorea alata*)	41.65
Yellow yam (*Dioscorea dumentarum*)	26.17
Jackfruit seed (*Artcapus heterophillus lam*)	51.65

The proximate composition of these feedstocks shown in Table 4.2 below indicate that wild cocoyam has (moisture content (69.73%) ; ash content (2.21%) ; fibre (1.23%) ; fat (0.56%) which compared favourably with Awokoya *et al* . (2012) who reported the following in their work (moisture content (10.0%); ash (2.01%); fibre (1.75%); fat (0.81%). The fat content is low in cocoyam starch which is good for better fermentation for ethanol production of feedstocks.

Jackfruit starch with (moisture content (70.63%); ash (1.47%); fibre (0.85%); fat (0.39%) comparatively was in conformity with the result of the study conducted by Bobbio *et al.* (2011) who obtained the following values in their study (moisture content (61.50%); ash (0.22%); fibre 0.73%); fat (0.43%); protein (0.57%). This variation can be attributed to variations in the soil types, agro-climatic and other micro and macro factors affecting the cultivation of the feedstocks, as well as method applied in the analysis of the starch composition (Okigbo, 1984; Ofoefule, 2012). However, these physicochemical properties are at variance with those reported by Tulyathan et al. (2002) with moisture content (8.57%); ash (3.92)% ; fibre (1.67%); fat (0.71%); protein (11.17%). Both the

ash and crude fibre are higher than that obtained in this study; however their values for moisture content can comparatively compare with that obtained by other researchers.

The result of the proximate composition of water yam (*Dioscorea alata*) starch obtained in this study also compared with that reported by Addy *et al.* (2012) with ash (9.06%)**,** crude fibre (0.8%), fat (0.2%), crude protein (1.2%), carbohydrate (97.60%), although their percentage of carbohydrate is higher than that under study. Also, Polycarp *et al*. (2012) reported the following values in their study (ash contents (2.45%); fibre (0.99%); fat (0.74%); protein (8.74%); carbohydrate (78.5%). These values share similarity with results obtained in this study but with higher percentage of the ash content of 5.34%.

Table 4.2. Proximate composition of the starch feedstock

Parameters (%)	**Cocoyam**	**Jackfruit**	**Water Yam**	**Yellow Yam**
Moisture	69.73	60.15	72.51	68.35
Ash	2.21	1.78	5.34	4.13
Crude fibre	0.83	1.23	2.36	2.17
Crude fat	0.61	0.56	0.54	0.43
Crude protein	2.33	2.17	6.18	4.16
Carbohydrates	83.14	76.15	65.61	75.32
Glucose	20.66	29.12	28.19	25.00

4.2 The phytochemical in the starch feedstocks

Phytochemicals occur naturally in plants. Table 4.3 shows the phytochemical in the starch of the feedstocks. They refer to those chemicals that have biological significance, but not established as essential nutrients (Liu, 2004). According to Wikipedia (2012), some are responsible for color others organoleptic properties,

such as the deep purple of blueberries and smell of garlic. They are non-nutritive plant chemicals that have protective or disease preventive properties. It has been observed that phytochemicals in freshly harvested plant foods may be destroyed or removed by modern processing techniques, including cooking. They further stated that some phytochemicals can reduce the gel hardness of starch, while some can facilitate the gelatinization of starch. Papp et al. (2007) also posited that absence or deficiency of phytochemicals in processed food may contribute to increased risk of preventable diseases. There are various types of phytochemicals each performing different function from the other.

Table 4.3: Qualitative determination of phytochemicals in the starch feedstocks

Parameters	**Cocoyam**	**Jackfruit**	**Water Yam**	**Yellow Yam**
Reducing Sugar	+	+	+	+
Saponins	+	+	+	+
Steroid	-	-	-	-
Tannins	+	+	+	+
Glycoside	-	-	-	-
Cynogenic	+	+	+	+
Flavonoids	+	+	+	+
Carbohydrate	+++	+ ++	+++	+++

4.3 Effect of slurry concentration of starch on gelatinization process of the feedstocks.

Gelatinization profile of the four feedstocks starches (cocoyam, jackfruit, water yam, and yellow yam) were investigated under four variants of water content. The consistency of the starch slurry determined the water volume that was used for the starting solution. The aim of this was to obtain a smooth paste on gelatinization instead of a lumpy paste. Thus, for cocoyam and jackfruit seed starches, the water content used were 2.0 – 4.0mg/l, while 1.0 – 5.0mg/l were used for water yam and yellow yam. It was obtained from the study that gelatinization temperature and reaction time generally increased with water content for all the starch feedstocks.

Gelatinization time ranged between 20-27 minutes with 2.0ml/g having the least gelatinization time in cocoyam. The starch gelatinized when the temperature approached 65^0C, however, not all the starch granules gelatinized at that temperature, but within the range of 22^0C difference.Results obtained from yellow yam (*Dioscorea dumentarum*) followed the same trend with the 1.0ml/g water content having the least gelatinization time within a short temperature range of 10^0C difference. For water yam (*Dioscorea alata*), the 1.0ml/g water content had the least gelatinization time within a short temperature difference of 12^0C. Jackfruit seed (*Artocarpus heterophyllus lam*) showed the least gelatinization time of 18 minutes with the temperature difference of 11^0C. Thus, the trend of the least gelatinization time of all the feedstocks followed this pattern yellow yam (10^0C) < jackfruit (11^0C) < water yam (12^0C) < cocoyam (22^0C).

General results indicate that gelatinization process to a larger extent is dependent on the content of slurry and temperature. The importance of completely dissolving the starches to ensure a smooth paste on gelatinization and cooling should be observed. If the starch is not completely dissolved, lumpy paste may result at the end of the process. Also, the texture of the gel formed from the various starch

variants was observed to be affected by the volume of water used. The thickness of the gel was observed to decrease as the water content increased. The rate of cooking of the respective gelatinized starch followed the same trend.

Retrogradation which is the process by which gelatinized starch gradually begins to re-organize its components to return to its former molecular structure while being cooled, differed and decreased in the order: jackfruit > water yam > yellow yam > cocoyam.This may be explained by the magnitude of the amylase/amylopectin ratio of the starch feedstock (*http://www.montignac.com/en/ig-fac-modif/php.gelatinization*) reported that the higher the amylase contents of the starch the greater the effectiveness of the retrogradation process, and also the degree of gelatinization is proportional to the amount of amylose in the starch. In this study, the results of the amylase/amylopectin contents of the different starches show that cocoyam had the least amylase content. This was followed by yellow yam, water yam, and jackfruit which had the highest amylase content. Thus, this offers explanation why the retrogradation of jackfruit starch was the highest and the degree of gelatinization lowest

4.4 Modelling of gelatinization time against water content

Gelatinization time was modeled against water content for all the feedstocks using Microsoft Excel Version 2013 for regression analysis. The relationship is graphically presented in Figures 4.1- 4.4. Meaningful correlation occur between R^2=+1 and R^2=-1 which is the limit giving perfect correlation (Njoku *et al*., 2006; Little and Hills, 1972). This represents the proportion of variance and that shows the level to which other variants can be predicted by the model.

The Jackfruit seed variant gave the best R^2 values (96.6%) as shown in Fig. 4.2 indicating that other water contents not utilized in the study can be predicted as well. This was closely followed by cocoyam with R^2 value of 96.5%, water yam

with R^2=94% and Yellow yam having R^2 value of 91.8%, regardless the fact that the incremental level of water content for water yam was 1ml/g while that of cocoyam and jackfruit were 0.5ml/g. Yellow yam gave the least R^2 value amongst other feedstock studied.

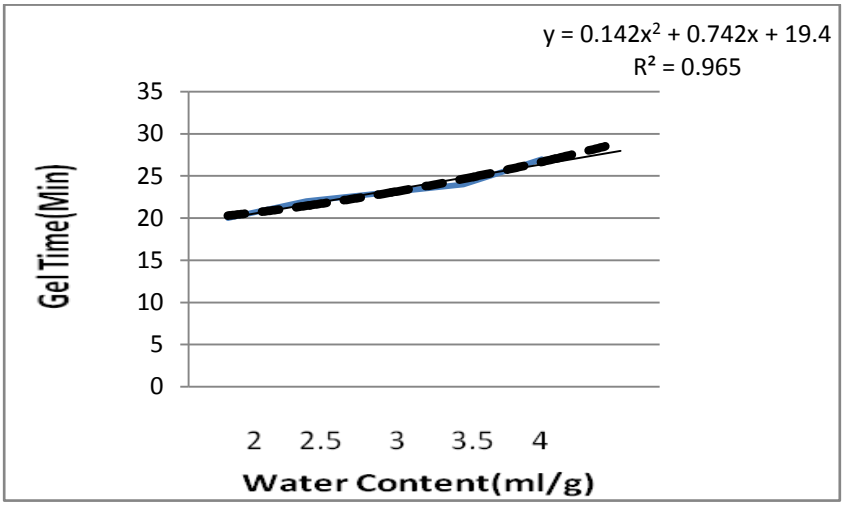

Fig. 4.1: Plot of variation in gelatinization time with water content in wild cocoyam

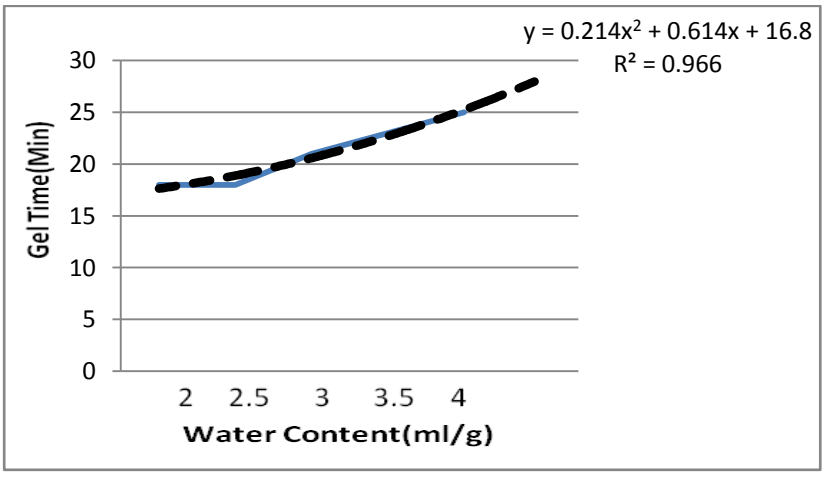

Fig. 4.2: Plot of variation in gelatinization time with water content in jackfruit seed starch

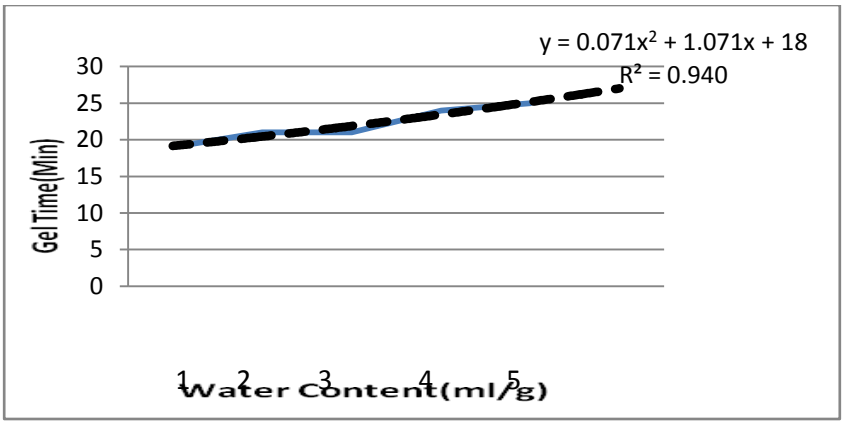

Fig. 4.3: Plot of variation in gelatinization time with water content in water yam

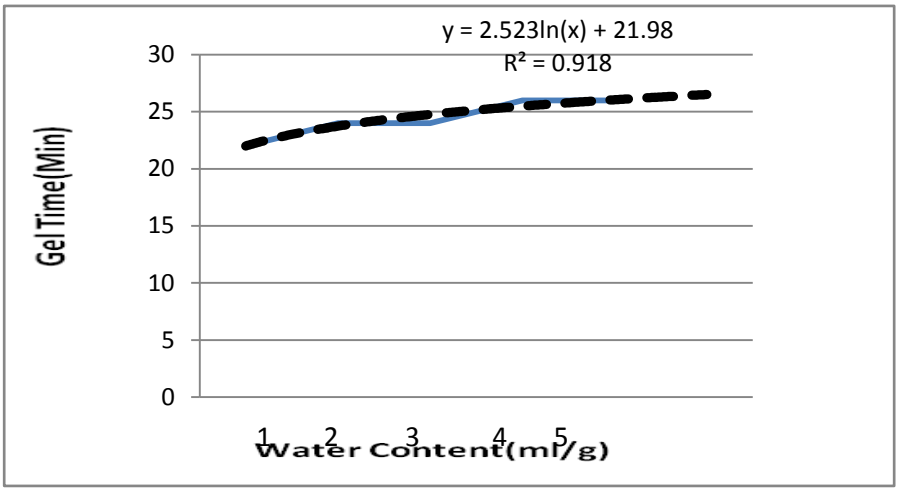

Fig. 4.4: Plot of variation in gelatinization time with water content in yellow yam

4.5 Optimization of percentage reducing sugar yield using Response Surface Methodology.

4.5.1 Optimization of percentage reducing sugar yield for water yam starch.

The optimization process of the reducing sugar yield (RSY) was done using the Central Composite Design (CCD). Four important factors which are temperature, time, enzyme concentration and water quantity were used as the independent variables where their combined effects were examined while the yield of the reducing sugar was the dependent variable or the response. This was done to determine the best conditions for optimum yield of the reducing sugar. Using the

CCD involves varying the independent variables at five different levels (-1, 0, +1). In this work, a set of 30 experiments were performed consisting of 16 core points, 8 star like points and 6 centre points or null points. The distance of the star like point α used was 1.5. The experiments were performed in random to avoid systematic error.

4.5.2 Statistical analysis of the optimization process using water yam

The reducing sugar yield results of the optimization process of water yam were given in Tables 4.4 to 4.5. It shows the combined effects of time (A), enzyme concentration (B), water quantity (C) and temperature (D). The highest percentage of yield reducing sugar was 122.23mg/mg occurring at a temperature of 70^{o}C, time of 2hrs, enzyme concentration of 0.10g/g and water quantity of 2ml/g. Design Expert 8.0.7.1 trial version was used to analyze the results.

Table 4.4 Optimization results for percentage reducing sugar yield using water yam starch.

Std	Factor 1 A: time hrs	Factor 2 B: enzyme conc. (g/g)	Factor 3 C: Water qty (ml)	Factor 4 C: Temperature. ^{0}C	Response D: Reducing sugar Mg/mg
1	2.50	0.20	2.50	55.00	90.15
2	2.50	0.20	3.25	55.00	105.24
3	2.50	0.20	1.75	55.00	91.64
4	2.00	0.30	2.00	40.00	105.14

5	2.00	0.10	4.00	40.00	78.34
6	3.00	0.10	4.00	40.00	90.36
7	2.00	0.30	4.00	40.00	98.76
8	3.00	0.30	4.00	40.00	110.23
9	2.00	0.10	2.00	70.00	122.23
10	3.00	0.10	2.00	70.00	120.14
11	2.00	0.30	2.00	70.00	86.23
12	3.00	0.30	2.00	70.00	122.56
13	2.00	0.10	4.00	70.00	93.21
14	3.00	0.10	4.00	70.00	89.17
15	2.00	0.30	4.00	70.00	73.45
16	3.00	0.30	4.00	70.00	105.77
17	1.75	0.20	3.00	55.00	101.34
18	3.25	0.20	3.00	55.00	99.45
19	2.50	0.05	3.00	55.00	97.11
20	2.50	0.35	3.00	55.00	86.22
21	2.50	0.20	1.50	55.00	93.11
22	2.50	0.20	4.50	55.00	103.11
23	2.50	0.20	3.00	32.50	74.89.
24	2.50	0.20	3.00	77.50	101.12
25	2.50	0.20	3.00	55.00	63.13
26	2.50	0.20	3.00	55.00	75.21
27	2.50	0.20	3.00	55.00	63.13
28	2.50	0.20	3.00	55.00	63.13
29	2.50	0.20	3.00	55.00	63.13
30	2.50	0.20	3.00	55.00	63.13

Table 4.5. Summary of P-values for percentage reducing sugar yield.

Source	Sequential p-value	Lack of Fit p-value	Adjusted R-square	Predicted R-Squared	Predicted R-Squared
Linear	0.2631	0.0034	0.0522	-0.1219	
2FI	0.2293	0.0039	0.1549	-0.1646	
Quadratic	0.002.	0.0241	0.6323	0.1080	Suggested

Cubic	0.7808	0.0043	0.5207	-10.6982	Aliased

Table 4.6. Lack of Fit Test for percentage reducing sugar yield.

Source	Sum of Squares	df	Mean Square	F Value		p-value prob > F
Mean vs Total	2.354E	1	2.354E + 005			
Linear vs Mean	1693.47	4	423.37	1.40		0.2631
2FI vs linear	2438.83	6	406.47	1.51		0.2293
Quadratic vs 2F13365.53	4	841.38	7.17	0.0020		
Cubic vs Quadratic	689.51	8	86.19	0.56	Suggested	
Residual 1071.25	7	153.04			0.7808	Alia
Total2. 446E + 005	30	8154.74				

Table 4.7. Model Summary Statistics for percentage reducing sugar yield.

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	17.40	0.1829	0.0522	-0.1219	10386.85	
2F1	16.43	0.4463	0.1549	-0.1646	10782	
Quadratic	10.83	0.8098	0.6323	0.1080	82	Suggested
Cubic	12.37	0.8843	0.5207	-10.6982	8259 051.083E + 005	Aliased

4.5.3 ANOVA analysis for percentage reducing sugar yield for water yam

Design Expert 8.0.7.1 trial version was used to analyze the results. The results were shown below. The summary of P-values indicates that a quadratic model

fitted the ANOVA analysis and hence it was suggested. The linear and 2FI models were not suggested. The Cubic model is always aliased because the CCD does not contain enough runs to support a full cubic model. A significance level of 95% was used hence all terms whose P-value are less than 0.05 are considered significant. The lack of fit test and model summary for percentage reducing sugar yield were also presented in Tables 4.6 and 4.7 respectively while Table 4.8 shows the analysis of variance (ANOVA) for water yam.

Table 4.8. Analysis of variance (ANOVA) for water yam reducing sugar yield

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F
Mode	5282.43	14	377.32	2.12	0.0807
A – time	2.23	1	2.23	0.012	0.9125
B – enzym conc.	127.13	1	127.13	0.71	0.4114
C – water qty.	534.53	1	534.53	3.00	0.1037
D-temp	1010.88	1	1010.88	5.68	0.3009
AB	465.91	1	465.91	2.62	0.1266
AC	778.41	1	778.41	4.37	0.0540
AD	4.12	1	4.12	0.023	0.8811
BC	26.37	1	26.37	0.22	0.6465
CD	34.69	1	34.69	0.15	0.7058
A^2	42.22	1	42.22	0.19	0.6652
B^2	557.46	1	557.46	0.24	0.6334
C^2	601.48	1	601.48	3.13	0.0972
D^2	310.52	1	310.52	3.38	0.0860
Residual	2671.23	15	178.08	-	-
Lack of fit	2267.89	10	226.79	2.81-	0.1327
Pure error	403.34	5	80.67	-	
Cor Total	7953.66	29	-	-	

Std. Dev.=13.34; R-Squared= 0.9098; Mean=103.54;Adj. R-Squared= 0.9178

C.V. % = 12.89; Pred R-Squared = -0.8453; PREES 14079.30; Adeq Precision= 5.828

The adjusted R^2 =0.9178 was in close agreement with the predicted R^2 =0.8453. The adequate precision measures the signal to noise ratio and compares the range of the predicted value at the design points to the average prediction error. The adquate predicion ratio above 4 indicates adequate model efficacy (Kumar *et al.*, 2007). Hence, the adquate precision ratios of 5.828 indicates adquate model efficacy. Also, a PRESS value of 14079.30 indicates an adquate signal implying that the models can be used to navigate the design space.The coefficient of regression R^2 was used to validate the fitness of the model equation. For percentage reducing sugar yield for water yam, the R^2 has a high value of 0.9098 showing that 91% of the variability in the response can be explained by the model. This implies that the prediction of experimental data is quite satisfactory. The quadratic model equations obtained for the reducing sugar percentage for water yam is:

$$Y_{\text{Reducing sugar}} = +88.91 + 0.33\,A - 2.49B + 5.11C + 7.02D - 5.40AB + 6.98AC + 0.51AD + 1.56BC + 1.28BD - 1.47\,CD + 1.93A^2 + 7.00B^2 + 7.27C^2 + 5.22D^2 \quad (4.1)$$

In a regression equation, when an independent variable has a positive sign, it means that an increase in the variable will cause an increase in the response while a negative sign will result in a decrease in the response (Kumur *et al.*, 2008). Hence, an increase in temperature, time, enzyme concentration and water quantity will cause an increase in the percentage reducing sugar yield. Time and temperature will have more significant effect in the increment of the response since their coefficients were higher.

A combination of the actual experimental response and the predicted response from the mathematical equations are given in Tables 4.9 below where it is seen that there is a close correlation between the actual experimental response and the predicted response. This comfirms the effectiveness of the percentage reducing sugar.

Final model equation:

$$Y_{Reducing\ sugar} = +88.91 - 2.49B + 5.11C + 7.02D - 5.40AB + 6.98AC + 1.56BC + 1.28BD - 1.47\ CD + 1.93A^2 + 7.00B^2 + 7.27C^2 + 5.22D^2 \quad (4.2)$$

Table 4.9: Actual and predicted values of the percentage reducing sugar for water yam starch.

Std	Factor 1 A: time hrs	Factor 2 B: enzyme conc. g/g	Factor 3 C:water qty. ml/g	Factor 4 D: temperature ^0C	Experimental value	Predicted value
1	2.50	0.20	2.50	55.00	90.15	103.82
2	2.50	0.20	3.25	55.00	105.24	100.31
3	2.50	0.20	1.75	55.00	91.64	103.94
4	2.00	0.30	2.00	40.00	105.14	78.84
5	2.00	0.10	4.00	40.00	78.34	99.90
6	3.00	0.10	4.00	40.00	90.36	124.29
7	2.00	0.30	4.00	40.00	98.76	106.27
8	3.00	0.30	4.00	40.00	110.23	109.07
9	2.00	0.10	2.00	70.00	122.23	117.23
10	3.00	0.10	2.00	70.00	120.14	115.74
11	2.00	0.30	2.00	70.00	86.23	122.48
12	3.00	0.30	2.00	70.00	122.56	99.41
13	2.00	0.10	4.00	70.00	93.21	107.42
14	3.00	0.10	4.00	70.00	89.17	133.84
15	2.00	0.30	4.00	70.00	73.45	118.92
16	3.00	0.30	4.00	70.00	105.77	123.75
17	1.75	0.20	3.00	55.00	101.34	92.75
18	3.25	0.20	3.00	55.00	99.45	93.74
19	2.50	0.05	3.00	55.00	97.11	108.39
20	2.50	0.35	3.00	55.00	86.22	100.92
21	2.50	0.20	1.50	55.00	93.11	97.61
22	2.50	0.20	4.50	55.00	103.11	112.93
23	2.50	0.20	3.00	32.50	74.89.	90.13
24	2.50	0.20	3.00	77.50	101.12	111.19
25	2.50	0.20	3.00	55.00	63.13	88.91
26	2.50	0.20	3.00	55.00	75.21	88.91
27	2.50	0.20	3.00	55.00	63.13	88.91
28	2.50	0.20	3.00	55.00	63.13	88.91
29	2.50	0.20	3.00	55.00	63.13	88.91
30	2.50	0.20	3.00	55.00	63.13	88.91

The Normal plot of Residuals (Figures 4.5 and 4.6) and the Predicted vs Actual plots (Figures 4.7 and 4.8) were used to check whether the points will follow a straight line

in which conclusion will be drawn that the residuals follow a normal distribution. Hence, since from the figures, it is observed that the points were closely distributed to the striaght line of the plot, this confirms the good relationship between the experimental values and the predicted values of the response though some small scatter like an "S" shape is always expected. These plots equally confirm that the selected model was adequate in predicting the response of the experimental values.

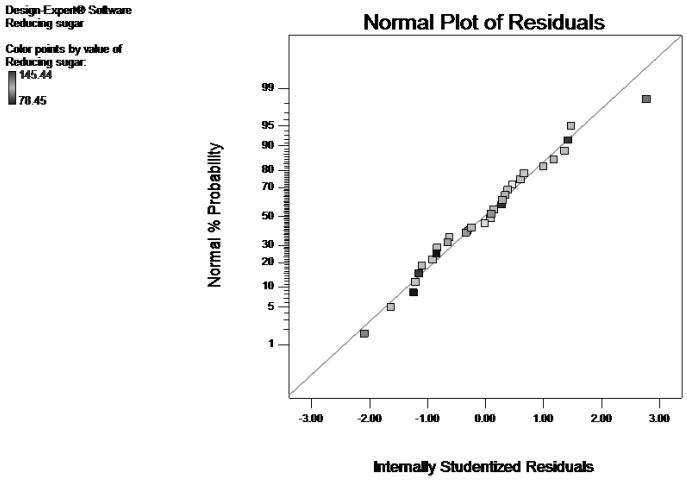

Fig. 4.5: Normal plot of residuals for reducing sugar yield for water yam

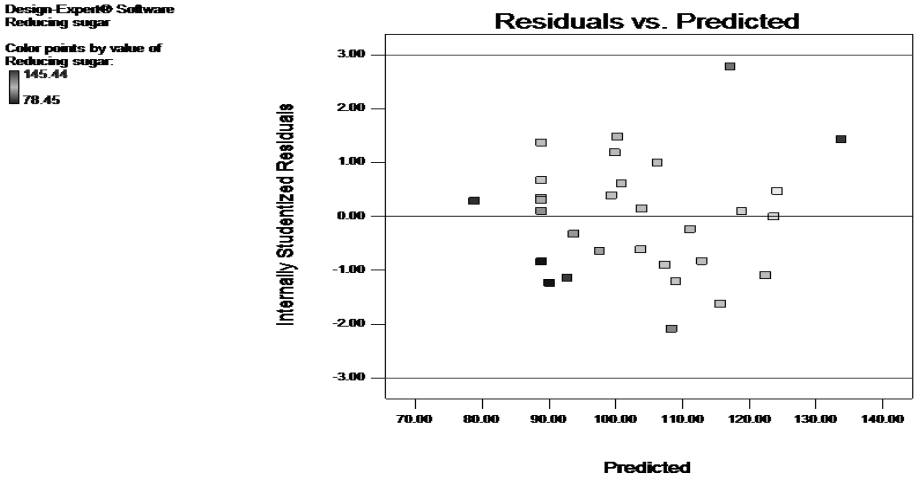

Fig. 4.6: Plot of Predicted vs Actual for reducing sugar yield for water yam

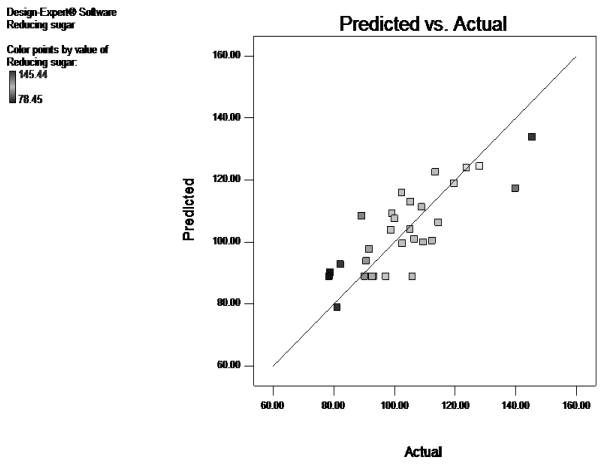

Fig.4.7: Plot of Predicted values vs actaul

4.5.4 The Three Dimensional (3-D) response surface plots for percentage reducing sugar for water yam

The 3-D response surface plots are graphical representation of the interactive effects of any two or more variable factors. Response surface plots as a function of two factors at a time, maintaining all other factors at fixed levels are more helpful in understanding both the main and the interaction effects of these two factors. These plots can be easily obtained by calculating from the model, the values taken by one factor where the second varies with constraint of a given Y value. The response surface curves were plotted to understand the interaction of the variables and to determine the optimum level of each variable for maximum response.

The nature of the response surface curves shows the interaction between the variables. The elliptical shape of the curve indicates good interaction of the two variables and circular shape indicates no interaction between the variables. From the figures, it was observed that the elliptical natures of the contour in graphs are in the mutual depicted interactions of all the variables. There was a relative significant interaction between every two variables.

4.6 Optimization of reducing sugar yield for Jackfruit seed starch

The optimization process of the reducing sugar yield (RSY) was done using the Central Composite Design (CCD). Four important factors which are temperature, time, enzyme concentration and water quantity were used as the independent variables where their combined effects were examined while the yield of the reducing sugar was the dependent variable or the response. This was done to determine the best conditions for optimum yield of the reducing sugar. Using the CCD involves varying the independent variables at five different levels (-1, 0, +1). In this work, a set of 30 experiments were performed consisting of 16 core points, 8 star like points and 6 centre points or null points. The distance of the star like point α used was 1.5. The experiments were performed in random to avoid systematic error.

4.6.1 Statistical analysis of the optimization process for percentage reducing sugar yield using jackfruit seed starch.

The reducing sugar yield results of the optimization process using jackfruit seed starch were given in Tables 4.10 to 4.14. It shows the combined effects of time (A), enzyme concentration (B), water quantity (C) and temperature (D). The highest percentage of yield reducing sugar jackfruit seed starch was 142.58mg/mg occurring at a temperature of 70^{o}C, time of 2hrs, enzyme concentration of 0.10g/g and water quantity of 2ml/g. Design Expert 8.0.7.1 trial version was used to analyze the results.

Table 4.10: Optimization results for reducing sugar yield using jackfruit seed starch

Std	A-Time(hrs)	B-Enzy.conc. (g/g)	C-Water qty. (ml/g)	D-Temp, (^{o}C)	Experimental value
1	2.50	0.20	3.25	55	114.56
2	2.50	0.20	2.50	55	117.71
3	2.00	0.10	3.00	40	102.13
4	2.50	0.35	2.50	55	78.33
5	3.00	0.30	3.00	40	121.47
6	2.00	0.10	2.00	40	131.57
7	2.50	0.20	2.50	32.50	107.71
8	3.00	0.10	2.00	40	132.76
9	3.00	0.30	3.00	70	142.58
10	2.50	0.20	2.50	55	99.10
11	3.00	0.30	2.00	70	116.17
12	2.50	0.20	2.50	55	105.15
13	3.25	0.20	2.50	55	112.56
14	2.00	0,10	3.00	70	117.41
15	2.50	0.20	2.50	55	125.74
16	3.00	0.10	3.00	70	128.58
17	3.00	0.10	3.00	40	80.91
18	2.50	0.20	1.75	55	87.66
19	2.00	0.30	2.00	70	99.13
20	2.00	0.30	3.00	70	107.43
21	1.75	0,20	2.50	55	89.47
22	2,50	0.20	2.50	55	103.51
23	2.50	0.05	2.50	55	76.61
24	2.00	0.30	3.00	40	106.22
25	3.00	0.10	2.00	70	96.35
26	2.50	0.20	2.50	55	89.15
27	2.50	0.20	2.50	55	87.45

28	2.00	0.10	2.00	70	89.23
29	2.00	0.30	2.00	40	95.41
30	3.00	030	2.00	40	100.21

Table 4.11: Summary of P-values for percentage reducing sugar for jackfruit seed starch.

Source	Sequential Source	Adjusted R-Squared	Predicted R-Squared	R-Squared	Remarks
Linear	0.3945	0.0043	0.0089	-0.2924	
2FI	0.3131	0.0044	0.0711	-1.1615	
Quadratic	0.0026	0.9130	0.8904	0.	Suggested
Cubic	0.9137	0.0031	0.3615	-18.8516	Aliased

Table 4.12: Lack of Fit Test for percentage reducing sugar for jackfruit seed starch.

Source	Sum of Square	Df	Mean Square	F-Value	p-value Prob>F	Remarks
Linear	7100.89	20	355.04	13.78	0.0043	
2FI	5020.76	14	358.63	13.92	0.0044	
Quadratic	1707.63	10	170.76	6.63	0.0249	Suggested
Cubic	1175.31	7	587.66	22.80	0.0031	Aliased
Pure error	128.85	5	25.77			

Table 4.13:Model Summary Statistics for percentage reducing sugar for jackfruit seed starch.

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS

Linear	17.01	0.1456	0.0089	-0.2924	10936.07	
2FI	16.46	0.3914	0.0711	-1.1615	18289.71	
Quadratic	11.06	0.9230	0.9150	0.83670	367.51	Suggested
Cubic	13.65	0.8459	0.3615	-18.8516	1.680E+005	Aliased

Table 4.14: Analysis of variance (ANOVA) for percentage reducing sugar for jackfruit seed starch

Source	**Sum of Squares**	**df**	Mean value	**Fvalue Value**	**p-value** Prob>F
Model	6625.2414	473.23	3.87	0.0068	*A-time*
24.01 *1*	*24.01*	*0.20*	*0.6642*		
B-enzyme conc.	*112.11*	*1*	*112.11*	*0.92*	*0.3538*
C-water qty.	*739.56*	*1*	*739.56*	*6.04*	*0.0266*
D-temperature	*356.31*	*1*	*356.31*	*2.91*	*0.1086*
AB	*21.28*	*1*	*21.28*	*0.17*	*0.6827*
AC	*870.10*	*1*	*870.10*	*7.11*	*0.0176*
AD	*234.93*	*1*	*234.93*	*1.92*	*0.1862*
BC	*440.48*	*1*	*440.48*	*3.60*	*0.0773*
BD	*292.15*	*1*	*292.15*	*2.39*	*0.1432*
CD	*221.19*	*1*	*221.19*	*1.81*	*0.1989*
A^2	*50.19*	*1*	*50.19*	*0.41*	*0.5317*
B^2	*1264.56*	*1*	*1264.56*	*10.33*	*0.0058*
C^2	*644.22*	*1*	*644.22*	*5.26*	*0.0367*
D^2	*315.90*	*1*	*315.90*	*2.58*	*0.1291*
Residual	1836.47	15	122.43		
Lack of Fit	*1707.63*	*10*	*170.76*	*6.63*	*0.0249*

significant			
Pure Error	*128.85*	*5*	*25.77*
Cor Total	8461.72	29	

Std. Dev.= 11.06; R- Squared = 0.9230 Mean=105.41; Adj R-Squared= 0.9150
C.V. % = 10.50; Pred R-Squared= 0.8904; PRESS= 9028.51; Adeq Precision= 6.117

In Table 4.10, the adjusted R^2 =0.9150 which was in close agreement with the predicted R^2 =0.8904. The adequate precision measures the signal to noise ratio and compares the range of the predicted value at the design points to the average prediction error. The adquate predicion ratio above 4 indicates adequate model efficacy (Kumar *et al.*, 2007). Also,the adquate precision ratios of 6.117 indicates adquate model efficacy. Also, a PRESS value of 9028.51 indicates an adquate signal implying that the models can be used to navigate the design space.

The coefficient of regression indicates the degree to which the fitness of the model equation is validated. In this work, R2=0.9230 which shows about 92% variability of the model equation. For percentage reducing sugar yield for jackfruit seed starch, the R^2 has a high value of 0.9230 showing that 92% of the variability in the response can be explained by the model. This implies that the prediction of experimental data is quite satisfactory. The quadratic model equations obtained for the reducing sugar yield for jackfruit seed is presented in Equation 4.3 below:

$$Y_{\text{Reducing sugar}} = +88.03 -1.08A -2.34 B +6.01 C +4.17D +1.15A B +7.37A C -3.83 A D +5.25 BC+4.27BD-3.72CD+2.10 A^2 +10.54B^2 +7.52C^2 +5.27D^2 \quad (4.3)$$

In a regression equation, when an independent variable has a positive sign, it means that an increase in the variable will cause an increase in the response while a negative sign will result in a decrease in the response (Kumur *et al.*, 2008). Hence, an increase in temperature, time, enzyme concentration and water quantity will cause an increase in the percentage reducing sugar yield. Time and temperature

will have more significant effect in the increment of the response since their coefficients were higher.

Table 4.15: Experimented and Predicted values of reducing sugar yield for jackfruit seed starch

Std	A-Time(hrs)	B-Enzyme conc. (g/g)	C-Water qty. (ml/g)	D-Temp, (^{o}C)	Experimental value	Predicted value
1	2.50	0.20	3.25	55	114.56	117.21
2	2.50	0.20	2.50	55	117.71	105.21
3	2.00	0.10	3.00	40	102.13	91.18
4	2.50	0.35	2.50	55	78.33	84.24
5	3.00	0.30	3.00	40	121.47	111.41
6	2.00	0.10	2.00	40	131.57	129.36
7	2.50	0.20	2.50	32.50	107.71	106.38
8	3.00	0.10	2.00	40	132.76	128.83
9	3.00	0.30	3.00	70	142.58	132.10
10	2.50	0.20	2.50	55	99.10	105.22
11	3.00	0.30	2.00	70	116.17	123.17
12	2.50	0.20	2.50	55	105.15	100.90
13	3.25	0.20	2.50	55	112.56	111.43
14	2.00	0,10	3.00	70	117.41	114.05
15	2.50	0.20	2.50	55	125.74	123.49
16	3.00	0.10	3.00	70	128.58	130.72
17	3.00	0.10	3.00	40	80.91	94.32

18	2.50	0.20	1.75	55	87.66	91.13
19	2.00	0.30	2.00	70	99.13	115.26
20	2.00	0.30	3.00	70	107.43	108.24
21	1.75	0,20	2.50	55	89.47	95.95
22	2,50	0.20	2.50	55	103.51	113.97
23	2.50	0.05	2.50	55	76.61	93.63
24	2.00	0.30	3.00	40	106.22	106.14
25	3.00	0.10	2.00	70	96.35	88.03
26	2.50	0.20	2.50	55	89.15	88.03
27	2.50	0.20	2.50	55	87.45	88.03
28	2.00	0.10	2.00	70	89.23	88.03
29	2.00	0.30	2.00	40	95.41	88.03
30	3.00	030	2.00	40	100.21	88.03

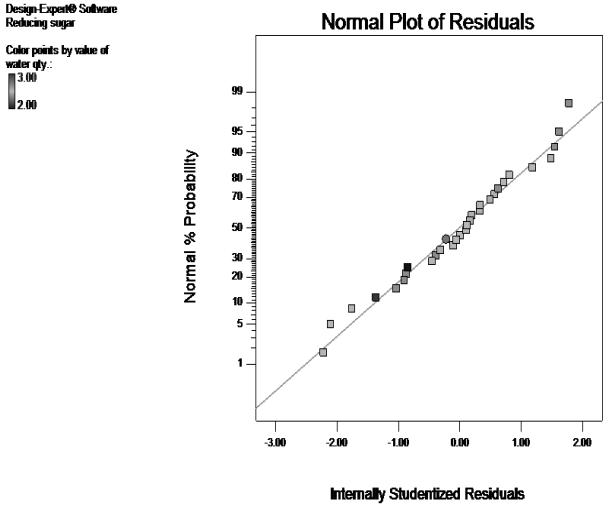

Fig. 4.8: Normal plot of Residual for reducing sugar yield for jackfruit seed

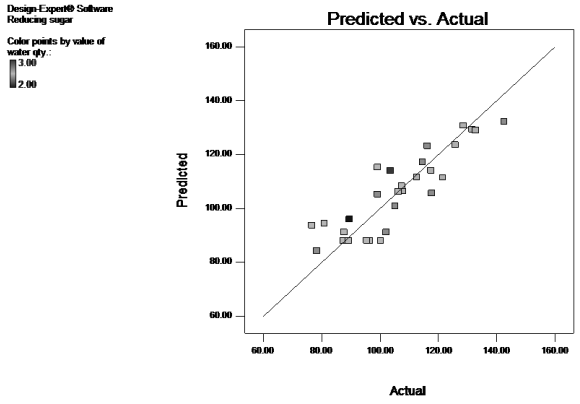

Fig. 4.9: Plot of Predicted vs Actual for reducing sugar yield for jackfruit seed

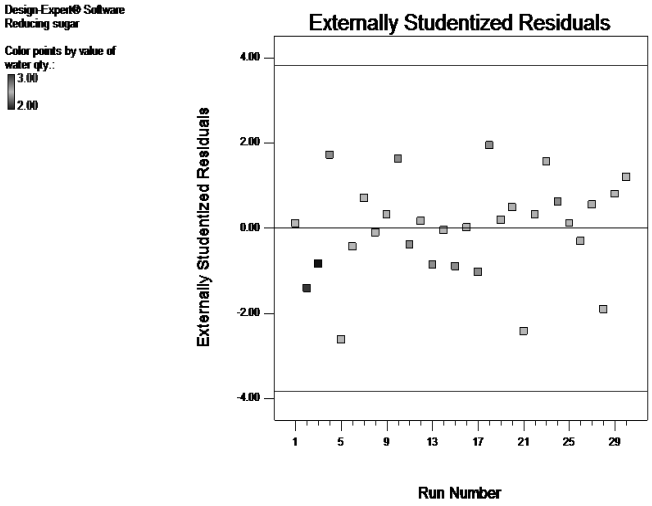

Fig. 4.10: Normal plot of Residual for reducing sugar yield for jackfruit seed

4.7 3D Surface plots for the optimization of reducing sugar yield for jackfruit seed starch

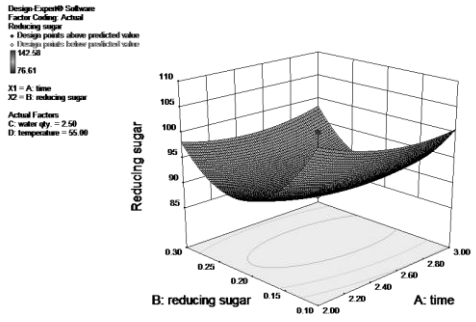

Fig. 4.11: 3D Surface plot for reducing sugar yield showing combine effects of reducing sugar and time on jackfruit seed starch

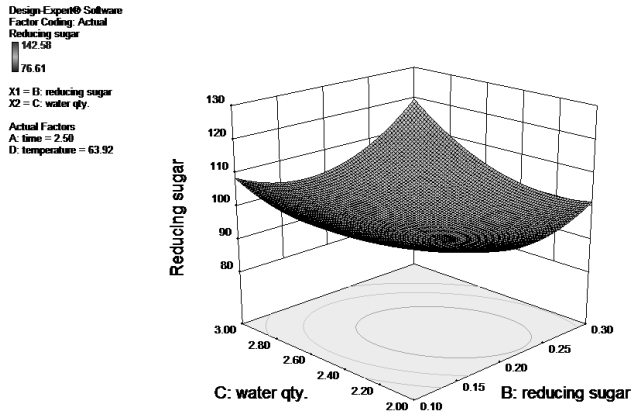

Fig. 4.12: 3D Surface plot for reducing sugar yield showing combine effects of reducing sugar and water quantity on jackfruit seed starch

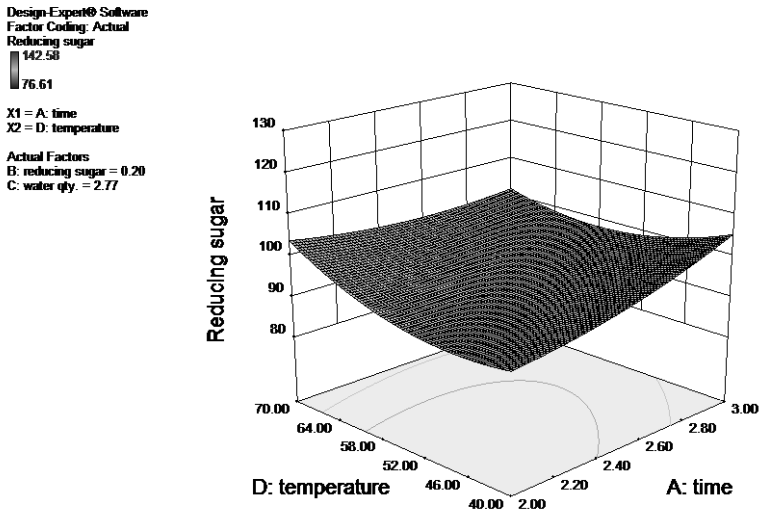

Fig. 4.13: 3D Surface plot for reducing sugar yield showing combine effects of temperature and time on jackfruit seed starch

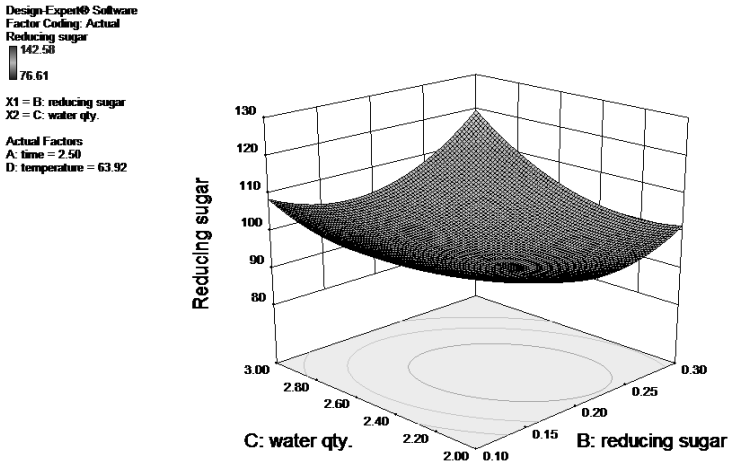

Fig. 4.14: 3D Surface plot for reducing sugar yield showing combine effects of water quantity and reducing sugar on jackfruit seed starch

4.8 Optimization of percentage reducing sugar yield for wild cocoyam starch.

The optimization process of the reducing sugar yield (RSY) for wild cocoyam starch was done using the Central Composite Design (CCD). Four important

factors which are temperature, time, enzyme concentration and water quantity were used as the independent variables where their combined effects were examined while the yield of the reducing sugar was the dependent variable or the response. This was done to determine the best conditions for optimum yield of the reducing sugar using the CCD involves varying the independent variables at five different levels (-1, 0, +1). In this work, a set of 30 experiments were performed consisting of 16 core points, 8 star like points and 6 centre points or null points. The distance of the star like point α used was 1.5. The experiments were performed in random to avoid systematic error.

4.8.1 Statistical analysis of the optimization process using wild cocoyam starch

The reducing sugar yield results of the optimization process using wild cocoyam were given in Tables 4.16 to 4.21. It shows the combined effects of time (A), enzyme concentration (B), water quantity (C) and temperature (D). The highest percentage of yield reducing sugar was 166.15mg/mg occurring at a temperature of 70^{o}C, time of 3hrs, enzyme concentration of 0.30g/g and water quantity of 3ml/g. Design Expert 8.0.7.1 trial version was used to analyze the results.

Table 4.16: Optimization results for percentage reducing sugar yield for wild cocoyam starch

Std	A-Time(hrs)	B-Enzyme conc. (g/g)	C-Water qty. (ml/g)	D-Temp, (oC)	Actual value
1	2.50	0.20	3.25	55	129.56
2	2.50	0.20	2.50	55	12743
3	2.00	0.10	3.00	40	98.12
4	2.50	0.35	2.50	55	97.71
5	3.00	0.30	3.00	40	87.23
6	2.00	0.10	2.00	40	80.14
7	2.50	0.20	2.50	32.50	8031
8	3.00	0.10	2.00	40	93.78
9	3.00	0.30	3.00	70	166.15
10	2.50	0.20	2.50	55	127.43
11	3.00	0.30	2.00	70	113.32
12	2.50	0.20	22.50	55	108.53
13	3.25	0.20	2.50	55	129.12
14	2.00	0,10	3.00	70	116.15
15	2.50	0.20	2.50	55	127.43

16	**3.00**	**0.10**	**3.00**	**70**	**114.21**
17	**3.00**	**0.10**	**3.00**	**40**	**123.78**
18	**2.50**	**0.20**	**1.75**	**55**	**118.54**
19	**2.00**	**0.30**	**2.00**	**70**	**116.15**
20	**2.00**	**0.30**	**3.00**	**70**	**70.33**
21	**1.75**	**0,20**	**2.50**	**55**	**109.13**
22	**2,50**	**0.20**	**2.50**	**55**	**127.43**
23	**2.50**	**0.05**	**2.50**	**55**	**98.21**
24	**2.00**	**0.30**	**3.00**	**40**	**89.32**
25	**3.00**	**0.10**	**2.00**	**70**	**124.34**
26	**2.50**	**0.20**	**2.50**	**55**	**127.43**
27	**2.50**	**0.20**	**2.50**	**55**	**127.43**
28	**2.00**	**0.10**	**2.00**	**70**	**98.34**
29	**2.00**	**0.30**	**2.00**	**40**	**101.23**
30	**3.00**	**030**	**2.00**	**40**	**103.20**

Table 4.17: Lack of fit for percentage reducing sugar for wild cocoyam starch.

Source	Sequential p-value	Lack of Fit p-value	Adjusted R- Square	Predicted R-Square	Remarks
Linear	**0.1508**		**0.1050**	**-0.0999**	
2FI	**0.5610**		**0.0670**	**-0.7991**	
Quadratic	**0.0002**		**0.9712**	**-0.0249**	**Suggested**
Cubic	**0.5901**		**0.6736**	**-13.9162**	**Aliased**

Table 4.18: Lack of fit for percentage reducing sugar for wild cocoyam.

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob>F

Linear	64496.00	20	324.80		
2FI	5146.33	14	367.60		
Quadratic	**1309.59**	**10**	**130.96**		
Cubic	663.32	2	331.666		
Pure Error	0.000	5	0.000		

Table 4.19: Model Summary Statistics for percentage reducing sugar for wild cocoyam

Source	Std.Dev	**R-Squared**	Adjusted R-Squared	Predicted R-Squared	Press	Remaks
Linear	16.12	0.2284	0.1050	-0.0999	9259.98	
2FI	16.46	0.3887	0.0670	-0.7991	15146.43	
Quadratic	9.34	0.9334	0.9136	0.9136	8628.87	Suggested
Quadratic	9.73	0.9743	0.9836	-13.9162	1.256E+005	Aliased

Table 4.20: Analysis of variance (ANOVA) for percentage reducing sugar for wild cocoyam

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F	Remarks
Model	7109.53	14	507.82	5.82	0.0008	Significant
A-Time	905.50	1	905.50	10.37	0.0057	
B-Enzymconc.	135.22	1	135.22	1.55	0.2324	
C- Water qty	0.081	1	0.081	9.298E-004	0.9761	
D-Temp.	882.32	1	882.32	10.11	0.0062	
AB	26.16	1	26.16	0.30	0.5021	
AC	5127	1	5127	0.59	0.4554	
AD	48.44	1	48.44	0.55	0.4679	
BC	1001.09	1	1001.09	11.47	0.0041	

BD	31.02	1	31.02	0.36	0.5600	
CD	191.68	1	191.68	2.20	0.1591	
A^2	20.42	1	20.42	0.23	0.6356	
B^2	1313.94	1	1313.94	15.05	0.0015	
C^2	8.20	1	8.20	0.094	0.7634	
D^2	1726.84	1	1726.84	19.78	0.0005	
Residuals	1309.59	15	87.31			
Lack of fit	1309.59	10	130.96			
Pure error	0.000	5	0.000			
Cor total	8419.12	29				

Std. Dev.=9.34; R-Squared= 0.9334; Mean= 109.38; Adj R-Squared= 0.9214; C.V. %= 8.54; Pred R-Squared= 0.9136; PRESS= 8628.87; SAdeq Precision= 7.8

The adjusted R^2=0.9214 was in close agreement with the predicted R^2 =0.9136. The adequate precision measures the signal to noise ratio and compares the range of the predicted value at the design points to the average prediction error. The adquate predicion ratio above 4 indicates adequate model efficacy (Kumar *et al.*, 2007). Hence, the adquate precision ratios of 7.8 indicates adquate model efficacy. Also, a PRESS value of 8628.87 indicates an adquate signal implying that the models can be used to navigate the design space.

The coefficient of regression R^2 = 0.9334 was used to validate the fitness of the model equation. For percentage reducing sugar yield for jackfruit seed starch, the R^2 has a high value of 0.9334 showing that 93% of the variability in the response can be explained by the model. This implies that the prediction of experimental data is quite satisfactory. The quadratic model equations obtained for the reducing sugar percentage is:

$$Y_{\text{Reducing sugar}} = +125.48 +6.65A -2.57B +0.063C +6.56D -1.28AB +1.79AC +1.74AD - 7.91BC -1.39BD -3.46CD -1.34A^2 -10.75B^2 +0.85C^2 -12.32D^2 \quad (4.4)$$

Table 4.21: Actual and Predicted values of percentage reducing sugar yield for wild cocoyam.

Std	A-Time(hrs)	B-Enzyme conc. (g/g)	C-Water qty. (ml/g)	D-Temp. (oC)	Experimental value	Predicted value
1	2.50	0.20	3.25	55	129.56	80.71
2	2.50	0.20	2.50	55	12743	89.50
3	2.00	0.10	3.00	40	98.12	96.74
4	2.50	0.35	2.50	55	97.71	100.41
5	3.00	0.30	3.00	40	87.23	100.00
6	2.00	0.10	2.00	40	80.14	115.95
7	2.50	0.20	2.50	32.50	8031	84.38
8	3.00	0.10	2.00	40	93.78	95.22
9	3.00	0.30	3.00	70	166.15	100.06
10	2.50	0.20	2.50	55	127.43	115.81
11	3.00	0.30	2.00	70	113.32	110.51
12	2.50	0.20	2.50	55	108.53	121.15
13	3.25	0.20	2.50	55	129.12	105.15
14	2.00	0,10	3.00	70	116.15	128.41
15	2.50	0.20	2.50	55	127.43	84.32
16	3.00	0.10	3.00	70	114.21	102.11
17	3.00	0.10	3.00	40	123.78	112,50
18	2.50	0.20	1.75	55	118.54	132.44
19	2.00	0.30	2.00	70	116.15	105.15
20	2.00	0.30	3.00	70	70.33	97.45
21	1.75	0,20	2.50	55	109.13	127.30
22	2,50	0.20	2.50	55	127.43	127.49
23	2.50	0.05	2.50	55	98.21	87.92
24	2.00	0.30	3.00	40	89.32	107.60
25	3.00	0.10	2.00	70	124.34	125.48
26	2.50	0.20	2.50	55	127.43	125.48
27	2.50	0.20	2.50	55	127.43	125.48
28	2.00	0.10	2.00	70	98.34	125.48
29	2.00	0.30	2.00	40	101.23	125.48
30	3.00	030	2.00	40	103.20	125.48

The Normal plot of Predicted vs Actual for wild cocoyam starch (Fig. 4.15) was used to check whether the points will follow a straight line in which conclusion was drawn that the residuals follow a normal distribution. Hence, since from the Normal plot of Residuals for cocoyam (Fig. 4.16) and Plot of Residue vs Run (Fig.4.17), it was observed that the points were closely distributed in each case to the striaght line of the plot, this confirms the good relationship between the experimental values and the predicted values of the response though some small scatter like an "S" shape is always expected. These plots equally confirm that the selected model was adequate in predicting the response variables in the experimental values.

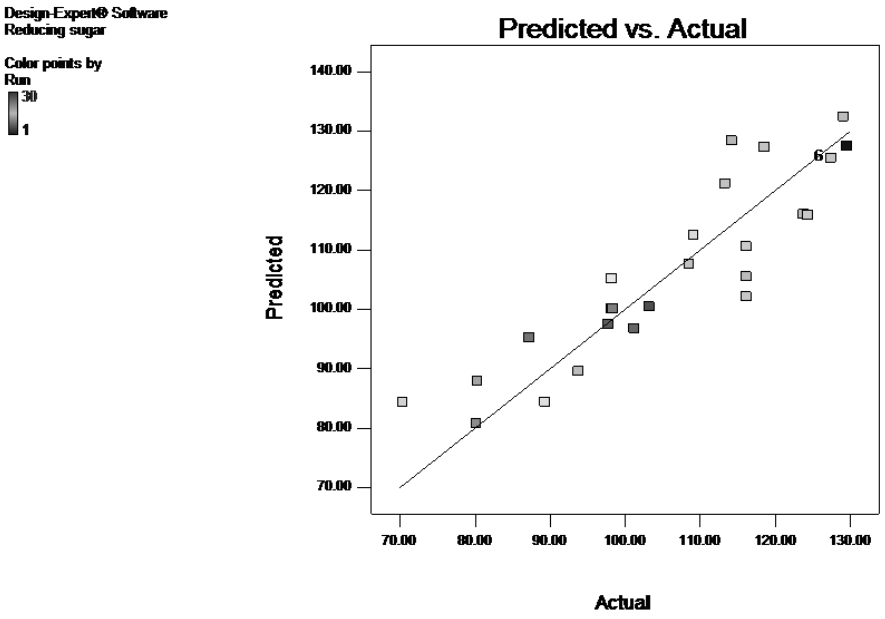

Fig. 4.15: Normal plot of Predicted vs Actual for wild cocoyam starch

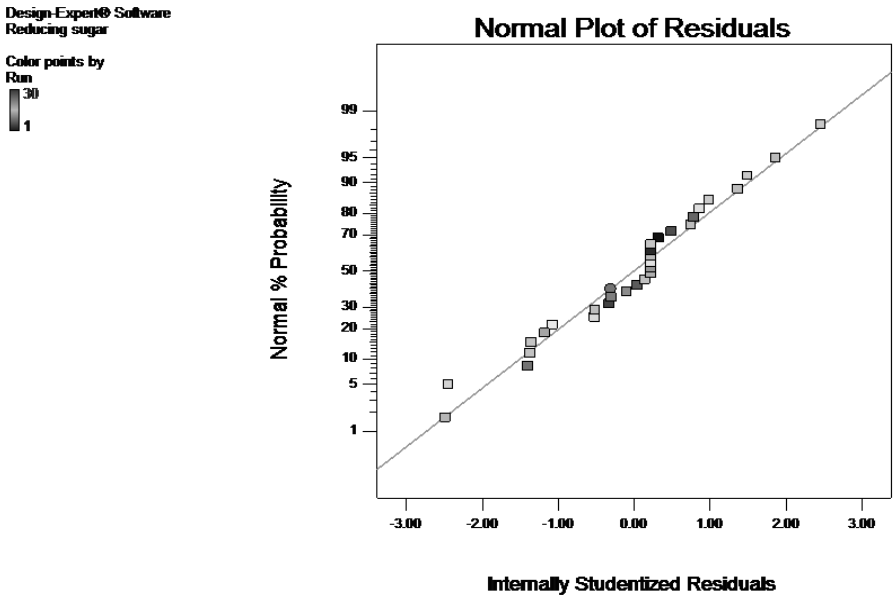

Fig. 4.16: Normal plot of Residuals for cocoyam

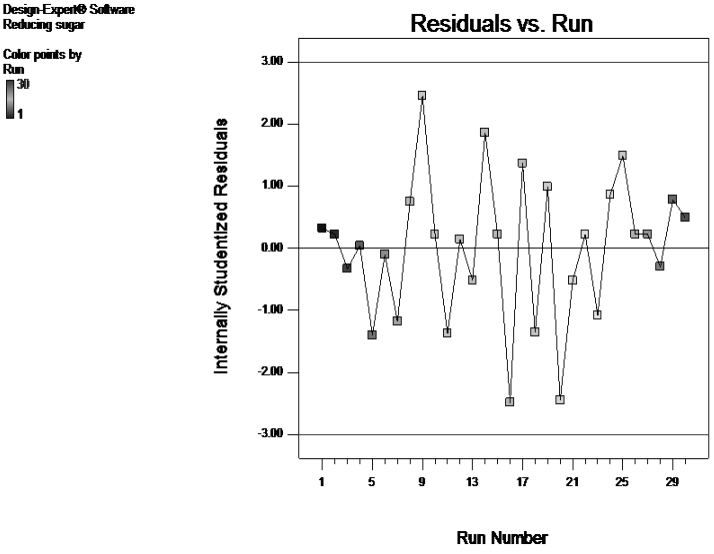

Fig. 4.17: Plot of Residue vs Run for cocoyam starch.

4.8.2 The Three Dimensional (3-D) response surface plots for reducing sugar yield for wild cocoyam starch

The 3-D response surface plots for wild cocoyam starch are presented in Figures 4.18 to 4.23. The 3-D response surface plots are graphical representation of the interactive effects of any two variables the factors for Response surface estimation

for maximum percentage reducing sugar yield. Response surface plots as a function of two factors at a time, maintaining all other factors at fixed levels are more helpful in understanding both the main and the interaction effects of these two factors. These plots can be easily obtained by calculating from the model, the values taken by one factor where the second varies with constraint of a given Y value. The response surface curves were plotted to understand the interaction of the variables and to determine the optimum level of each variable for maximum response.

The nature of the response surface curves shows the interaction between the variables. The elliptical shape of the curve indicates good interaction of the two variables and circular shape indicates no interaction between the variables. From figures, it was observed that the elliptical nature of the contour in graphs the mutual depicted interactions of all the variables. There was a relative significant interaction between every two variables, and there was a maximum predicted yield as indicated by the surface confined in the smallest ellipse in the contour diagrams.

3D Surface plots for optimization of reducing sugar yield for wild cocoyam

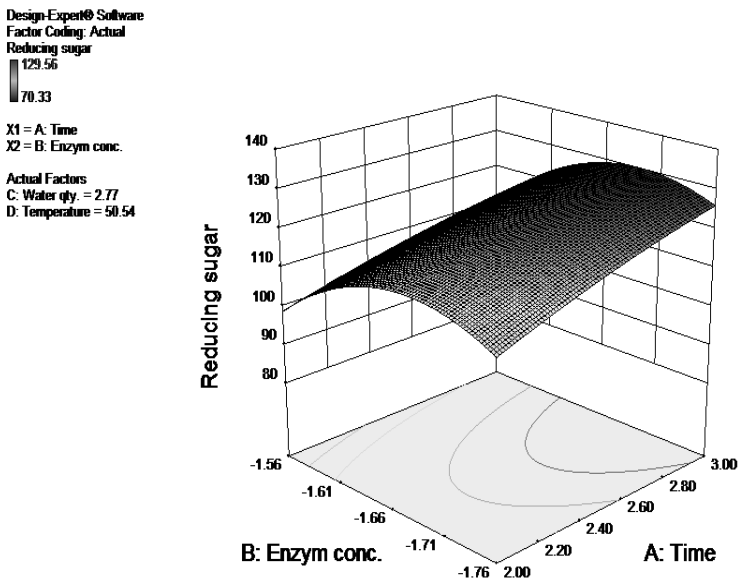

Fig. 4.18: 3D Surface plot of reducing sugar yield showing combined effect of temperature and water quantity on wild cocoyam starch

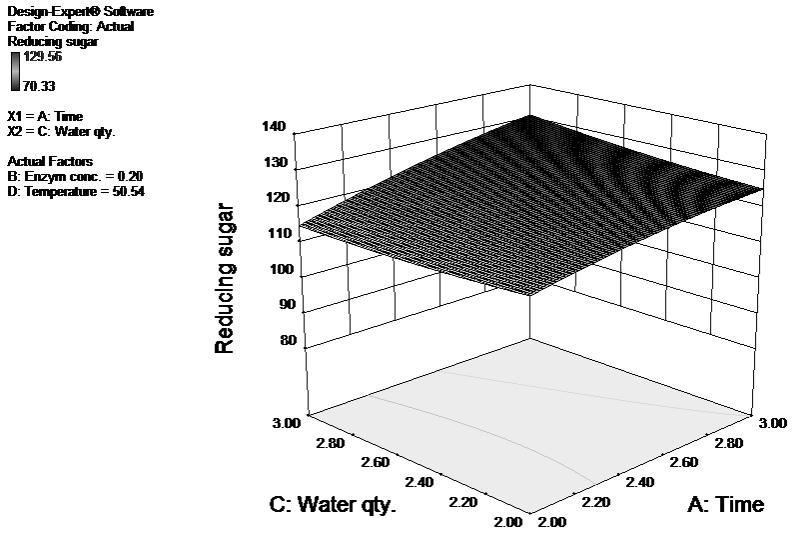

Fig. 4.19: 3D Surface plot of reducing sugar yield showing combined effect of temperature and water quantity on wild cocoyam starch

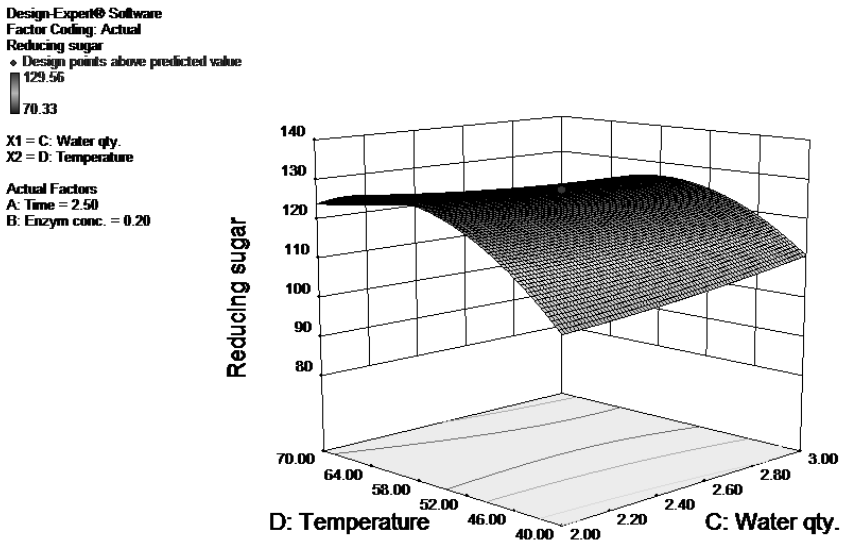

Fig. 4.20: 3D Surface plot of reducing sugar yield showing combined effect of temperature and water quantity on wild cocoyam starch

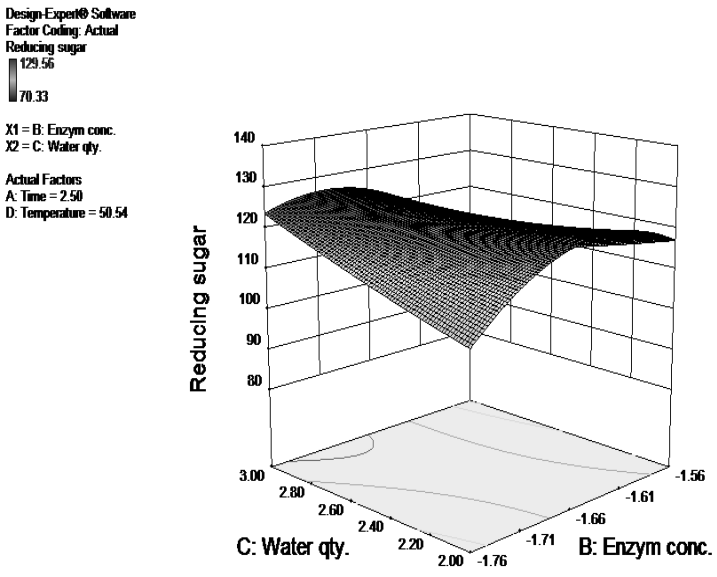

Fig. 4.21: 3D Surface plot of reducing sugar yield showing combined effect of water quantity and enzyme concentration on wild cocoyam starch

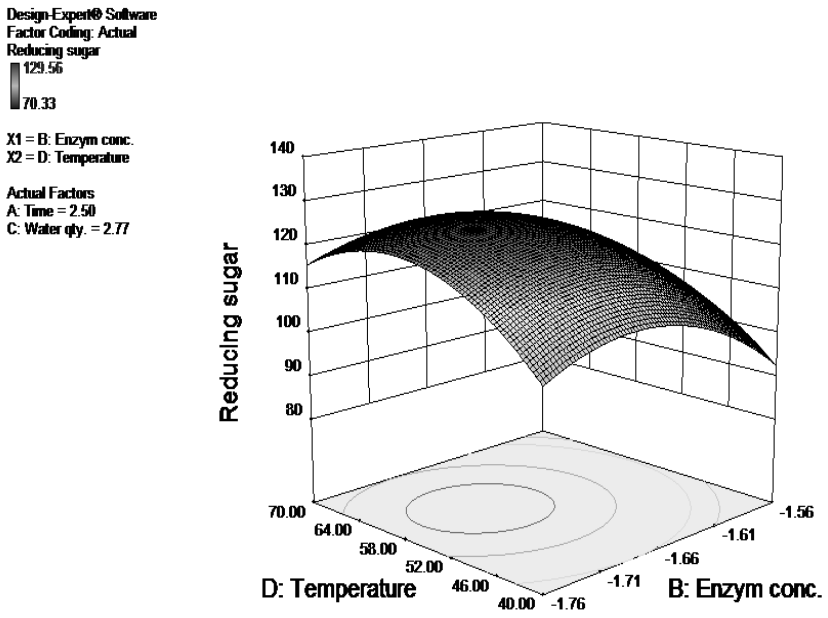

Fig.4.22: 3D Surface plot of reducing sugar showing combined effect of temperature and enzyme concentration on wild cocoyam starch

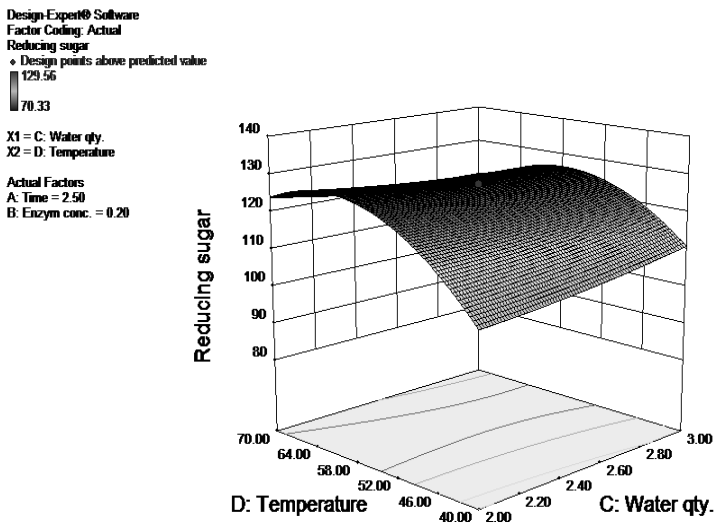

Fig. 4.23: 3D Surface plot of reducing sugar yield showing combined effect of temperature and water quantity on wild cocoyam starch

4.9 Optimization of percentage reducing sugar yield for yellow yam starch.

The optimization process of the reducing sugar yield (RSY) was done using the Central Composite Design (CCD). Four important factors which are temperature, time, enzyme concentration and water quantity were used as the independent variables where their combined effects were examined while the yield of the reducing sugar was the dependent variable or the response. This was done to determine the best conditions for optimum yield of the reducing sugar. Using the CCD involves varying the independent variables at five different levels (-1, 0, +1). In this work, a set of 30 experiments were performed consisting of 16 core points, 8 star like points and 6 centre points or null points. The distance of the star like point α used was 1.5. The experiments were performed in random to avoid systematic error.

4.9.1 Statistical analysis of the optimization process using yellow yam

The reducing sugar yield results of the optimization process using yellow yam were given in Tables 4.20 to 4.24. It shows the combined effects of time (A),

enzyme concentration (B), water quantity (C) and temperature (D). The highest percentage of yield reducing sugar was 122.23mg/mg occurring at a temperature of 70^{o}C, time of 2hrs, enzyme concentration of 0.10g/g and water quantity of 2ml/g. Design Expert 8.0.7.1 trial version was used to analyze the result.

Table 4.22: Actual and predicted values of the percentage reducing sugar for yellow yam starch

Std	Factor 1 A: time hrs	Factor 2 B: enzyme conc. g/g	Factor 3 C:water qty. ml/g	Factor 4 D: temperature ^{0}C	Response D: reducing sugar Mg/mg
1	2.50	0.20	2.50	55.00	75.23
2	2.50	0.20	3.25	55.00	83.45
3	2.50	0.20	1.75	55.00	75.23
4	2.00	0.30	2.00	40.00	85.78
5	2.00	0.10	4.00	40.00	78.34
6	3.00	0.10	4.00	40.00	90.36
7	2.00	0.30	4.00	40.00	98.76
8	3.00	0.30	4.00	40.00	110.23
9	2.00	0.10	2.00	70.00	122.23
10	3.00	0.10	2.00	70.00	120.14
11	2.00	0.30	2.00	70.00	86.23
12	3.00	0.30	2.00	70.00	122.56
13	2.00	0.10	4.00	70.00	93.21
14	3.00	0.10	4.00	70.00	89.17
15	2.00	0.30	4.00	70.00	73.45
16	3.00	0.30	4.00	70.00	105.77
17	1.75	0.20	3.00	55.00	101.34
18	3.25	0.20	3.00	55.00	99.45
19	2.50	0.05	3.00	55.00	97.11
20	2.50	0.35	3.00	55.00	86.22
21	2.50	0.20	1.50	55.00	93.11
22	2.50	0.20	4.50	55.00	103.11
23	2.50	0.20	3.00	32.50	74.89
24	2.50	0.20	3.00	77.50	101.12
25	2.50	0.20	3.00	55.00	63.13
26	2.50	0.20	3.00	55.00	75.12
27	2.50	0.20	3.00	55.00	63.13
28	2.50	0.20	3.00	55.00	63.13
29	2.50	0.20	3.00	55.00	63.13
30	2.50	0.20	3.00	55.00	63.13

Table 4.23: Experimental and Predicted values of reducing sugar yield for yellow yam

Std	Factor 1 A: time hrs	Factor 2 B: enzyme conc. g/g	Factor 3 C:water qty. ml/g	Factor 4 D: temp. ^{0}C	Experimental value	Predicted value
1	2.50	0.20	2.50	55.00	75.23	81.82
2	2.50	0.20	3.25	55.00	83.45	79.82
3	2.50	0.20	1.75	55.00	75.23	72.61
4	2.00	0.30	2.00	40.00	85.78	89.74
5	2.00	0.10	4.00	40.00	78.34	90.26
6	3.00	0.10	4.00	40.00	90.36	87.95
7	2.00	0.30	4.00	40.00	98.76	98.14
8	3.00	0.30	4.00	40.00	110.23	114.97
9	2.00	0.10	2.00	70.00	122.23	122.75
10	3.00	0.10	2.00	70.00	120.14	125.81
11	2.00	0.30	2.00	70.00	86.23	93.69
12	3.00	0.30	2.00	70.00	122.56	115.90
13	2.00	0.10	4.00	70.00	93.21	94.30
14	3.00	0.10	4.00	70.00	89.17	97.05
15	2.00	0.30	4.00	70.00	73.45	82.34
16	3.00	0.30	4.00	70.00	105.77	104.23
17	1.75	0.20	3.00	55.00	101.34	83.77
18	3.25	0.20	3.00	55.00	99.45	98.69
19	2.50	0.05	3.00	55.00	97.11	83.27
20	2.50	0.35	3.00	55.00	86.22	81.74
21	2.50	0.20	1.50	55.00	93.11	90.16
22	2.50	0.20	4.50	55.00	103.11	87.73
23	2.50	0.20	3.00	32.50	74.89	67.52
24	2.50	0.20	3.00	77.50	101.12	90.16
25	2.50	0.20	3.00	55.00	63.13	70.49
26	2.50	0.20	3.00	55.00	75.12	70.49
27	2.50	0.20	3.00	55.00	63.13	70.49
28	2.50	0.20	3.00	55.00	63.13	70.49
29	2.50	0.20	3.00	55.00	63.13	70.49
30	2.50	0.20	3.00	55.00	63.13	70.49

Table 4.24: Summary of P-values for percentage reducing sugar yield for yellow yam

Sequential R-Squared	Lack of Fit R-Squared	Adjusted	Predicted	Source	p-value
Linear	0.2631	0.0034	0.0522	-0.1219	
2FI	0.2293	0.0039	0.1549	-0.1646	
Quadratic	0.0020	0.0241	0.6323	0.1080	Suggested
Cubic	0.7808	0.0043	0.5207	-10.6982	Aliased

Table 4.25: Lack of Fit Test for percentage reducing sugar yield for yellow yam

Source	Sum of Squares	df	Mean Square	F Value	Prob > F		p-value
Linear	7443.51	20	372.18	15.30	0.0034		
2FI	5004.68	14	357.48	14.70	0.0039	Quadratic	
1639.15	10	163.92	6.74	0.0241	Suggested	Cubic	949.64

Table 4.26: Model Summary Statistics for reducing sugar yield for yellow yam

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	17.40	0.1829	0.0522	-0.1219	10386.85	
2FI	16.43	0.4463	0.1549	-0.1646	10782.82	
Quadratic	10.83	0.9178	0.7323	0.1080	8259.05	Suggested
Cubic	12.37	0.8843	0.5207	-10.6982	1.083E+005	Aliased

4.9.2 ANOVA analysis for reducing sugar yield for yellow yam

Design Expert 8.0.7.1 trial version was used to analyze the results. The results were shown below. The summary of P-values indicates that a quadratic model

fitted the ANOVA analysis and hence it was suggested. The linear and 2FI models were not suggested. The Cubic model is always aliased because the CCD does not contain enough runs to support a full cubic model. A significance level of 81% was used hence all terms whose P-value are less than 0.05 are considered significant as shown in Table 4.24. The lack of fit test and model summary test for reducing sugar yield were also presented in Tables 4.25 and 4.26 respectively, while ANOVA analysis for reducing sugar yield for yellow yam starch is presented in Table 4.27 below.

Table 4.27: Analysis of variance (ANOVA) for reducing sugar yield for yellow yam

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	7497.82	14	535.56	4.56	0.0030	significant
A- time	*-506.97*	*1*	*506.97*	*4.320.*	*0553.33*	
C-water qty.	*13.38*	*1*	*13.38*	*0.11*	*0.7404*	
D-temperature	*1167.80*	*1*	*1167.80*	*9.95*	*0.0066*	
AB	*366.34*	*1*	*366.34*	*3.12*	*0.0976*	
AC	*0.096*	*1*	*0.096*	*8.187E-004*	*0.9776*	
AD	*25.65*	*1*	*25.65*	*0.22*	*0.6469*	
BC	*292.24*	*1*	*292.24*	*2.49*	*0.1355*	
BD	*393.63*	*1*	*393.63*	*3.35*	*0.0870*	
CD	*1360.87*	*1*	*1360.87*	*11.59*	*0.0039*	
A^2	*966.89*	*1*	*966.89*	*8.24*	*0.0117*	
B^2	*324.26*	*1*	*324.26*	*2.76*	*0.1173*	
C^2	*765.59*	*1*	*765.59*	*6.52*	*0.0220*	
D^2	*156.76*	*1*	*156.76*	*1.34*	*0.2659*	
Residual	1760.76	15	117.38			
Lack of Fit	*1639.15*	*10*	*163.92*	*6.74*	*0.0241*	*significant*
Pure Error	*121.61*	*5*	*24.32*			
Cor Total	9258.58	29				

Std. Dev.=10.83; R-Squared=0.9254; Mean= 88.58; Adj R-Squared= 0.9208

C.V. %=12.23; Pred R-Squared= 0.8323; PRESS= 8416.05; Adeq Precision= 7.609

The adjusted R^2 =0.9208 was in close agreement with the predicted R^2 =0.8323. The adequate precision measures the signal to noise ratio and compares the range of the predicted value at the design points to the average prediction error. The adquate predicion ratio above 4 indicates adequate model efficacy (Kumar *et al.*, 2007). Hence, the adquate precision ratios of 7.608 indicates adquate model efficacy. Also, a PRESS value of 8416.05 indicates an adquate signal implying that the models can be used to navigate the design space.

The coefficient of regression R^2 was used to validate the fitness of the model equation. For percentage reducing sugar yield for water yam, the R^2 has a high value of 0.9254 showing that 92.54% of the variability in the response can be explained by the model. This implies that the prediction of experimental data is quite satisfactory. The quadratic model equation obtained for the reducing sugar yield is for wild cocoyam is:

$$Y_{\text{Reducing sugar}} = +70.49 +4.97A -0.51B -0.81C +7.55D +4.79AB -0.077AC +1.27AD +4.27BC -4.96BD -9.22CD +9.22A^2 +5.34B^2 +8.20C^2 +3.71D^2 \quad (4.5)$$

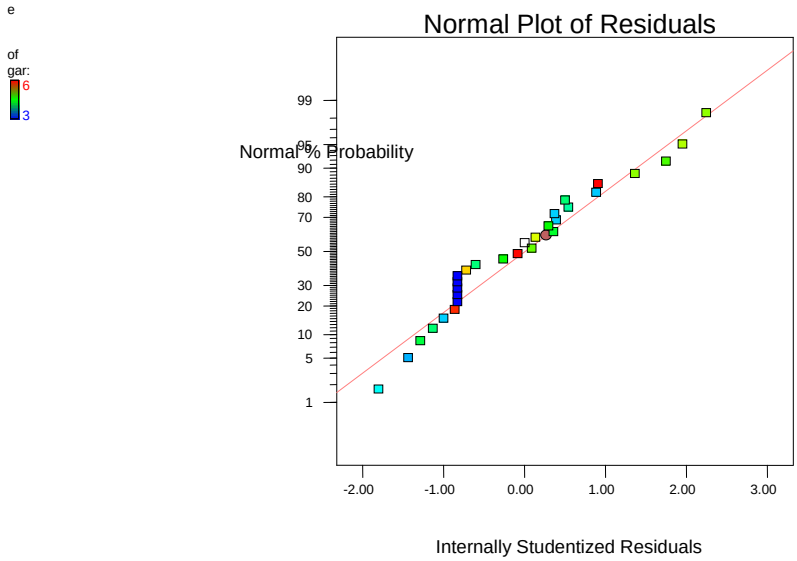

Fig. 4.24: Normal plot of Residual for reducing sugar yield for yellow yam starch

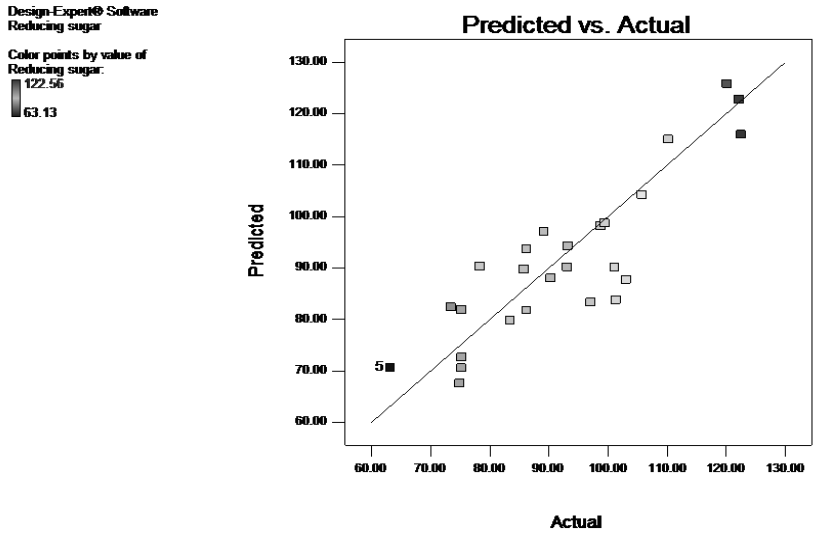

Fig. 4.25: Normal plot of Residual for reducing sugar yield for yellow yam

4.9.3 3 D Surface plots for optimization of reducing sugar yield for yellow yam

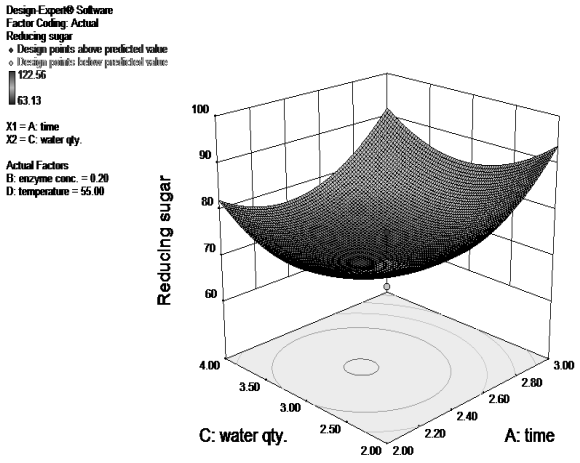

Fig. 4.26: 3D Surface plot of reducing sugar yield showing combined effect of water quantity and enzyme on yellow yam starch

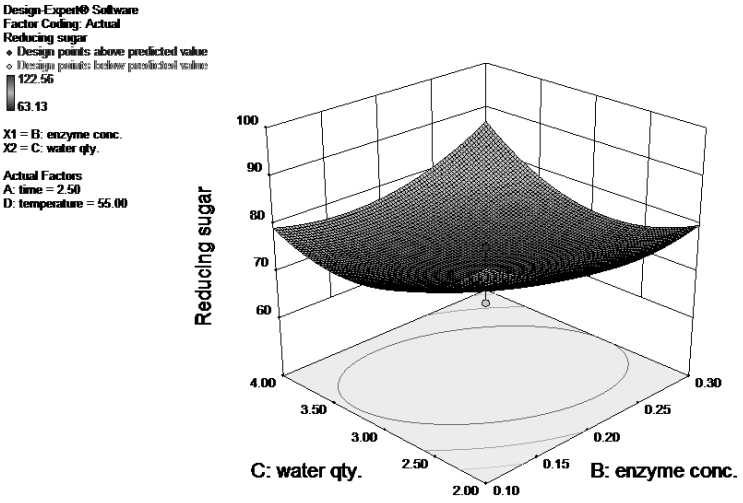

Fig. 4.27: 3D Surface plot of reducing sugar yield showing combined effect of water quantity and enzyme on yellow yam starch

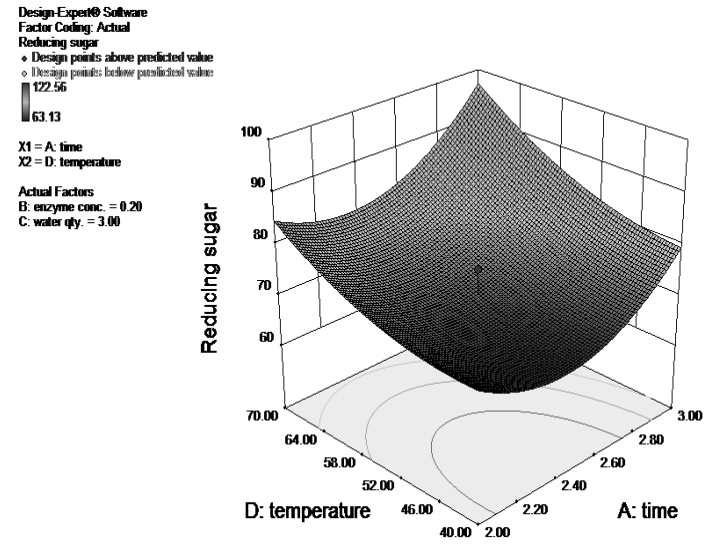

Fig. 4.28: 3D Surface plot of reducing sugar yield showing combined effect of temperature and time on yellow yam starch

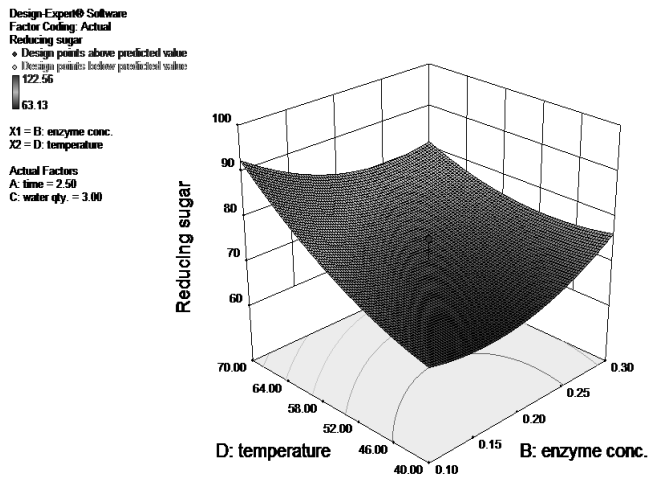

Fig. 4.29: 3D Surface plot for reducing sugar yield showing combined effect of temperature and enzyme concentration on yellow yam starch

4.10 Validation of model predicted result

The derived model was validated by carrying out a statistical analysis (Correlation), comparison with standard model (Regression model) and deviational analysis.

1). Comparison with standard model (Regression Model)

The comparison of the Adjusted R^2 and the Predicted R^2 shown in Table 4.28 below show that they are in close agreement with each other. These evaluated results indicate that the derived model predictions are significantly reliable and hence valid considering the proximate agreement with the results from actual experiment and regression model.

Table 4.28: showing comparison of Adjusted R^2 and Predicted R^2 values for the feedstocks

Feedstock	Adjusted R^2	Predicted R^2
Wild cocoyam	0.9214	0.9136
Jackfruit seed	0.9150	0.8904
Water yam	0.9178	0.8453

Yellow yam	0.9208	0.8323

2). Comparison with Adequate Presision ratio.

Adequate Precision ratio measures the signal to noise ratio and compares the range of the predicted values at the design points to the average prediction error. Kumar *et al*. (2007) reported that an adequate precision ratio above 4 indicates adequate model efficacy. The values of adequate precision ratio obtained in this study as shown in Table 4.29 below are all above 4 indicating adequate model efficacy.

Table 4.29: Showing Adequate Precision Ratio

Feedstock	Adequate precision ratio
Wild cocoyam	7.8
Jackfruit seed	6.117
Water yam	5.828
Yellow yam	7.609

3). Comparison with Coefficient of Regression (R^2)

The coefficient of regression R^2 was used to validate the fitness of the model equations. Results from the study show that the R^2 value for wild cocoyam, jackfruit seed, water yam and yellow yam were 0.9334, 0.9230, 0.9098, and 0.9254 respectively. This shows that 93.34%, 92.30%, 90.98% and 92.54% (for wild cocoyam, jackfruit seed, water yam and yellow yam respectively) of the variability in the responses can be explained or accounted for by the models implying that the prediction of the experimental data are quite satisfactory.

4). Comparison with the P-values.

Kumar *et al.* (2007) reported that when in a regression equation , an independent variable has a positive sign, it indicates that an increase in the variable will cause

an increase in the response while a negative sign will result to decrease in the response.

Values of P less than 0.05 indicate that the model term is significant. In this study, for wild cocoyam, jackfruit seed, water yam and yellow yam from the P values it was found that, among the test variables used in the study of the optimization of percentage reducing sugar yield, that by eliminating the insignificant terms, the final model equations becomes as expressed in Equations 4.6 to 4.9

1). Wild cocoyam:

$Y_{\text{Reducing sugar}} = 125.48 + 6.65A - 2.57B + 6.56D - 1.28AB + 1.79AC + 1.74AD - 7.91BC - 1.39BD - 3.46CD - 1.34A^2 - 10.75B^2 - 12.32D^2$ (4.6)

2). Jackfruit seed:

$Y_{\text{Reducing sugar}} = 88.03 - 1.08A - 2.34B + 6.01C + 4.17D + 1.15AB + 7.37AC - 3.83AD + 5.25BC + 4.27BD - 3.72CD + 2.10A^2 + 10.54B^2 + 7.52C^2 + 5.27D^2$ (4.7)

3). Water yam:

$Y_{\text{Reducing sugar}} = 88.91 - 2.49B + 5.11C + 7.02D - 5.40AB + 6.98AC + 1.56BC + 1.28BD - 1.47CD + 1.93A^2 + 7.00B^2 + 7.27C^2 + 5.22D^2$ (4.8)

4).Yellow yam:

$Y_{\text{Reducing sugar}} = 70.49 + 4.97A + 7.55D + 4.79AB + 1.27AD + 4.27BC - 4.96BD - 9.22CD + 9.22A^2 + 5.34B^2 + 8.20C^2 + 3.71D^2$ (4.9)

5). Comparison with Press value.

Also, the Press values obtained in this study are listed in Table 4.30 below. Their values indicate adequate signal implying that the models can be used to navigate their respective design space.

Table 4.30 showing Press values

Feedstock	Press value
Wild cocoyam	8628.87
Jackfruit seed	9028.51

Water yam	14079.30
Yellow yam	8416.05

4.11 Optimization of biogas yield from the broth of wild cocoyam and jackfruit seed wastes co-digested with cow paunch using Response Surface Methodology

The optimization process of biogas yield was done using the Central Composite Design (CCD). The effects of important independent variables which were water, inoculums and time were individualy and combined effects were examined while the yield of the biogas was the dependent variable or the response. This was done to determine the best conditions for optimum biogas yield. Using the CCD involves varying the independent variables at five different levels ($-\alpha$, -1, 0, +1, $+\alpha$). In this work, a set of 30 experiments were performed consisting of 16 core points, 8 star like points and 6 centre points or null points. The distance of the star like point α used was 1.5. The experiments were performed in random to avoid systematic error.

4.11.1 Statistical analysis of the optimization process using wild cocoyam and jackfruit seed

The biogas yield results of the optimization process using wild cocoyam and Jackfruit seed wastes were given in Tables 4.31 to 4.32. It shows the combined effects of wild cocoyam and Jackfruit seed wastes. The highest cumulative biogas yield were 136ml/l occurring at hydraulic retention time of 40days, with 10ml inoculums,150 ml of water and 75gr and122.56ml/l occurring hydraulic retention time of 40days, with 10ml inoculums,150 ml of water with 75gr of the mixture of wild cocoyam jackfruit seed waste and cow paunch respectively. Design Expert 8.0.7.1 trial version was used to analyze the results.

Table 4.31: Optimization results for cumulative biogas yield from wild cocoyam waste

Run	Std	COY+CP (gr)	Water (ml)	Inoculums (ml)	Time (days)	Experimental Biogas Yield (ml)
14	1	75	50	30	40	90.15
25	2	50	100	20	25	105.24
1	3	25	50	10	10	91.64
8	4	75	150	30	10	105.14
5	5	25	50	30	10	78.34
17	6	0	100	20	25	90.36
22	7	50	100	40	25	98.76
4	8	75	150	10	10	110.23
2	9	75	50	10	10	122.23
26	10	50	100	20	25	120.14
24	11	50	100	20	55	86.23
16	12	75	150	30	40	122.56
10	13	75	50	10	40	93.21
19	14	50	0	20	25	89.17
27	15	50	100	20	25	73.45
12	16	75	150	10	40	105.77
6	17	75	50	30	10	101.34
18	18	100	100	20	25	99.45
29	19	50	100	20	25	97.11
3	20	25	150	10	10	86.22
13	21	25	50	30	40	93.11
11	22	25	150	10	40	103.11
30	23	50	100	20	25	74.89.
28	24	50	100	20	25	101.12
7	25	25	150	30	10	63.13
9	26	25	50	10	40	75.21
23	27	50	100	20	-5	63.13

20	28	50	200	20	25	63.13
15	29	25	150	30	40	63.13
21	30	50	100	30	40	63.13

Table 4.32: Optimization results for cumulative biogas yield from jackfruit seed waste

Std	COY+CP (gr)	Water (ml)	Inoculums (ml)	Time (days)	Experimental Biogas Yield (ml)
1	75	50	30	40	93.11
2	50	100	20	25	90.15
3	25	50	10	10	120.14
4	75	150	30	10	90.36
5	25	50	30	10	86.22
6	0	100	20	25	63.13
7	50	100	40	25	73.45
8	75	150	10	10	74.89
9	75	50	10	10	63.13
10	50	100	20	25	89.17
11	50	100	20	55	93.21
12	75	150	30	40	86.23
13	75	50	10	40	112.56
14	50	0	20	25	103.11
15	50	100	20	25	101.34
16	75	150	10	40	122.23
17	75	50	30	10	97.11
18	100	100	20	25	98.76
19	50	100	20	25	75.21
20	25	150	10	10	99.45
21	25	50	30	40	78.34
22	25	150	10	40	110.23
23	50	100	20	25	105.77
24	50	100	20	25	91.64
25	25	150	30	10	63.13
26	25	50	10	40	63.13

27	50	100	20	-5	105.14
28	50	200	20	25	101.12
29	25	150	30	40	105.14
30	50	100	30	40	105.14

Table 4.33: Summary of P-values for cumulative biogas yield from wild cocoyam waste

Source	Sequential p-value	Lack of Fit p-value	Adjusted R-Square	Predicted R-Squared	
Linear	< 0.0001	0.0621	0.6338	0.5213	
2FI	0.0344	0.1249	0.7492	0.5981	
Quadratic	0.0325	0.2607	0.8360	0.5898	Suggested
Cubic	0.2544	0.3086	0.8796	-0.5962	Alias

Table 4.34: Summary of P-values for cumulative biogas yield from jackfruit seed waste

Source	Sequential p-value	Lack of Fit p-value	Adjusted R-Squared	Predicted R-Squared	
Linear	0.6100	0.8211	-0.0456	-0.2577	
2FI	0.0895	0.9452	0.1868	-0.2522	
Quadratic	0.7329	0.9147	0.0921	-0.5399	Suggested
Cubic	0.7484	0.9381	-0.1461	-0.3933	Aliased

Table 4.35: Lack of Fit Test for cumulative biogas yield from wild cocoyam waste

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Linear	8215.87	20	410.79	4.10	0.0621	
2FI	4035.49	14	288.25	2.87	0.1249	
Quadratic	1841.08	10	184.11	1.84	0.2607	Suggested
Cubic	301.08	2	150.54	1.50	0.3086	Aliased
Pure Error	501.50	5	100.30			

Table 4.36: Lack of Fit Test for cumulative biogas yield from jackfruit seed waste

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Linear	5848.02	20	292.40	0.59	0.8211	
2FI	2435.82	14	173.99	0.35	0.9452	
Quadratic	1850.68	10	185.07	0.37	0.9147	Suggested
Cubic	64.60	2	32.30	0.065	0.9381	Aliased
Pure Error	2496.19	5	499.24			

Table 4.37: Model Summary Statistics for cumulative biogas yield from wild cocoyam waste

Source	Std. Dev.	Adjusted R-Squared	Predicted R-Squared	R-Squared	PRESS	
Linear	18.67	0.6843	0.6338	0.5213	13219.41	
2FI	15.45	0.8357	0.7492	0.5981	11097.85	
Quadratic	12.50	0.9152	0.8360	0.5898	11326.80	Suggested
Cubic	10.71	0.9709	0.8796	-0.5962	44078.16	Aliased

Table 4.38: Model Summary Statistics for cumulative biogas yield from jackfruit seed waste

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	18.27	0.0986	-0.0456	-0.2577	11642.11	
2FI	16.11	0.4672	0.1868	-0.2522	11591.59	
Quadratic	17.02	0.9304	0.8021	0.8399	14254.43	Suggested
Cubic	19.13	0.7234	-0.1461	-0.3933	12897.12	Aliased

4.11.2 ANOVA analysis for cumulative biogas yield from wild cocoyam and jackfruit seed wastes

Design Expert 8.0.7.1 trial version was used to analyze the results. The results were shown below. The summary of P-values indicates that a quadratic model fitted the ANOVA analysis and hence it was suggested. The linear and 2FI models were not suggested. The Cubic model is always aliased because the CCD does not contain enough runs to support a full cubic model. A significance level of 95% was used hence all terms whose P-value are less than 0.05 are considered significant.

Table 4.39: Analysis of variance (ANOVA) for cumulative biogas yield from wild cocoyam waste

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	25271.28	14	1805.09	11.56	< 0.0001	significant
A-COY+CP	*2838.38*	*1*	*2838.38*	*18.17*	*0.0007*	
B-Water	*330.04*	*1*	*330.04*	*2.11*	*0.1666*	
C-Innoculum	*376.04*	*1*	*376.04*	*2.41*	*0.1416*	
D-Time	*15352.04*	*1*	*15352.04*	*98.30*	*< 0.0001*	
AB	*138.06*	*1*	*138.06*	*0.88*	*0.3620*	
AC	*217.56*	*1*	*217.56*	*1.39*	*0.2563*	
AD	*473.06*	*1*	*473.06*	*3.03*	*0.1023*	
BC	*451.56*	*1*	*451.56*	*2.89*	*0.1097*	
BD	*1827.56*	*1*	*1827.56*	*11.70*	*0.0038*	
CD	*1072.56*	*1*	*1072.56*	*6.87*	*0.0193*	
A^2	*1207.65*	*1*	*1207.65*	*7.73*	*0.0140*	
B^2	*358.36*	*1*	*358.36*	*2.29*	*0.1506*	
C^2	*125.07*	*1*	*125.07*	*0.80*	*0.3850*	
D^2	*414.07*	*1*	*414.07*	*2.65*	*0.1243*	
Residual	2342.58	15	156.17			
Lack of Fit	*1841.08*	*10*	*184.11*	*1.84*	*0.2607*	*not sigr*
Pure Error	*501.50*	*5*	*100.30*			
Cor Total	27613.87	29				

The Model F-value of 11.56 implies that the model is significant.

Std. Dev.	12.50	R-Squared	0.9152	
Mean	64.73	Adj R-Squared	0.8360	
C.V. %	19.31	Pred R-Squared	0.5898	
PRESS	11326.80	Adeq Precision	11.656	

Table 4.40: Analysis of variance (ANOVA) for cumulative biogas yield from jackfruit seed waste.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	4324.72	10	432.47	1.67	0.1624	not sign
A-jackfruit+CP	*38.99*	*1*	*38.99*	*0.15*	*0.7026*	
B-Inoculums	*414.59*	*1*	*414.59*	*1.60*	*0.2216*	
C-Water	*302.53*	*1*	*302.53*	*1.17*	*0.2938*	
D-Time	*156.42*	*1*	*156.42*	*0.60*	*0.4472*	
AB	*1.58*	*1*	*1.58*	*6.092E-003*	*0.9386*	
AC	*2.67*	*1*	*2.67*	*0.010*	*0.9204*	
AD	*350.53*	*1*	*350.53*	*1.35*	*0.2596*	
BC	*206.57*	*1*	*206.57*	*0.80*	*0.3835*	
BD	*0.089*	*1*	*0.089*	*3.410E-004*	*0.9855*	
CD	*2850.76*	*1*	*2850.76*	*10.98*	*0.0036*	
Residual	4932.01	19	259.58			
Lack of Fit	*2435.82*	*14*	*173.99*	*0.35*	*0.9452*	*not sign*
Pure Error	*2496.19*	*5*	*499.24*			
Cor Total	9256.73	29				
Std. Dev.	16.11		R-Squared	0.9530		
Mean	90.99		Adj R-Squared	0.1868		
C.V. %	17.71		Pred R-Squared	0.9304		
PRESS	11591.59		Adeq Precision	4.999		

For wild cocoyam, the Adjusted R^2 =0.9098 was in close agreement with the Predicted R^2 =0.8453 and also Jackfruit seed waste with R^2=0.9304 as well was in close agreement with the Predicted R^2=0.8399. The Adequate Precision measures the signal to noise ratio and compares the range of the predicted value at the design points to the average prediction error. The Adquate Predicion ratio above 4 indicates adequate model efficacy (Kumar *et al.*, 2007). Hence, the Adquate Precision ratios of 5.828 for wild cocoyam indicates adquate model efficacy as well the value of 6.117 for jackfruit seed waste. Also, the PRESS value of 14079.30 and 11591.59 for wild cocoyam and jackfruit seed wastes respectively indicate adquate signal implying that the models can be used to navigate the design space.

The coefficient of regression R^2 was used to validate the fitness of the model equation. For cumulative biogas yields, for wild cocoyam the R^2 has a high value of 0.9152 while jackfruit seed with R^2=0.9304 showing that 91% and 93% for the two feedstock's variability in the response can be explained by the model. This implies that the predictions of experimental data are quite satisfactory. The quadratic model equations obtained for the cumulative biogas yield for wild cocoyam and jackfruit seed wastes respectively are:

$$Y_{Biogas\ yield} = +88.91 + 0.33\,A - 2.49B + 5.11C + 7.02D - 5.40AB + 6.98AC + 0.51AD + 1.56BC + 1.28BD - 1.47\,CD + 1.93A^2 + 7.00B^2 + 7.27C^2 + 5.22D^2 \quad (4.10)$$

$$Y_{Biogas} = +90.99 - 1.27A + 4.16B + 3.55C + 2.55\,D + 0.31AB - 0.41AC + 4.68AD - 3.59 - 0.074BD + 13.35CD \quad (4.11)$$

In a regression equation, when an independent variable has a positive sign, it means that an increase in the variable will cause an increase in the response while a negative sign will result in a decrease in the response (Kumur *et al.*, 2008). Hence, an increase in substrates, inoculums, water ad time will cause an increase in

cumulative biogas yield. Variables with higher coefficients will have more significant effect in the increment of the response.

Values of P less than 0.05 indicate the model term is significant. For wild cocoyam and jackfruit seed from the P values it was found that, among the test variables used in the study that eliminating the insignificant terms, the final model equations becomes as expressed in Equations 4.12 to 4.13 below.

1). Wild cocoyam

$$Y_{Biogas\ yield} = 88.91 - 2.49B + 5.11C + 7.02D - 5.40AB + 6.98AC + 0.51AD + 1.56BC + 1.28BD + 1.93A^2 + 7.00B^2 + 7.27C^2 + 5.22D^2 \quad (4.12)$$

2). Jackfruit seed

$$Y_{Biogas} = 90.99 - 1.27A + 4.16B + 3.55C + 2.55D + 4.68AD - 3.59 - 0.074BD + 13.35CD \quad (4.13)$$

A combination of the actual experimental response and the predicted response from the mathematical equations are given in Tables 4.41 and 4.42 below it can be seen that there is a close correlation between the actual experimental response and the predicted response. This comfirms the effectiveness of the cumulative biogas yield.

Table 4.41: Actual and predicted values of the cumulative biogas yield from wild cocoya waste.

Run	Std	COY+CP (gr)	Water (ml)	Inoculums (ml)	Time (days)	Experimental Biogas Yield (ml)	Predicted Biogas Yield (ml)
14	1	75	50	30	40	36.12	26.08
25	2	50	100	20	25	38.05	35.46
1	3	25	50	10	10	29.23	28.63
8	4	75	150	30	10	32.34	26.25
5	5	25	50	30	10	42.22	53.63
17	6	0	100	20	25	87.06	77.75
22	7	50	100	40	25	28.11	34.92
4	8	75	150	10	10	55.24	47.29
2	9	75	50	10	10	52.34	60.79
26	10	50	100	20	25	108.15	91.92
24	11	50	100	20	55	106.45	106.08
16	12	75	150	30	40	136.44	125.46
10	13	75	50	10	40	59.34	55.58

19	14	50	0	20	25	100.54	101.46
27	15	50	100	20	25	76,15	79.63
12	16	75	150	10	40	113.66	113.75
6	17	75	50	30	10	75.26	62.29
18	18	100	100	20	25	85.54	105.79
29	19	50	100	20	25	30.10	35.63
3	20	25	150	10	10	48.00	50.46
13	21	25	50	30	40	44.43	58.13
11	22	25	150	10	40	80.23	73.96
30	23	50	100	20	25	18.46	22.46
28	24	50	100	20	25	120.2	123.63
7	25	25	150	30	10	65.12	57.50
9	26	25	50	10	40	44.00	57.50
23	27	50	100	20	-5	68.34	57.50
20	28	50	200	20	25	48.12	57.50
15	29	25	150	30	40	65.32	57.50
21	30	50	100	30	40	55.31	57.50

Table 4.42: Actual and predicted values of the cumulative biogas yield for jackfruit seed waste.

Std	COY+CP (gr)	Water (ml)	Inoculums (ml)	Time (days)	Experimental Biogas Yield (ml)	Predicted value
1	75	50	30	40	93.11	96.28
2	50	100	20	25	90.15	84.55
3	25	50	10	10	120.14	111.30
4	75	150	30	10	90.36	100.83
5	25	50	30	10	86.22	84.68
6	0	100	20	25	63.13	71.33
7	50	100	40	25	73.45	85.33
8	75	150	10	10	74.89	73.23
9	75	50	10	10	63.13	65.47
10	50	100	20	25	89.17	72.47
11	50	100	20	55	93.21	80.20
12	75	150	30	40	86.23	88.45
13	75	50	10	40	122.56	107.27
14	50	0	20	25	103.11	112.64
15	50	100	20	25	101.34	107.27
16	75	150	10	40	122.23	114.25

17	75	50	30	10	97.11	93.54
18	100	100	20	25	98.76	88.45
19	50	100	20	25	75.21	82.68
20	25	150	10	10	99.45	99.39
21	25	50	30	40	78.34	83.89
22	25	150	10	40	110.23	98.10
23	50	100	20	25	105.77	85.89
24	50	100	20	25	91.64	96.10
25	25	150	30	10	63.13	90.99
26	25	50	10	40	63.13	90.99
27	50	100	20	-5	105.14	90.99
28	50	200	20	25	101.12	90.99
29	25	150	30	40	105.14	90.99
30	50	100	30	40	105.14	90.99

Fig. 4.45: Plot showing effect of time on TDS on the substrates

4.12.3 Effect of time on pH

Figure 4.30: shows the effect of time on pH. It is observed that pH value decreases with increase in hydraulic retention time. This may be attributed to the fact that the acid forming bacteria are breaking down the organic matter and producing volatile fatty acids resulting to increase in acidity of the digesting material, thus leading to decrease in pH value. pH is a lagging indicator in anaerobic digestion but proper buffering can correct the problem. Buffering is the digester's ability to resist pH change. Methanogens are sensitive to the acid concentration within the digester and their growth can be inhibited by acidic conditions. Verma (2002) pointed out that an optimum pH value for anaerobic treatment lies between 5.5 and 8.5.

Methanogens need a pH range between 6.5 and 7.8 whereas the acid-producing bacteria have optimum value between 5 and 6. In this study, pH range in all the digesters lie within the optimal value as posited by Verma (2002). Generally, result show that pH value decreases with increase in hydraulic retention time (HRT). pH stability in this study can be accounted for by the high level of protein content and

other micro-molecule present in the residue which has some buffer effect (Dinamarca *et al*., 2003).

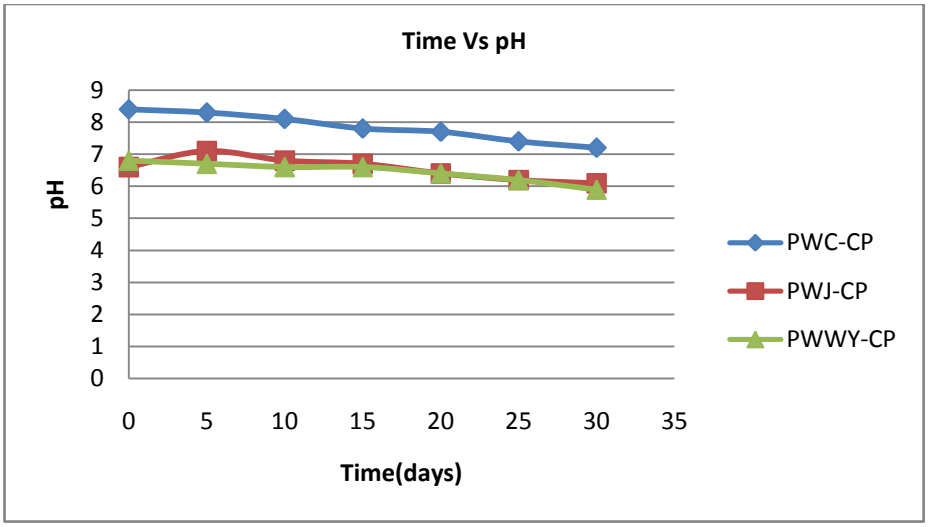

Fig. 4.30: Plot showing effect of time on pH on the substrates

4.12.4 Effect of time on Chemical Oxygen Demand

Figure 4.47 is the plot of the effects of chemical oxygen demand (COD) with time where it was also observed that generally, there is a decrease in the COD level with time. Initially, pure waste of wild cocoyam mixed with cow paunch (PWC-CP) has the highest COD concentration followed by that of pure waste of jackfruit mixed with cow paunch manure (PWJ=CP) and pure waste of water yam mixed with cow paunch manure (PWWY-CP).

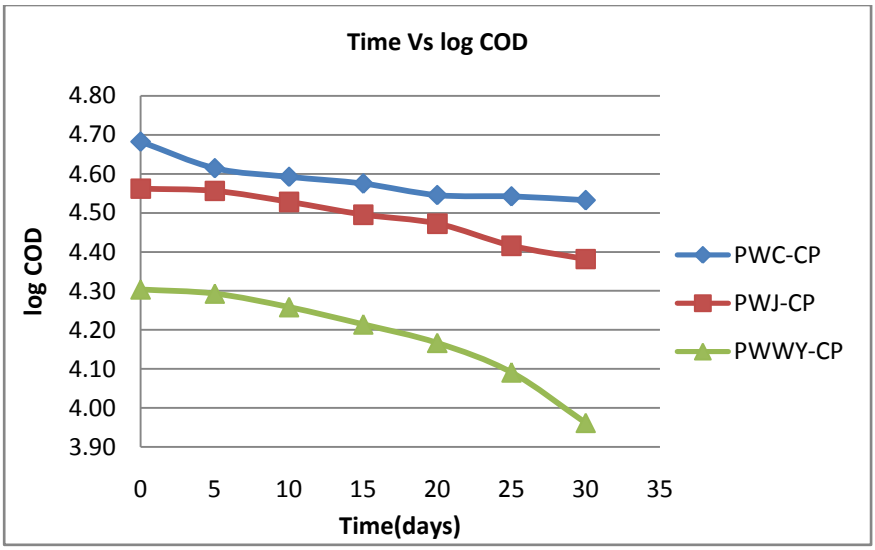

Fig 4.30: Plot showing effects of time on log COD of the substrates.

4.12.5 Effect of Time on Total Viable Count

Figure 4.32 is the plot of the variation of total viable count (TVC) with time for the three substrates. It was observed that at the beginning, the nutrients in the feedstocks were utilized by the bacteria. This provided them with sufficient energy which they use for reproduction and hence their population increased at a very high rate. The substrates consumption at high rate until it became insufficient. This caused the metabolism of the bacteria to slow down and hence, resulting to decrease in the mass of bacteria and they began to die off

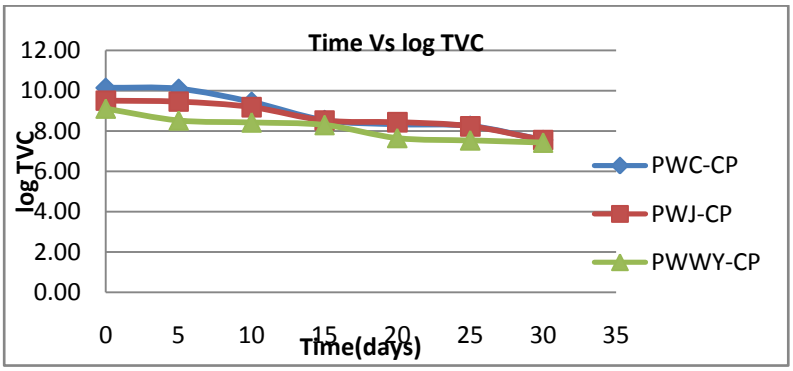

Fig 4.31: Plot showing effects of time on Total Viable Count (TVC) on the substrates.

4.12.6 Effect of time on temperature

The effect of temperature with time on the substrates digestion is presented in Figure 4.32. It is observed that during the period of digestion, the temperature never reached the thermophilic level (between 40 to 55^{o}C) but was always maintained at between the mesophilic level 26 and 35^{o}C) and below. This is probably because of the nature of the substrates and that the anaerobic digestion proceeded much more rapidly at that temperature (Mccarty, 1964)

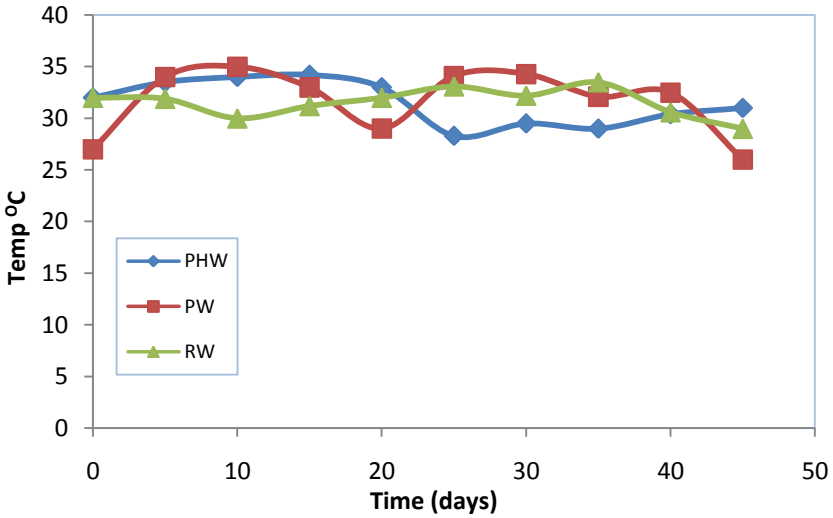

Fig 4.32: Plot showing effects of time on temperature.

4.12.7 Effect of time on net specific growth rate

The plot of the variations in the net specific growth rate of the micro-organisms μ_{net} with time is presented on Figure 4.33 for the anaerobic digestion of the substrates. μ_{net} was observed generally to decrease continually with time especially in the PWC-CP and PWJ-CP which were relatively high initially. This is predicated to the facts that as the nutrients reduce, the micro-organisms that depend on them begin to die off.

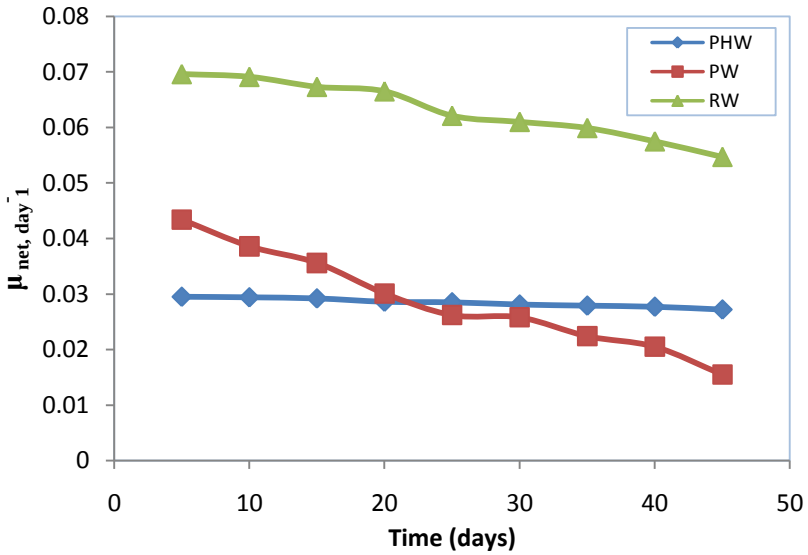

Fig 4.33: Effects of net specific growth rate of the micro-organisms μ_{net} with time on the wastewaters

4.12.8 Determination of the maximum rate of substrate utilization K and the half-velocity constant K_s

The linear plot of $\frac{1}{U}$ against $\frac{1}{S_e}$ was used to calculate the maximum rate of substrate utilization K and the half-velocity constant K_s from the intercept and the slope respectively as shown in Fig. 4.34 and 4.35 for wild cocoyam and jackfruit seed wastes respectively. The values of K were 0.0289 day^{-1} ,0.0231 day^{-1} ,while that of K_s were 28.561mg/l , 29.77mg/l and the correlation coefficients of the linear plot were 0.928, 0.934 for wild cocoyam and jackfruit seed wastes respectively. The small values of K suggest that for effective digestion of substrates, the reactor used must be very large and inoculants required. This confirms that the digesting micro-organisms will need high retention time to regenerate after being inactivated and therefore inoculation will be needed for complete digestion of the substrates.

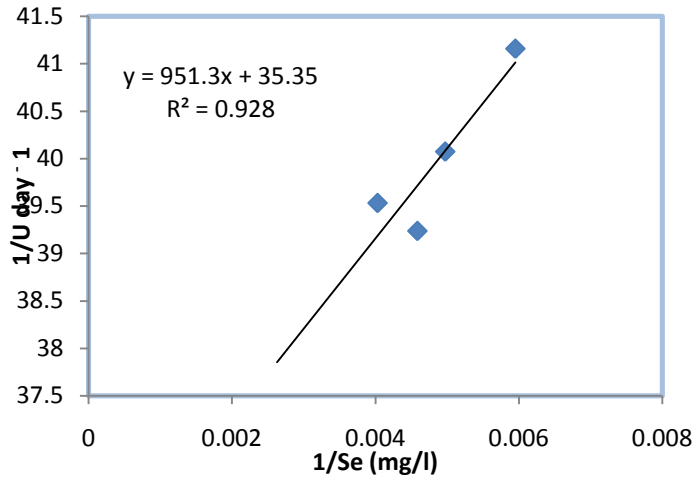

Fig. 4.34: Plot for determination of K and K_s for wild cocoyam waste digestion

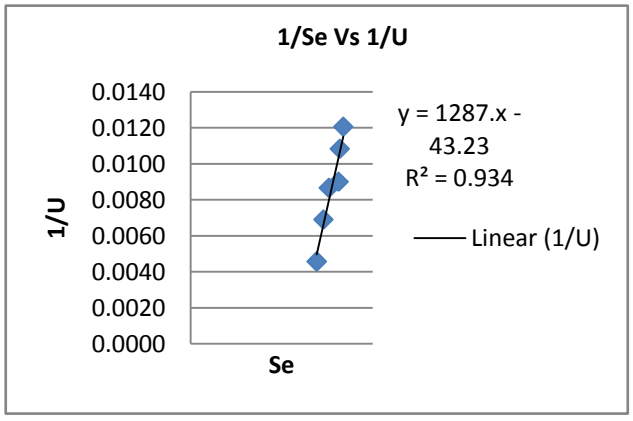

Fig. 4.35: Plot for determination of K and K_s for jackfruit seed waste digestion.

4.13 Kinetics analysis of the substrates digestion

4.13.1 Determination of the first-order inactivation rate coefficient or rate constant K

The kinetic orders of the substrates anaerobic digestion were investigated by plotting $-ln(S_e/S_o)$ against time as shown in Fig. 4.36. The first-order plot obtained gave a correlation coefficient of 0.9554 which indicated that the PWC-CP digestion followed a first-order reaction. From the slope of the plot, the first-order rate constant K_1 was determined as 0.0532 day^{-1}. This showed that the maximum constant rate of the PWC-CP digestion is greater than that of PWJ-CP. The micro-organisms must first become acclimatized to their surrounding environment and to the food provided. (Peavey *et al.*, 1985).

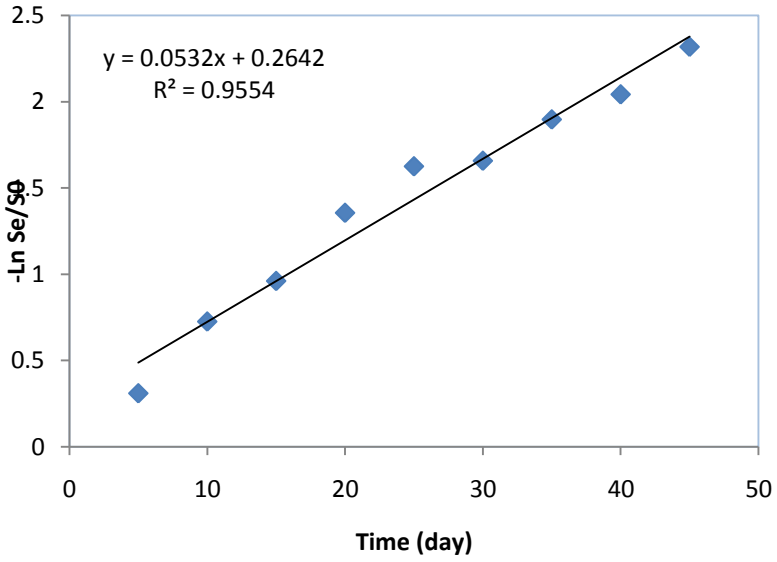

Fig. 4.36: First order kinetic plot for wild cocoyam digestion

4.13.2 Determination of the maximum rate of substrate utilization K and the half-velocity constant K_s

The linear plot of $\frac{1}{U}$ against $\frac{1}{S_e}$ was used to determine the maximum rate of substrate utilization K and the half-velocity constant K_s. The R-squared values were 0.9314, 0.9448 and 0.9180 for wild cocoyam, jackfruit seed and water yam respectively showing the conformity of the linear plots as shown in Fig. 4.37 to 4.38 The slope which was $\frac{K_s}{K}$ and the

intercept which was $\frac{1}{K}$ were used to calculate the K and K_s constants as 0.0730 day^{-1} , 0.0325 day^{-1},and 0.0377 day^{-1} and 26.704 mg/l, 42.47mg/l,48.47mg/l for wild cocoyam, jackfruit seed and water yam respectively. The small value of K imply that the micro-organisms required more time to regenerate which is the reason for their sluggish performance and hence the need for inoculation for better performance (Nwabanne *et al*., 2009). A large digester is required since inoculants would be added for more effective digestion.

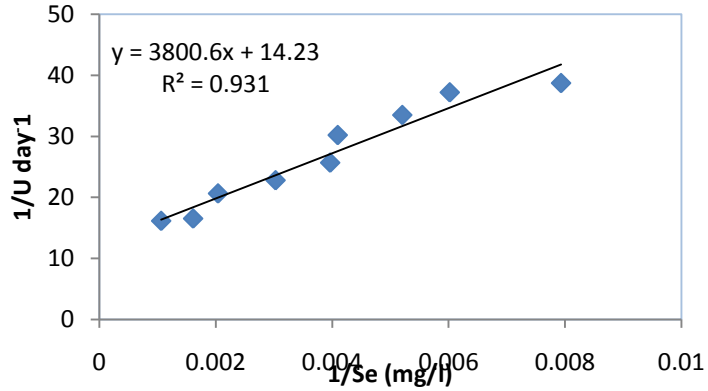

Fig. 4.37: Plot for determination of K and K_s for wild cocoyam digestion.

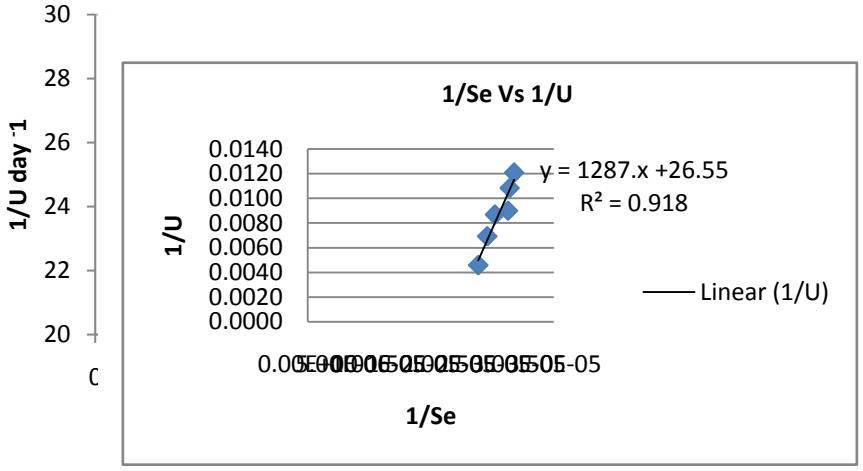

Fig. 4.38: Plot for determination of K and K_s for jackfruit seed digestion

4.13.3 Determination biomass yield (Y) and endogenous decay coefficient (K_d)of the substrates digestion.

To determine the biomass yield Y and endogenous decay coefficient K_d of the substrates digestion, the linear graph of the inverse mean cell residence, $1/\theta$ against the specific substrate rate of utilization, U was plotted as shown in Figs. 4.39 to 4.41. The correlation coefficients were 0.9342, 0.9310 and 0.9130 for wild cocoyam, jackfruit seed and water yam respectively. From the slope and intercept of the plot, the biomass yield Y were obtained as 0.6102 mg/mg, 0.0452mg/mg and 0.0467mg/mg while the endogenous decay coefficient K_d were 0.0062 day^{-1}, 0.0451 day^{-1}, and 0.0512 day^{-1} for wild cocoyam, jackfruit seed and water yam, respectively.

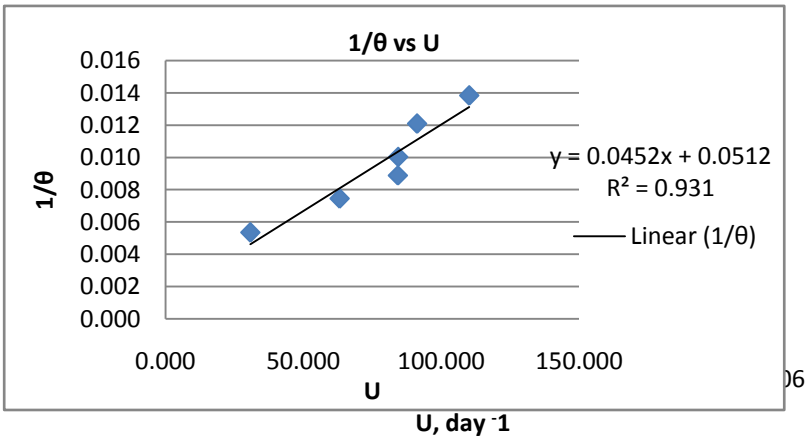

Fig. 4.39: Plot for determination of Y and K_d for water yam digestion

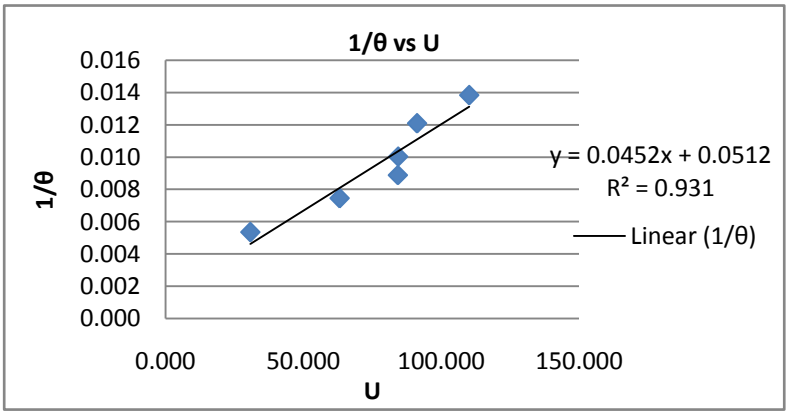

Fig. 4.40: Plot for determination of Y and K_d for jackfruit seed waste digestion.

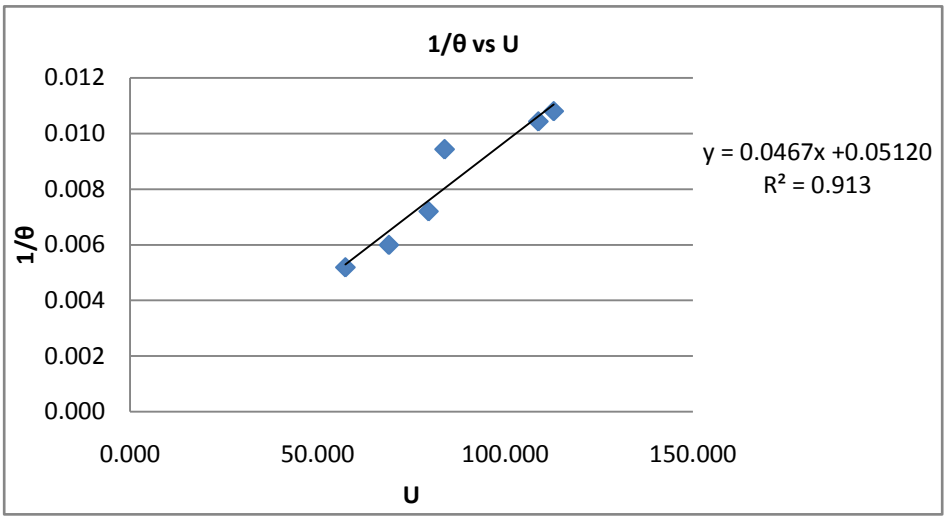

Fig. 4.41: Plot for determination of Y and K_d for water yam digestion

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The starches of wild cocoyam, jackfruit, water yam and yellow yam which have largely been reported to be underutilized were studied for their biofuels potentials. The concerns for food shortage with regards to their use for bioethanol production would be alleviated with their incorporation into the bioethanol feedstock base.

Results obtained from the study indicate that:

(i) Wild cocoyam (*Xanthosoma sagittifolium)* ranked first amongst the feedstocks in all the parameters investigated. Jackfruit and water yam gave good results and compete favourably with each other. Generally, the result show that cocoyam, jackfruit and water yam are very good primary feedstocks for bio-ethanol and biogas production while yellow yam can be supplementary feedstock.

(ii) The study offers a good data base to plant breeders who study the genotypes of the crops.

(iii) Co-digesting the wastes with cow paunch manure would give better yields of biogas thereby, supplementing the energy supply to the industry and providing an effective waste management system.

(iv) The study also shows that biogas can be produced from the crops studied with a considerable rate of decrease in the values of COD, BOD and pH. Thus, the methanogens gradually converts the organic acids into methane gas and CO_2, which indicates that the waste has better anaerobic biodegradability, therefore achieves a waste to resource utilization.

5.2 Recommendations

1. Mechanized cultivation of the crops to boost the feedstock base of the bio-ethanol industries reduce dependent on crops used for humans and animals.
2. Enhance the exploitation of marginal lands.
3. Exploitation of the spent slurry as source of revenue for the industries and cheap fertilizer for agricultural purposes.
4. Inclusion of alternative energy policy in the national plan as a source of energy crops.

5.3 Contribution to knowledge

1. The study provides information on the potentials of the chosen crops for bio-fuels production which will enrich the feedstock lists for industrialists on bio-fuels production.
2. This study provides information on the use of some of the crops facing extinction in bio-fuel production.
3. This study has provided three published journal papers listed below while six others are still in press.

i). Umeghalu, I.C.E., Nwuba, E.I.U., Okonkwo, I.F., Ngini, J.O. and Ajaefobi, J. (2015). Kinetics of biogas production from jackfruit waste co-digested with cow paunch in batch mode. Journals of Agricultural Advances No. 4 (3):pp 34-41. www.sjournals. com

ii). Umeghalu, I.C.E., Nwuba, E.I.U., Onukwuli, D.O., Okonkwo, I.F. and Ngini, J.O. (2015). Kinetics of biogas production from fermentation broth of wild cocoyam co-digested with cow paunch in batch mode. Scientific Journals of Pure and Applied Sciences. 4(3): pp98-104.

iii).Umeghalu, I.C.E., Okonkwo, I.F., Ngini, J.O. and Okoye, C.C. (2015). Performance evaluation of biogas yield from jackfruit waste co-digested with cow

paunch and poultry droppings in batch mode. International Journal of Agriculture and Biosciences . 4(1): pp35-37. www.ijagbio.com

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