THE EFFECT OF ACID PRETREATMENT ON CASSAVA PEELS AND SAWDUST AS FEEDSTOCK FOR THE PRODUCTION OF BIOETHANOL

BY

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CERTIFICATION

This is to certify that this research project is an original work carried out by **EDOKPAYI OSASERE OSEMUDIAMEN** under our supervision in fulfilment of the requirements for the award of Bachelor of Engineering (B.Eng.) Degree in Chemical Engineering, in Chemical Engineering Department of the University of Benin, Benin City, Edo State, Nigeria.

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DEDICATION

This report is dedicated to God almighty for his abundant mercies and grace, also to family and friends for their support throughout my studies.

ACKNOWLEDGEMENTS

I want to appreciate God for the strength to carry out this research work. I also want to acknowledge my wonderful parents Mr. and Mrs. Victor Edokpayi for their support throughout the process of acquiring this degree in chemical engineering. Also, I would love to thank my elder brother (Aisosa Edokpayi) for his encouragement and guidance in the course of this work. I sincerely appreciate Mrs. Ekhator Omosede for her motherly care and support during my study in school. I would not fail to acknowledge my amiable supervisor, Engr. Prof. E. O. Aluyor for his astute leadership, corrections and guidance. I also want to thank Engr. Fred at Luco Scientific Laboratory, Benin City, for all his encouragement and readiness to assist even in inconvenient situations. For guiding and assisting in all practical steps of this work. I want to specially thank Miss Success Osaruonamen Osamwonyi for her assistance and leadership in the course of my research work.

ABSTRACT

This study investigated the effect of process parameters, temperature, hydrolysis time, and acid concentration in the pretreatment of cassava peels and sawdust on sugar yield. The pretreatment steps were performed using the Box Behnken full factorial of a central composite design (CCD) in a response methodology (RSM) with DESIGN EXPERT. Dilute acid was applied in the pretreatment process to enhance the generation of fermentable sugar yields by the DNS method. Forty six (46) experimental runs were carried out at parameters range of; temperature (30 – 100°C), HCl concentration (1 – 5%w/w), and time (15 – 90 mins). The concentration of the reducing sugar produced for each was used to study the effect of variation of process parameters. The results reveal that temperature, time and acid concentration significantly affected sugar yield. The optimum amount of the reducing sugar yield 6327.77 mg/L was obtained at 5/5g, 65°C, 52.5min, 3%w/w.

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LIST OF ABBREVIATIONS

ABBREVIATION	MEANING
ANOVA	Analysis of Variance
CCD	Central Composite Design
DNS	Dinitrosalicylic Acid
DOE	Design of Experiments
GHG	Greenhouse gases
HMF	Hydroxymethylfurfural
LCW	Lignocellulose Waste
OVAT	One Variable At a Time
RSM	Response Surface Methodology

CHAPTER ONE

1.0 BACKGROUND OF STUDY

1.1 Introduction

The society of the 21st century is facing considerable challenges related to the increase in population and in the demand for food and energy, the depletion of fossil resources, oil price fluctuations, energy security and climate change. New policies addressing these issues are being introduced to improve sustainability, and reduce the negative effects on the environment resulting from human activities. Global concern about climate change and the consequent need to diminish greenhouse gas emissions have encouraged the use of bioethanol as a gasoline replacement or additive (Balatetal, 2007), this is because cellulosic ethanol and ethanol produced from other biomass resources have the potential to cut greenhouse gas emissions by 86% (Wyman et al., 1994). Increase in world‘s energy demand and the progressive depletion of oil reserves motivate the search for alternative energy resources, especially for those derived from renewable materials such as biomass (Mandel, *et al.*, 2009). Expanding current biofuel production from sugar and starch-based crops has raised concerns about competition with crops cultivated for food and natural resources, such as water and productive land (sun 2000).

Man’s agricultural activities have led to the production of large quantities of agricultural waste biomass that tends to occupy and pollute the environment. These wastes biomass consist of cellulose, hemicellulose, lignin and other materials called extractive. Among all the constituents of agricultural wastes biomass, cellulose constitutes the highest percentage because it is a strong elastic material that forms the cell wall of nearly all plants (kulkarni et al, 2015). The conversion of the cellulose and hemi-cellulose from waste materials to sugars, these sugars can serve as feedstock in the production fuel ethanol and will substantially reduce the amount of waste.

Renewable biomass fuels such as bioethanol, biodiesel, bio-hydrogen, etc., derived from sugarcane, cassava, sawdust, corn, switch grass, algae, etc, can replace petroleum-based fuels. Bioethanol, an ethanol liquid which is a clean fuel for combustion engines, is a readily available substitute for fossil fuels since it can be derived from plant-based materials which are renewable. The total consumption of bioethanol in 2008 was more than 65,000 million liters and the usage is growing rapidly because it has already replaced 5.4 % gasoline usage in 2013 (Danmaliki *et al.*, 2016).

Ethanol also known as alcohol is a colorless, flammable, volatile liquid with a molecular formula of C_2H_6O. It has a molar mass of 46.07 g/mole, a density of 0.789 g/cm^3, a melting point of −114 °C, and a boiling point of 78.37°C (Chechet, 2016). It is widely used as a solvent, a fuel, and a raw material for the production of other useful chemicals that have wide applications in the industry. It is also consumed as alcohol beverage, for household heating, and applied as an antiseptic. It is produced from ethylene hydration and fermentation of sugars, starch, lignocellulosic materials, or hydrocarbon-based bioethanol production.

Bioethanol is at present the most widely used liquid biofuel for motor vehicles (Sarkar *et al.*, 2012), The importance of ethanol is increasing due to a number of reasons such as global warming and climate change. Ethanol which is a renewable source of energy, which can be produced domestically, has been subject to many research. Bioethanol has been receiving widespread interest at the international, national and regional levels. (Sarkar *et al.*, 2012). It is highly advantaged over other conventional fuels; it has higher octane rating and it is safer to use because of its clean and proper burning quality. National policies are being framed to replace the use of oil with renewable biofuels such as biodiesel and bioethanol (Jahid et al., 2018). However, the production of ethanol from lignocellulosic food crops is a challenge due to the availability of food at such large scale and to the continual use of these feedstock can lead to food and fuel controversy. Also the use of agroresidues, forest residues or MSW etc. would produce insufficient amount of ethanol (Jahid et al., 2018), hence the reason why other agricultural waste are explored as feed stocks which are consumed at a higher rate and generate a large amount of sugary and fibrous waste (Otulugbu, 2012).

Feedstock pretreatment has been recognized as a necessary upstream process to remove lignin and enhance the porosity of the lignocellulosic biomass before the enzymatic process (Zhu & Pan, 2010; Kumar et al., 2009). Lignocellulose hydrolysis using dilute acid solutions has been studied intensively and reviewed by (Steinbach et al., 2017). Most experimental studies are performed in batch systems, where intermediate compounds like sugar undergo successive reactions (Lenihan et al., 2010). The formation of intermediates can be examined in detail using a semi-continuous reaction setup, providing new insights into the acid hydrolysis process. The selection of optimal lignocellulose pretreatment conditions is essential for maximizing the product's sugar yield. Furthermore, minimizing production costs is vital for the industrialization of a process. As a result,

an optimal balance should be found between high yields at high reaction conditions and, on the other hand, simple processes at short reaction times, resulting in small equipment size and investment.

1.2 Aim and Objectives

The research project aimed to investigate the effect of acid pretreatment of cassava peels and sawdust using Box Behnken Design.

The objectives of the research study were:

1. To evaluate the variations of pretreatment conditions such as time, temperature, and acid concentration on cassava peels and sawdust.
2. To use a suitable model to generate optimum pretreatment conditions using the DESIGN EXPERT software package.
3. To estimate the fermentable sugar yield in the cassava peels and sawdust by the DNS method.

1.3 Scope of Work

This study was carried out to investigate the effect of different process parameters on the pretreatment of cassava peels and sawdust and how they affect sugar yield and to assess the suitability of cassava peels and sawdust for producing bioethanol to reduce waste in the environment.

1.4 Problem Statement

The adverse effect caused by mineral fuels over time to the ecosystem especially in Nigeria where exploration, drilling and refining of petroleum has become a practice, as it cannot be overemphasized as they took stake on humans and plants, a compromise is therefore reached to proffer solution to this threats posed by fossil fuels by initiating an alternative form of fuel from plant origin [biomass] whose features can suit the ecosystem and can even work with mineral fuels.
An increase in the annual weight percent carbon emitted into the atmosphere driving climate change has stimulated researchers to produce bioethanol used as biomass fuel from various raw materials or feedstock.

Many of these agro-wastes are allowed to rot away not utilized (kulkarni et al, 2015). These wastes end up in open dumps or drainage system which is hazardous to surface water and human health. Hence, the need for the advancement of better alternative fuel and source of fuel such as bioethanol and agricultural waste respectively.

1.5 Study Relevance

Meeting the growing need for energy, heating, and industrial operations, as well as providing materials for the industry in a sustainable manner, is one of the society's major issues in the 21st century. Ethanol is a chemical substance that belongs to the 'Alcohol' class of organic molecules. Ethanol can be employed as an ingredient in alcoholic beverages, can be used as solvent, germicide, antifreeze, fuel and a flexible intermediary for other organic molecules (Otulugbu, 2012).

Ethanol is a colorless, volatile, flammable liquid with a faint odor. It has a scorching taste in dilute aqueous solution. Ethanol has been produced by fermenting sugars since prehistoric times. This technique still produces all ethanol drinks and more than half of industrial ethanol. The sole distinction between bioethanol and synthetic ethanol is that synthetic ethanol is made from fossil raw materials, whilst bioethanol is made from modern materials (Broni-Bediako et al., 2017). Chemically, they are the same substance, with the only change being the isotopic composition of carbon atoms. Bio-ethanol is thought to be one of the most promising renewable fuels. It's used in medications, cosmetics, and industrial goods, and it's becoming more popular every year. Bio-ethanol manufacturing has recently become a focus of interest due to rising pricing and global environmental concerns (Oliveira *et al.*, 2017).

Bioethanol feedstock can be divided into three categories: sugar-based feedstock, starchy materials, and lignocellulose biomass. Sugar-based feedstock is a good source of fermentable sugars and can be used to make ethanol by direct fermentation. When compared to processes that use starchy materials or lignocellulose biomass as raw materials, direct fermentation of sugars offers lower ethanol production costs (Razmovski, 2012). The analysis presented in this study will convey valuable information for future research exploring the benefits of utilizing cassava peels and sawdust for biofuel production.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Renewable energy

Renewable energy is energy in which its source is gotten from renewable resources which can be naturally replenished on a regular basis. Its source is unlimited natural resources that can be replaced within a short period of time (Gorjian, 2017). Renewable energy resources provide a significant opportunity for improved energy efficiency across a different part of the world. An increased deployment of renewable energy would create an avenue for significant energy security and economic benefits. According to REN21's 2017 report, renewable energy contributed 19.3% to the world's global energy consumption and 24.5% to the generation of electricity in 2015 and 2016 respectively. Climate change and global warming concerns has led to an increased support for renewable energy as the governments of different countries are adopting policies that favour renewable energy.

2.1.1 Different forms of Renewable energy

There are different forms of Renewables in the world today which can be used to generate electricity and energy for the manufacturing and transport sectors of various countries in the world. Some of these renewables include:

- Solar energy
- Wind energy
- Geothermal energy
- Hydropower
- Ocean energy
- Bioenergy which are Biofuels

2.2 Biofuels

Biofuels are gaseous, liquid or solid fuel gotten from biological vegetation also referred to as "biomass". This biomass are vegetation or plants in which through photosynthesis captures CO_2 which are used by plants, these plants and its products in turn are used as feedstock for biofuel production. Various plants and its materials are used for biofuel production such as Sugar crops,

wood and its by products, agricultural wastes and residues, animal wastes etc. Biofuels are currently one of the most important sources of renewable energy for road transportation as they are blended with gasoline and used in Internal Compression engines. The use of biofuels is rapidly growing around the world and a debate between its supporters and critics is at its peak. There is an increasing demand for energy which is projected to double by the mid-21st century, it is expected that biofuels would be and important contributor to this energy mix and would quench the thirst of this high demand for energy (Gadonneix, Barnés De Castro, & Drouin, 2009). There are numerous reasons for the interest in biofuels which is currently spreading around the world and driving the increase in production of biofuels .The reasons include the need to have different sustainable source of energy, counter increasing crude oil prices, curb the environmental pollution from agricultural waste product and actively address the important issue of global warming. Currently, two countries Brazil and USA are the major producers of biofuels and they account for over 80% of global biofuels production which is majorly bioethanol (Gadonneix et al., 2009).

2.3 Generations of biofuels

This refers to the different approaches of bioethanol production over the centuries worldwide. Currently, there are two generations of bioethanol production i.e. first and second generations and a third and fourth generation still under development.

2.3.1 First Generation Biofuel

First generation biofuels, also called conventional biofuels, are gotten from sugar, starch or vegetable oil. First generation biofuels are produced using well-understood technologies and processes, like fermentation, distillation and transesterification (NW, 2007). These processes have been in use for hundreds of years in many uses, such as making alcohol. Sugars and starches are fermented to produce majorly ethanol, and in smaller quantities, butanol and propanol. Global production of first-generation bio-ethanol in 2006 was about 51 billion litres, with Brazil (from sugar cane) and the United States (from maize) each contributing about 18 billion litres, or 35 per cent of the total (Eric D. Larson, 2008). China and India contributed 11 per cent to global ethanol production in 2006, and production levels were much lower in other countries, with feedstocks that include cane, corn, and several other sugar or starch crops (sugar beets, wheat, potatoes) (Eric D. Larson, 2008).

2.3.2 Second Generation Biofuel

Second generation ethanol feedstock are majorly from agricultural wastes such as corn stover, sugarcane bagasse and also from wood, grasses or the non-edible parts of plants. It is produced from lignocelluloses, a structural material that consists much of the mass of plants. The ethanol is not gotten from the starch component like the first generation ethanol, but from the lignocellulosic part of the feedstock (IEA, 2011). There are large sources of lignocellulose are available including non-food wild plants which grow in non-cultivated and non-arable lands. The second generation ethanol feedstocks overcome the two main challenges for the first generation feedstock: adverse effects on food prices and inability to scale. Lignocellulosic materials including agricultural wastes, forestry residues, grasses and woody materials have great potential for bio-fuel production (Anwar et al., 2014).

2.3.3 Third Generation Biofuel

These biofuels include specific production of biodiesel and other algal-based biofuels like ethanol and biogas (Aro, 2016). Third generation biofuels research and development is largely focused on algae as a raw material. Early research demonstrated that energy yields from a given surface area are far greater from algae than from plants currently used in producing biofuels (Bowyer *et al.*, 2018). Microalgae have the potential to yield 15-300 times more oil for the production of biodiesel than the traditional crops on area basis (Hameed, 2018b).

2.3.4 Fourth Generation Biofuel

Fourth generation biofuel (FGB) uses genetically modified (GM) algae to enhance biofuel production. Although GM algae biofuel is a well-known alternative to fossil fuels, the potential environmental and health-related risks are still of great concern (Abdullah *et al.*, 2019).

2.4 Different forms of Biofuel

Biofuel comes in different forms and these forms of biofuel are processed differently from each other and they all have different properties and energy content. The different forms of biofuel include the following which are;

- Bioethanol
- Biodiesel

- Biogas
- Biobutanol

2.4.1 Bioethanol

Ethanol also called alcohol is a colorless, flammable, volatile liquid with a molecular formula of C_2H_6O. It has a molar mass of 46.07 g/mole, a density of 0.789 g/cm3, a melting point of −114°C, and a boiling point of 78.37 °C. It is widely used as a solvent, a fuel, and as a raw material for the production of other useful chemicals that have wide applications in the industry. It is also consumed as alcohol beverage, for household heating, and applied as an antiseptic. It is used as a substitute to petrol, can also blended with petrol to reduce the amount of petroleum consumed. ethanol is produced from ethylene hydration and fermentation of sugars, starch, lignocellulosic materials, or hydrocarbon-based ethanol production (Danmaliki *et al.*, 2016).

2.4.2 Biodiesel

Biodiesel is a fuel derived from biological materials such as vegetable oils, animal fats or oil from algae. Biodiesel is produced from a process called Transesterification, which involves a chemical reaction of vegetable or waste oil and ethanol and methanol. Biodiesel properties are similar to the properties of fossil diesel fuel, which can be used in a standard diesel engine without modification. The most common biofuel in Europe is Biodiesel (Gujral, 2014). In a number of countries, 5% biodiesel blend is widely used and is available at thousands of gas stations. Biodiesel is an oxygenated fuel, meaning that it contains a reduced carbon content and higher hydrogen and oxygen content than conventional diesel (Gujral, 2014). This improves the combustion of biodiesel and reduces the particulate emissions from un-burnt carbon.

2.4.3 Biogas

Biogas can be made by the biodegradation of organic material such as human and animal waste product by bacterial decomposition of this organic matter in the absence of air (Shaaban & Petinrin, 2014). Some of the feedstock used for biogas production are animal dung, industrial wastes, household wastes, food wastes, green wastes, and agricultural residues. The mixture of different types of wastes would lead to the production of more biogas energy (Shaaban & Petinrin, 2014). Biogas is produced by anaerobically digesting anaerobic organisms or methanogen which digest materials in a bioreactor. Biogas is basically methane CH_4 and carbon (iv) oxide CO_2 and may

contain small amounts of hydrogen sulphide H_2S, moisture and siloxanes. Biogas can be compressed and used to power vehicles. It is suitable for a variety of application in the agricultural, household, and industrial sectors.

2.4.4 Butanol

Butanol is an alcohol which can be gotten or produced through petrochemical processes, can also be produced, like ethanol, through fermentation of sugars derived from starch and agricultural crops. 1-Butanol (butyl alcohol or n-butanol) is a four carbon straight chained alcohol with a molecular formula of C_4H_9OH, molecular weight of 74.12g and boiling point of 118 C. 1-Butanol is an important chemical precursor for paints, polymers and plastics. In 2008, the global market for 1-butanol was 2.8 million, estimated to be worth approximately $5 billion (Green, 2008).

2.5 Properties of ethanol

2.5.1 Physical properties

Ethanol at standard temperature and pressure is volatile, flammable, clear, colourless liquid. Its odour is pleasant, familiar and has a characteristic sweet taste when diluted with water. The physical and chemical properties of ethanol are solemnly dependent upon the hydroxyl group. These two properties are to a greater extent responsible for the abnormal physical behaviour of lower molecular weight alcohols as compared to hydrocarbons of equivalent weight (Fredrickso et al., 1983). Infrared spectrographic studies have shown that, in the liquid state, hydrogen bonds are formed by the attraction of the hydroxyl hydrogen of one molecule and the hydroxyl oxygen of a second molecule. The net effect of this bonding is to make liquid alcohol behave as though it were largely dimerized. This behaviour is analogous to the behaviour of water, which however is more strongly bonded and appears to exist in liquid clusters of more than two molecules (Tsao GT et al., 1975).

2.5.2 Chemical properties

The chemical properties of ethyl alcohol are predominantly the function of the hydroxyl group and the reactions involved includes dehydration, dehydrogenation, esterification and oxidation. Active metals, such as sodium, potassium and calcium can be used to replace the Hydrogenatom of the hydroxyl group with resulting metal ethoxide (ethylate) formation and Hydrogen gas evolution.

Sodium ethoxide is produced by the reaction of absolute ethyl alcohol and sodium or by refluxing absolute ethyl alcohol with anhydrous sodium hydroxide which addition of anhydrous acetone leads to the precipitates of the sodium ethoxide. This strong base submissively hydrolyses to give ethyl alcohol and sodium and hydroxyl ions (Blakebrough, et al., 1965).

2.6 Production of ethanol

Ethanol can be produced via two main methods; chemical synthesis and biological production (Adsul, 2005).

2.6.1 Chemical synthesis of ethanol

This is an option through which ethanol is been produced by hydration of ethylene an approach employed mostly by petrochemical industries. Ethanol can be produced from a reaction of ethane with steam in a reversible exothermic reaction. For each run, about 5% of ethane can be recovered. An overall 95% conversion rate can be achieved by removing the ethanol from the recycling mixture (Chandraken et al., 2005).

2.6.2 Biological production of ethanol (fermentation)

The techniques, approaches and steps involved in biological means of ethanol production of ethanol depend on the starting materials. Nevertheless, the yeast Saccharomyces cerevisiae, through Calvin cycle, converts the sugars to carbon dioxide and alcohol by fermentation. Sugar cane, citrus fruits, punk, and juice of trees contain simple sugars like glucose and fructose that can be fermented direct by microorganisms.While for starch and glycogen containing materials such as rye, corn, wheat and sorghum, saccharification of the carbohydrate before the subsequent microbial conversion to ethanol is needed. Both cellulosic and lignocellulosic substrates require pretreatment before fermentation (Aiba, 1973). Ethanol production from lignocellulosic biomass is still developing, even though many technologies for that are established (laser, 2002). The standard ethanol production approach in the U.S. today is the conversion of corn starch to ethanol at a significant rate of 2.7 gallons of ethanol per bushel of corn, a three-step procedure consisting of a wet and/or dry milling procedure in series with a hydrolysis and fermentation step. The milling step (wet or dry) in ethanol production is the most costly step. .

2.7 Bioethanol Production from Biomass

Lignocellulose biomass can be converted to a wide variety of biofuels and chemicals by proper conversion processes. Lignocellulose biomass must be pretreated to modify the structural properties and increase enzymatic digestibility. This step can also be carried out chemically using dilute sulphuric acid or other acids. The sugars produced are then fermented to ethanol via yeast fermentation (Olsson & Hahn-Hagerdal, 1996).

The reaction equation for fermentation of sugar into ethanol;

Photosynthesis

$$6CO_{2\,(g)} + 6H_2O_{\,(l)} + \text{light} \longrightarrow C_6H_{12}O_{6\,(l)} + 6O_{2\,(g)} \quad \ldots \text{(Eq 2-1)}$$

Fermentation

$$C_6H_{12}O_{6\,(l)} \longrightarrow 2C_2H_5OH_{\,(l)} + 2CO_{2\,(g)} + \text{Heat} \quad \ldots \text{(Eq 2-2)}$$

Combustion

$$C_2H_5OH_{\,(l)} + 3O_{2\,(g)} \longrightarrow 2CO_{2\,(g)} + 3H_2O_{\,(l)} + \text{Heat} \quad \ldots \text{(Eq 2-3)}$$

$$\text{Light} \longrightarrow \text{Heat}$$

2.8 Lignocellulosic Biomass

Lignocellulosic materials are the most promising feedstock as natural and renewable resource essential to the functioning of modern industrial societies (Anwar et al., 2014). Lignocellulose is the most abundant renewable biomass; its annual production has been estimated in 1×10^{10} MT worldwide (Alvira et al., 2010). Production of biofuels as transportation fuels from lignocellulosic biomass is a feasible alternative for improving energy security and reducing greenhouse effects on the environment. Unlike fossil fuels, which is gotten from plants that grew millions of years ago, biofuels are gotten from plants grown today. They have cleaner combustion than fossil fuels, and the short cycle of growing plants and burning fuel made from them does not add CO_2 to the atmosphere (Sarkar *et al.*, 2012) .

All plant materials majorly consist of cellulose, hemicellulose and lignin (Chen, 2014). Lignocellulosic materials including agricultural wastes, forestry residues, grasses and woody

materials have great potential for biofuel production. Typically, most of the agricultural lignocellulosic biomass is comprised of about 10-25% lignin, 20-30% hemicellulose, and 40-20% cellulose (Anwar et al., 2014).

For the conversion of biomass to fuel, the cellulose and hemicellulose must be broken down into their corresponding monomers (sugars), so that microorganisms can utilize them. Three major hydrolysis processes are typically used to produce a variety of sugars suitable for ethanol production: dilute acid, concentrated acid, and enzymatic hydrolysis.

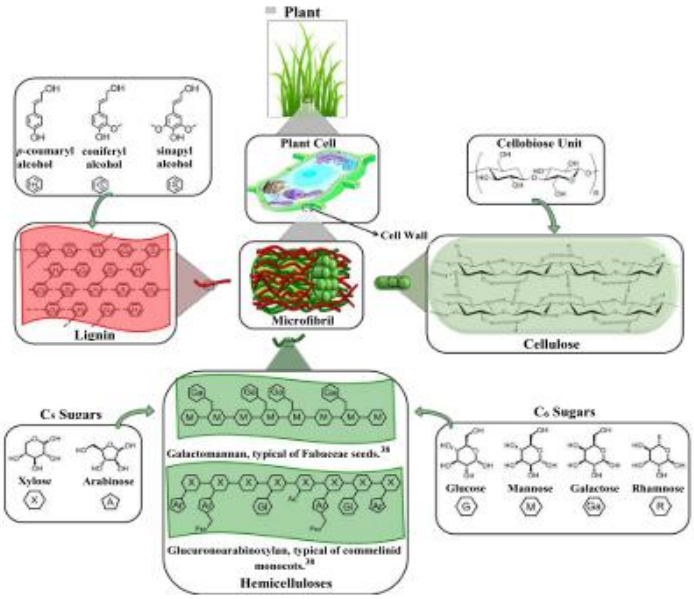


Figure 2.1. The main components and structure of lignocellulose. (Anwar et al,. 2014)

2.8.1 Physico-chemical characteristics of lignocellulosic biomass

Cellulose is a major structural component of plant cell walls, which is responsible for mechanical strength while hemicellulose macromolecules are often repeated polymers of pentoses and hexoses. Lignin contains three aromatic alcohols (coniferyl alcohol, sinapyl alcohol and p-coulmary alcohol) produced through a biosynthetic process and forms a protective seal around the other two components- cellulose and hemicellulose. In general the composition of lignocel- lulose highly depends on its source whether it is derived from the hardwood, softwood, or grasses.

2.8.2 Composition of Lignocellulosic Biomass

Lignocellulosic biomass contains the following components in polymeric form; cellulose, hemicellulose, lignin and minor amounts of other substances (Satarn, Lamamorphanth, & Kamwilaisak, 2014). Different plants contain varying ratios of lignocellulosic materials. 30-60% cellulose, 20-40% hemicellulose and 15-25% lignin are the percentage content on a moist- free basis (Meyer, 2016). Xylans and also be found in lignocellulosic biomass (W. Chen & Kuo, 2011). Lignocellulosic biomass is difficult to break down from starch to simple sugars due to the presence of a complex cell structure and majorly lignin (Hisano, Nandakumar, & Wang, 2009) The various compositions of lignocellulosic biomass are discussed in detail in subsequent sections.

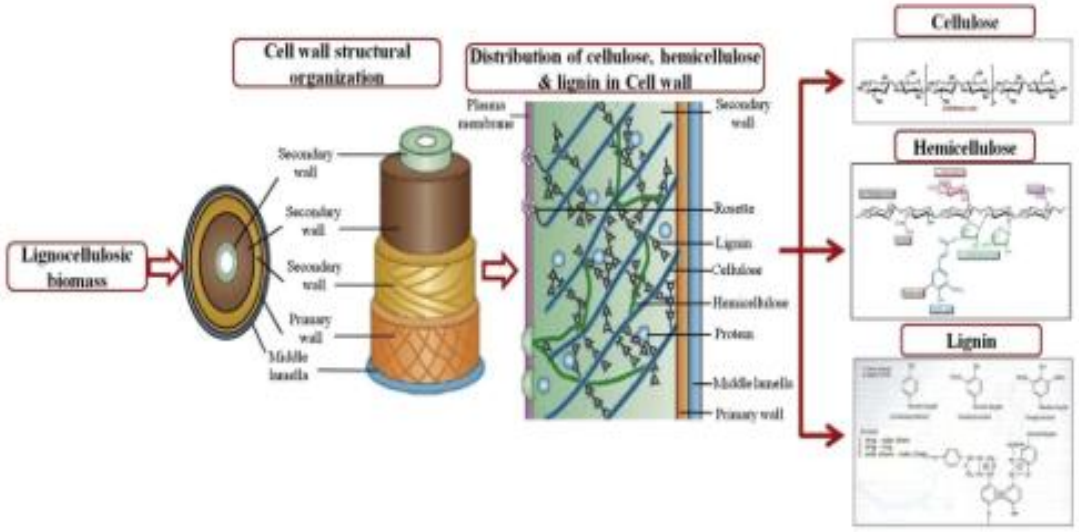


Figure 2.2 framework of lignocellulose; cellulose; hemicellulose and lignin. (Anwar et al., 2014)

2.8.2.1 Cellulose

Cellulose is a major structural component of plant cell walls, which provides mechanical strength while, hemicellulose macromolecules are often repeated polymers of pentose and hexoses. Lignin contains three aromatic alcohols (coniferyl alcohol, sinapyl alcohol and p- coumaryl alcohol) produced through a biosynthetic process and forms a protective seal around the other two components i.e., cellulose and hemicelluloses. Lignocellulosic materials including agricultural wastes, forestry residues, grasses and woody materials have great potential for bio-fuel pro- duction. Typically, most of the agricultural lignocellulosic biomass is comprised of about 10-25% lignin, 20-30% hemicellulose, and 40-50% cellulose (Anwar *et al.*, 2014). Cellulose is a highly stable polymer

consisting of glucose and attached with linear chains up to 12,000 residues. The major component of lignocellulosic biomass is cellulose. Unlike to glucose in other glucan polymers, the repeating unit of the cellulose chain is the disaccharide cellobiose (Isikgor and Becer, 2015).

2.8.2.2 Hemicellulose

Hemicellulose is the second most abundant polymer, unlike cellulose, hemicellulose has a random and amorphous structure, which is composed of several heteropolymers including xylan, galactomannan, glucuronoxylan, arabinoxylan, glucomannan and xyloglucan (Isikgor and Becer, 2015). There is a difference in composition in hemicelluloses; hardwood hemicelluloses contain mostly xylans, whereas softwood hemicelluloses contain mostly glucomannans. The heteropolymers of hemicellulose are composed of different 5- and 6-carbon monosaccharide units; pentoses (xylose, arabinose), hexoses (mannose, glucose, galactose) and acetylated sugars (Isikgor and Becer, 2015). Hemicelluloses are imbedded in the plant cell walls to form a complex network of bonds that provide structural strength by linking cellulose fibres into microfibrils and cross-linking with lignin.

2.8.2.3 Lignin

Lignin is the most complex and least fraction, representing about 10-25% of the biomass by weight (Anwar et al,. 2014). It has a long-chain, heterogeneous polymer which has a high quantity of phenyl-propane units most commonly linked by ether bonds. Lignin is a three dimensional polymer of phenylpropanoid units, it functions as the cellular glue which provides compressive strength to the plant tissue and the individual fibres, stiffness to the cell wall and resistance against insects and pathogens (Isikgor and Becer, 2015). It is found in all plant biomass, and classified byproduct or as a residue in bioethanol production. Lignin is comprised of complex and large polymer of phenyl-propane, methoxy groups and non- carbohydrate poly phenolic substance, which bind cell walls component together (Anwar et al,. 2014).

2.9 Pretreatment

The pretreatment of lignocellulosic biomass is a necessary step that must be taken in order breakdown biomass building blocks and provide the necessary surface area for enzymatic hydrolysis which would result in a high yield of reducing sugar for microbial fermentation (Kumar & Sharma, 2017; Meyer, 2016). The basic essence of pretreatment is to reduce the lignin content and the crystalline structure of the cellulose present in the lignocellulosic feedstock. There are

conditions which a pretreatment process must meet, some of which include; effectiveness over a wide range, recovery of lignocellulosic components in useable form in separate fractions and it should have a low capital and operational cost (Agbor et al., 2011).

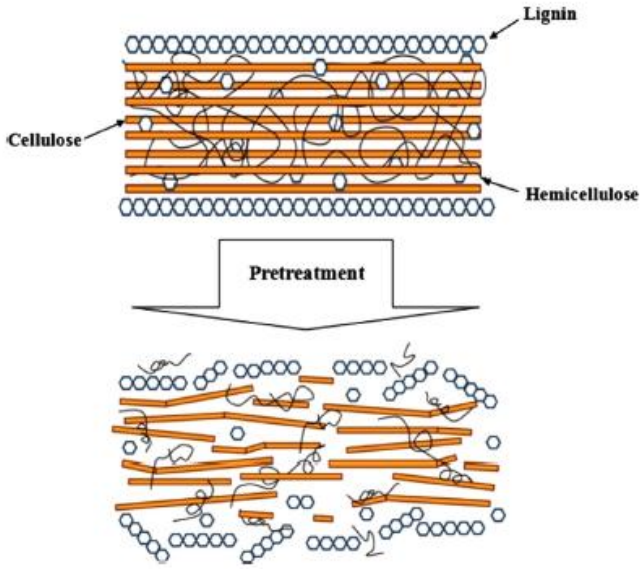


Figure 2.3 Schematic of pretreatment of a lignocellulosic biomass. Source:(Haghighi et al., 2013).

2.9.1 Pretreatment technologies for lignocellulosic biomass

Pretreatment technologies result in efficient lignin removal from the biomass structure and the breakdown of crystalline cellulose for easy enzymatic hydrolysis. Lignocellulosic biomass pretreatment is classified into: Biological and Non Biological pretreatment which is based on the type of pretreatment method to be used (Kumar & Sharma, 2017). Biological methods involves the usage of microorganisms such as rot fungi for lignin degradation (Meyer, 2016) but the rate of biological pretreatment is too slow for industrial purposes (Agbor et al., 2011). Non biological pretreatment method doesn't involve the use of microorganisms and can be divided into different methods which are; physical methods, chemical methods and physio-chemical methods (Kumar & Sharma, 2017).

2.9.1.1 Physical Pretreatment Method

2.9.1.1.1 Mechanical Extrusion

It is the most common biomass pretreatment method in which the feedstock materials are heated (>300°C) under shear mixing. This pretreatment process primarily yields gaseous products and char from pretreated lignocellulosic biomass residues (Shafizadeh & Bradbury, 1979) . This method requires a significant amount of high energy, making it expensive and challenging to scale up for industrial purposes (Zhu & Pan, 2010) . These studies clearly show that mechanical extrusion treatment affected the breakdown of cellulose and hemicellulose fractions from a wide range of lignocellulosic feedstocks; however, when combined with other pretreatment methods, mechanical extrusion performs better and may increase overall reducing sugar yields.

2.9.1.1.2 Milling

Mechanical grinding (milling) is used to reduce cellulose crystallinity. It primarily consists of chipping, grinding, and milling techniques. Chipping can reduce the biomass particle size to 10–30 mm, whereas grinding and milling can reduce the particle size to 0.2mm. However, studies have found that further reduction of biomass particle size below 0.4mm has no significant effect on hydrolysis rate and yield (Chang VS, 1997). The type and duration of milling, the type of biomass, and the final degree of polymerization determine the increase in specific surface area and cellulose crystallinity. To improve the digestibility of lignocellulosic materials, various milling methods such as two-roll milling, hammer milling, colloid milling, and vibratory milling are used (Taherzadeh & Karimi, 2008) . Vibratory ball milling is more effective than conventional milling in reducing cellulose crystallinity and improving the digestibility of spruce and aspen chips. Wet disk milling is also a popular mechanical pretreatment method due to its low energy consumption. Disk milling improves cellulose hydrolysis by producing fibers, and it is more efficient than hammer milling, which produces more delicate bundles (Zhua JY, 2009).

2.9.1.1.3 Pyrolysis

In biorefinery processes, pyrolysis has also been used for the pretreatment of lignocellulosic biomass. This is in contrast to bioethanol applications, where pyrolysis produces bio-oil from lignocellulosic feedstock. Although there have been few studies on pyrolysis to reduce sugar production, there have been few reports on using pyrolysis in the pretreatment of chemically pretreated biomass. Pyrolysis is classified as slow or fast based on the rate of heating. The amount

of the end product varies depending on the type of pyrolysis used, the characteristics of the biomass, and the reaction parameters. Pyrolysis is more efficient at lower temperatures when performed in the presence of oxygen. (Kumar & Wyman, 2009)

2.9.1.2 Chemical pretreatment

Chemical pretreatment methods involve the usage of dilute acid, alkali, ammonia, organic solvent, SO_2, CO_2 or other chemicals. These methods are easy in operation and have good conversion yields in short span of time.

2.9.1.2.1 Acid pretreatment

Acid pretreatment is considered as one of the most important techniques and aims for high yields of sugars from lignocellulosics. It is usually carried out by concentrated or diluted acids (usually between 0.2% and 2.5% w/w) at temperatures between 130°C and 210°C. Sulphuric acid is widely used for acid pretreatment among various types of acid such as hydrochloric, nitric and phosphoric acids (Cardona et al., 2009). Acid pretreatment can utilize either dilute or concentrated acids to improve cellulose hydrolysis (Balatet al., 2008). The acid medium attacks the polysaccharides, especially hemicelluloses which are easier to hydrolyse than cellulose (Cardona et al., 2009). However, acid pretreatment results in the production of various inhibitors like acetic acid, furfural and 5-hydroxymethylfurfural. These products are growth inhibitors of microorganisms. Hydrolysates to be used for fermentation therefore need to be detoxified. Moiseret al., (2005) reported higher hydrolysis yield from lignocellulose pretreated with diluted H2SO4 compared to other acids. A saccharification yield of 74% was obtained from wheat straw when subjected to 0.75%v/v of H_2SO_4 at 121°C for 1hr. (Sahaet al., 2005).

2.9.1.2.2 Alkaline Pretreatment

Alkaline pretreatment of lignocellulosics digests the lignin matrix and makes cellulose and hemicellulose available for enzymatic degradation (Pandey et al., 2000). Alkaline pretreatment processes utilize lower temperatures and pressures than other pretreatment technologies (Mosier et al., 2005b). Alkali treatment of lignocellulose disrupts the cell wall by dissolving hemicelluloses, lignin, and silica, by hydrolysinguronic and acetic esters, and by swelling cellulose. Crystallinity of cellulose is decreased due to swelling. By this process, the substrates can be fractionated into alkali-

soluble lignin, hemicelluloses and residue, which make it easy to utilize them for more valuable products. The end residue (mainly cellulose) can be used to produce either paper or cellulose derivatives (Cardona et al., 2009). Hydroxides of sodium, potassium, calcium and ammonium can be used in this process. Sun et al., (1995) studied the effectiveness of different alkaline solutions by analysing the delignification and dissolution of hemicellulose in wheat straw. They found that the optimal process condition was that using 1.5% NaOH for 144 hr. at 20°C, releasing 60% and 80% lignin and hemicellulose respectively.

2.9.1.2.3 Wet oxidation

In wet oxidation, the feedstock material is treated with water and either by air or oxygen at temperatures above 120°C. The water is added to the biomass at a ratio of 1L per 6g of biomass (Martín et al., 2007). The transfer of hemicelluloses from solid phase to the liquid phase is promoted in this technique. It does not hydrolyse the liberated hemicellulose molecules. The products of hemicellulose hydrolysis during wet oxidation are sugar oligomers (Cardona et al., 2009).

2.9.1.2.4 Organosolv pretreatment

Organic solvent or organosolv pulping processes are alternative methods for the delignification of lignocellulosic materials. The utilization of organic solvent/water mixtures eliminates the need to burn the liquor and allows the isolation of the lignin (by distillation of the organic solvent). Examples of such pretreatments include the use of 90% formic acid and that of pressurized carbon dioxide in combination (50% alcohol/water mixture and 50% carbon dioxide) (Cardona et al., 2009). Other various organic solvents which can be used for delignification are methanol, ethanol, acetic acid, formic acid and acetic acid and acetone etc. (Zhao et al., 2009). A combination of ammonia and ionic liquid pretreatments of rice straw resulted in 97% conversion of cellulose to glucose (Nguyen et al., 2010).

2.9.1.3 Physicochemical Pretreatment

Pretreatments that combine both chemical and physical processes are referred to physicochemical processes. Physicochemical processes are considerably more effective than physical methods of pretreatment. Different chemical agents employed during these processes are ozone, acids, alkali, peroxide and organic solvents. Several physicochemical methods are employed for pretreatment of

biomass before its saccharification, such as Ammonia Fiber Explosion (AFEX), Autohydrolysis (Steam Explosion), Acid Treatment.

2.9.1.3.1 Steam Explosion (Autohydrolysis)

Steam explosion is a thermo-mechanicochemical pretreatment which allows the breakdown of lignocellulosic structural components by the action of heating, formation of organic acids during the process, and shearing forces resulting in the expansion of the moisture. Two distinct stages compose the steam-explosion process: vapocracking and explosive decompression which include modification of the material components: hydrolysis of hemicellulosic components (mono- and oligosaccharides released), modification of the chemical structure of lignin, and modification of the cellulose crystallinity index, etc. These effects allow the opening of lignocellulosic structures and influence the enzymatic hydrolysis yield of the material (Jacquet *et al.*, 2015)

2.9.1.3.2 Ammonia Fibre Explosion (AFEX)

AFEX is one of the alkaline physico-chemical pretreatment processes. Here, the biomass is exposed to liquid ammonia at relatively high temperature (90-100°C) for a period of about 30min, followed by immediate reduction of pressure. The effective parameters in the AFEX process are ammonia loading, temperature, water loading, blowdown pressure, time and number of treatments. The AFEX process can either modify or effectively reduce the lignin fraction of the lignocellulosic materials, while the hemicellulose and cellulose fractions may remain intact. At optimum conditions, AFEX can significantly improve the enzymatic hydrolysis. The optimum conditions for AFEX depend on the lignocellulosic materials.

2.9.1.3.3 Carbon Dioxide Explosion

In attempts to develop improved lignocellulosic pretreatment techniques, the idea of using supercritical CO_2 explosion, which would have a lower temperature than steam explosion and possibly a reduced expense compared to ammonia explosion, was developed. Superficial fluid refers to a fluid that is in a gaseous form but is compressed at temperatures above its critical point to a liquid-like density. It was hypothesized that, because CO_2 forms carbonic acid when dissolved in water, the acid increases the hydrolysis rate. Carbon dioxide molecules are comparable in size to water and ammonia and should be able to penetrate small pores accessible to water and ammonia molecules. Carbon dioxide was suggested to be helpful in hydrolyzing hemicellulose as well

ascellulose. Moreover, the low temperature prevents any appreciable decomposition of monosaccharides by the acid.

2.9.1.4 Biological Pretreatment

Most pretreatment technologies require expensive instruments or equipment that have high energy requirements, depending on the process (Surya and Kumar, 2018). Biological treatment using various types of rot fungi, a safe and environmentally friendly method, is increasingly being advocated as a process that does not require high energy for lignin removal from a lignocellulosic biomass, despite extensive lignin degradation. In this method, microorganisms are used in disrupting the lignocellulosic biomass under moderate conditions without using any specific instrument for bioconversion. The lignocellulosic biomass delignification activity has been investigated for both bacteria and fungi, and rot fungi having wood-degrading enzymes proved to be highly useful in biomass conversions for biofuels production (Hameed, 2018a). The white rot, brown rot, and soft rot fungi are extensively used in the biological pretreatment of lignocellulosic biomass.

2.10 Cassava peels as Feedstock for Bioethanol Production

Cassava peels are an important environmental waste, notably in Nigeria, where cassava is widely grown. Current disposal techniques frequently entail open dumping or burning, which contributes to environmental degradation and health risks. Cassava peels that are not properly disposed of can contaminate land and water, emit toxic gasses, and lead to disease outbreaks. Cassava peels are widely accessible as a byproduct of cassava processing for food, animal feed, and industrial use. The amount of peels produced is enormous, particularly in areas with strong cassava output.

Cassava peels and wastewater can contaminate soil, lowering its fertility and production. Discharging liquid waste into water bodies can contaminate the water and destroy aquatic life. Burning cassava peels emits toxins into the air, leading to respiratory difficulties. Improper disposal practices can provide breeding grounds for disease vectors, increasing the risk of epidemics.

Anaerobic digestion of cassava peels can provide biogas, a renewable energy source. Cassava peels contain cellulose, hemicellulose, and lignin, all of which can be fermented into sugars. The peels also include nutrients like as crude protein, crude fiber, and ash. Pretreatment procedures, such as

acid or alkaline hydrolysis, are frequently required to break down the complex structure and liberate fermentable sugars from cellulose and hemicellulose.

2.11 Sawdust as Feedstock for Bioethanol Production

Sawdust is an abundant renewable resource on Earth, accounting for roughly half of global biomass. Sawdust contains lignocellulose and it is outside the human food chain, and its energy content often exceeds the world's primary energy requirements. These features make it an essential option as feedstock, a relatively inexpensive raw material, for bioethanol production and for developing other bio industries to face the international demand for the biofuel market. In 2008 it was estimated that 21800 tons of biomass were generated, and only 3% were used in pulp and paper industries (Zhang, 2008; Sánchez & Cardona, 2008; Rutz & Janssen, 2008).

2.12 Optimization

Optimizing refers to improving the performance of a system, a process, or a product in order to obtain the maximum benefit from it. The term optimization has been commonly used in analytical chemistry as a means of discovering conditions at which to apply a procedure that produces the best possible response. Traditionally, optimization in analytical chemistry has been carried out by monitoring the influence of one factor at a time on an experimental response. While only one parameter is changed, others are kept at a constant level (Sudha *et al.*, 2017). This optimization technique is called one-variable-at-a-time. Its major disadvantage is that it does not include the interactive effects among the variables studied. As a consequence, this technique does not depict the complete effects of the parameter on the response. Another disadvantage of the one-factor optimization is the increase in the number of experiments necessary to conduct the research, which leads to an increase of time and expenses as well as an increase in the consumption of reagents and materials. In order to overcome this problem, the optimization of analytical procedures has been carried out by using multivariate statistic techniques.

2.13 Design of Experiment (DOE)

Design of experiment involves the design of any task that aims to describe or explain the variation of information under conditions that are hypothesized to reflect the variation. Design of experiment helps in the understanding of cause and effect relationship in a system. This relationship can be understood by making certain changes to the input variables of a system and observing the effect of

these changes on the response variable or system output variable(Montgomery, 2017). However, in order to change the condition of the input variable to a system and observing its relative effect on the output variable, experiments have to be conducted. Experiments can therefore be defined as a test or series of runs in which purposeful changes are made to the input variables of a system so that the reasons for the changes that may be observed on the output variables may be observed and identified (Montgomery, 2017). Some of the objectives of experiment include;

- Determination of the input variables which are most influential on the response variable.
- Determination of where the controllable variable can be set so that the response can always be near its optimal value (Cavazzuti, 2012)

2.13.1 Principles of Design of Experiment

The basic principles of statistical method in DOE are replication, randomization and blocking. Replication is the repetition of the experiment in order to obtain a more accurate result (sample mean value) and to estimate the experimental error (sample standard deviation).It has two important properties:

- It allows the experimenter to obtain an estimate of the experimental error.
- If the sample mean is used to estimate the true mean response for one of the factor levels in an experiment, replication permits the experimenter to obtain a more accurate estimate of this parameter (Montgomery, 2017)

Randomization refers to the random determination of the order in which the individual runs of the experiments are to be performed. Randomization usually makes assumption valid(Montgomery, 2017). This principles holds that the condition in one run is independent on the conditions of the previous run and does not predict the conditions of subsequent runs (Cavazzuti, 2012).

Blocking refers to the arrangement of experiments in groups that are similar to one another. Blocking is a design technique used to improve the precision with which comparisons between factors of interest are made. An experimental block is a set of relatively homogenous conditions within which different conditions of the primary variables are compared(Telford, 2007). Aside the primary variables or controllable variables, a system has those variables that cannot or should not be held constant, these variables are termed “Noise variables” or “Nuisance factors”. These factors

may have effect on the experimental response but the experimenter is not interested in them. Blocking helps in the reduction or elimination of the variability transmitted by the nuisance factors by isolating the effect of a noise variable and ensuring it does not contaminate the evaluation of the effects of a primary variable (Telford, 2007).

2.13.2 Classes of Experimental Designs

There are several classes of experimental designs. Some of which include; Randomized Complete Block Design (RCBD), Central Composite Design (CCD), Box Behnken Design (BBD), full factorial design and fractional factorial design.

2.13.2.1 Randomized Complete Block Design

The randomized complete block design is one of the most widely used experimental designs. Since it is known that variability which arises as a result of nuisance factors affect experimental result, RCBD helps in eliminating these errors. Nuisance factors has been defined as design factors that have effect on the response variable of a system but these effects are of no interest to the experimenter. A nuisance factor may be unknown and uncontrolled, that is, the existence of such factor is unknown to the experimenter and this factor might be changing levels during the course of experiment. Randomization helps to guard against such factors. At times, nuisance factors may be known but uncontrollable, this implies that these factors take different values during each run of experiment. These values can be compensated for in the statistical analysis using Analysis of co-variance. However, if the nuisance factor is known and controllable, its effect on the statistical comparison among treatments can be systematically eliminated with the aid of a design technique called blocking (Montgomery, 2017).

2.13.2.2 Full Factorial Design

Experiments involve the study of the effect of two or more factors. Factorial design implies that in each complete trial of an experiment, all possible combinations of the levels of the factors involved are investigated. For the vast majority of factorial experiments, each factor has only two levels. For example, with two factors each taking two levels, a factorial experiment would have four treatment combinations in total, and is usually called a 2×2 factorial design.These levels are called high (h) and low (l) or represented as “+” or “-” (Cavazzuti, 2013). The effects of a factor is defined to be

the change in the response produced as a result of change in the level of the factor. This effect is called the main effect as it refers to the primary factor of interest in the experiment.

For a three-factors and two-level per factor experimental design, the table below shows the full factorial design for the experimental design.

Table 2.1 Example of 23 Full factorials experimental design (Cavazzuti, 2013)

Experiment Number	Factor level			Response variable	Two and three factor interactions			
	X_1	X_2	X_3		$X_1.X_2$	$X_1.X_3$	$X_2.X_3$	$X_1.X_2.X_3$
1	-1(*l*)	-1(*l*)	-1(*l*)	$y_{l,l,l}$	+1	+1	+1	-1
2	-1(*l*)	-1(*l*)	+1(*h*)	$y_{l,l,h}$	+1	-1	-1	+1
3	-1(*l*)	+1(*h*)	-1(*l*)	$y_{l,h,l}$	-1	+1	-1	+1
4	-1(*l*)	+1(*h*)	+1(*h*)	$y_{l,h,h}$	-1	-1	+1	-1
5	+1(*h*)	-1(*l*)	-1(*l*)	$y_{h,l,l}$	-1	-1	+1	+1
6	+1(*h*)	-1(*l*)	+1(*h*)	$y_{h,l,h}$	-1	+1	-1	-1
7	+1(*h*)	+1(*h*)	-1(*l*)	$y_{h,h,l}$	+1	-1	-1	-1
8	+1(*h*)	+1(*h*)	+1(*h*)	$y_{h,h,h}$	+1	+1	+1	+1

2.13.2.3 Fractional Factorial Design

In cases where the number of factors or variables affecting the response is more, it becomes difficult and time consuming to run a full factorial design. Fractional factorial design runs only a subset (half or one quarter of the sample size of a full factorial.) of full factorial experiments. This design is able to provide some good information about the main effects and interaction effects. The fractional factorial samples must be chosen in such a way that each factors has the same number of samples for each of its level (Cavazzuti, 2013; Telford, 2007). By considering a one-half fractional factorial

of a 2^K full factorial design, K=3 such that one-half will be equal to 2^{K-1}. The fractional factorial of this design is shown below.

One of the shortcomings of this design is the impossibility to distinguish between the main effects of X_3 (C) and $X_1.X_2$ (AB) interactive effect. I.e X_3 has been confounded with $X_1.X_2$(Cavazzuti, 2013).

Table 2.2 Example of 2^{3-1} fractional factorial design

Experimental number	Factor level			
	X_1 (A)	X_2 (B)	X_3(C)=$X_1.X_2$	$X_1.X_2.X_3$
1	-1	-1	+1	+1
2	-1	+1	-1	+1
3	+1	-1	-1	+1
4	+1	+1	+1	+1

2.13.2.4 Central Composite Design

A central composite design is a 2^Kfull factorial to which the central point and the star points are added. The star points are the sample points in which all the factors but one is set at the mean level "m". The value of the remaining factors is given in terms of distance from the central point. The distance of the star points from the central point can be chosen in different ways (Cavazzati 2013).

i. If it is set to 1, all the samples are placed on a hyper sphere centered in the central point (*central composite circumscribed*, or CCC). This method requires five levels for each factor, namely *ll*, *l*, *m*, *h*, *hh*. This is the most common method used by design of experiment software.

ii. If it is set to the value $\frac{\sqrt{K}}{K}$, the parameter remains on the same levels of the 2^K full factorial (*central composite faced*, or CCF). The method requires three levels for each factor, namely *l*, *m*, *h*.

iii. If it is set to the value $\frac{\sqrt{K}}{K}$, the parameter remains on the same levels of the 2^K full factorial (*central composite faced*, or CCF). The method requires three levels for each factor, namely *l*, *m*, *h*,

iv. If the distance is set to any other value, whether it is $<\frac{\sqrt{K}}{K}$ (star points inside thedesign space), <1 (star points inside the hyper sphere), or>1 (star points outside the hypersphere), we talk of *central composite scaled*, or CCS. The method requires five levels for each factor.

Table 2.3 Central composite design

Factorials	Response	Factors		
		X_1	X_2	X_3
i	*y (l, l, m)*	-1	-1	0
	y (h, l, m)	+1	-1	0
	y (l, h, m)	-1	+1	0
	y (h, h, m)	+1	+1	0
ii	*y (l, m, l)*	-1	0	-1
	y (h, m, l)	+1	0	-1
	y (l, m, h)	-1	0	+1
	y (h, m, h)	+1	0	+1

iii	*y (m, l, l)*	0	-1	-1
	y (m, h, l)	0	+1	-1
	y (m, l, h)	0	-1	+1
	y (m, h, h)	0	+1	+1
iv	*y (m, m, m)*	0	0	0

2.13.3 Box Behnken Design

Box Behnken designs are a class of rotatable or nearly rotatable second order (quadratic) independent designs based on incomplete three levels factorial designs (Ferreira et al., 2007). For three factors, its graphical representation can be seen as;

a. A cube that consist of the central point and the middle point of the edges
b. A figure of three interlocking *2×2* factorial designs and a central point.

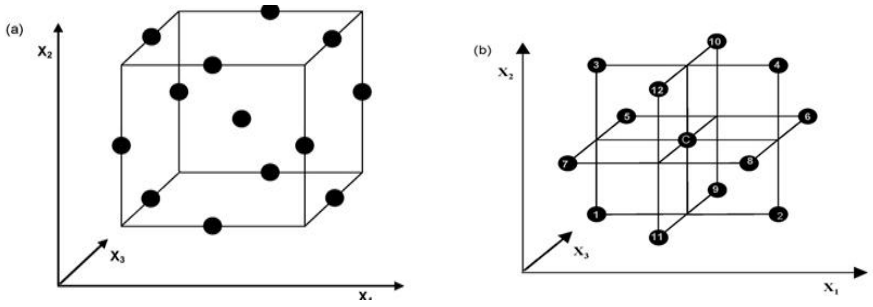


Figure 2.4 Box Behnken designs

They are built combining two levels factorial designs with incomplete block designs in a particular manner. In BBD, a block of sample corresponding to a two–level factorial design is repeated over different set of parameters with the parameters which are not included in the factorial design remaining at their mean level throughout the block. Considering the Box Behnken design with three factors , in this case a 2^2factorial is repeated three times;

i. On the first and the second parameters keeping the third parameter at the mean level (samples are: *llm*, *lhm*, *hlm*, *hhm*).
ii. On the first and the third parameters keeping the second parameter at the mean level (samples are: *lml*, *lmh*, *hml*, *hmh*).
iii. On the second and the third parameters keeping the first parameter at the mean level (samples: *mll*, *mlh*, *mhl*, *mhh*).
iv. Then the central point (*mmm*), is added (Cavazzuti, 2013)

2.14 RESPONSE SURFACE METHODOLOGY (RSM)

Response surface methodology is a collection of mathematical and statistical techniques that are based on the fit of empirical models to the experimental data obtained in relation to experimental designs. It explores the relationship between several input variables and response variables (Carley et al., 2004; Khuri and Mukhopadhyay, 2010). This technique is useful in the development, improvement and optimization of processes. This method involves running a series of full factorial experiments using Central Composite design or Box-Behnken design and mapping of responses to generate mathematical equations that describe how factors affect the response variable.

Some of the stages in the application of RSM as an optimization technique are;

i. The selection of independent variables with major effects on the system based on objective of the study and experience of the researcher.
ii. Choice of experimental design.
iii. The mathematical-statistical treatment of the obtained experimental data through the fit of a polynomial function.
iv. Evaluation of model's fitness.
v. The verification of the necessity and possibility of performing a displacement in direction to the optimal region
vi. Obtaining the optimum values for each studied variable (Bezerra et al., 2008).

In general, the response is a quantitative continuous variable and the mean response (objective function) is an unknown function of the levels of p factors and the mean response, when plotted as a function of the treatment combinations gives a response surface in p+1 dimensions (Dean et al., 2017).

In RSM, the unknown relationship between the response and input variable has been assumed to follow the polynomial model of the form

$$Y = f(x)\beta + \varepsilon$$

Where x = (X_1, X_2, X_3, …., X_k),$f(x)$, is a vector function of p elements that consists of powers and cross products of the powers of X_1, X_2, X_3, …., $X_{k.}$ β is a vector of p unknown constant coefficients called parameters while ε is a random experimental error.

Two important models employed in RSM which are special cases of the above equation are the first degree model

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \epsilon$$

And the second degree model

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{\cdot} \sum_{i<j} \beta_{ij} x_i x_j + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \epsilon$$

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

Table 3.1 Reagents and Raw Materials

MATERIALS	**USES**
Cassava peels	Biomass for carrying out this research

Saw dust	Biomass for carrying out this research
NaOH	For to neutralize the pretreated biomass
HCl	For pretreatment of the biomass
Distilled Water	For washing the cassava peels
DNS reagent	Testing for sugar yield

Table 3.2 Equipment/apparatus used for the experiment

S/N	**EQUIPMENTS**	**MAKE**	**USES**
1	Oven	Jinotech Sc. Co Ltd. Guandong, China	For drying the cassava peels
2	Weighing balance	OHAUS Pioneer	For measuring the mass of the biomass
3	Measuring cylinder	Pyrex (500 ml)	Measurement of specific volumes of solution
4	Beakers	Pyrex (250 ml)	For transfer and supply of measured volumes of liquids.
5	Droppers	3.0 ml rubber droppers	For dispensing liquids in very small quantities (droplets)
6	Filter paper	Whatman No.4	Separation of solute and filtrate from solution
8	Stirring rod		For proper mixing
9	pH paper.		For determining pH of solution
10	Hand gloves		This is used as protection for the hand
11	UV-Vis Spectrophotometer		Determination of absorbance of fermentable sugar content by DNS method

3.2 Methods

3.2.1 Collection of samples

The cassava peels were gotten from Ekehuan Army Barracks farm and the sawdust was gotten from Luco Laboratory.

3.2.2 Sample preparation

The cassava peels were washed with clean water to remove the soil and other unwanted materials and subjected to drying in an oven. The dried samples were grinded to powder form with a grinding machine at uselu market. The grinded samples were stored until the next stage of the process.

3.2.3 Pretreatment of samples

The purpose of the pretreatment was to remove as much lignin as possible, reduce cellulose crystallinity and increase the surface area of the materials. Forty six runs were carried out. Varying amount of the cassava peels powder and sawdust were measured in a beaker then poured into a conical flask. A mixture of varying quantities of water and hydrochloric acid to give varying acid concentrations were also measured and poured into the conical flask containing the biomass. The mixture was heated on a heating/magnetic at varying temperatures and time according to the variables of the stated run, then the heated samples were left to cool.

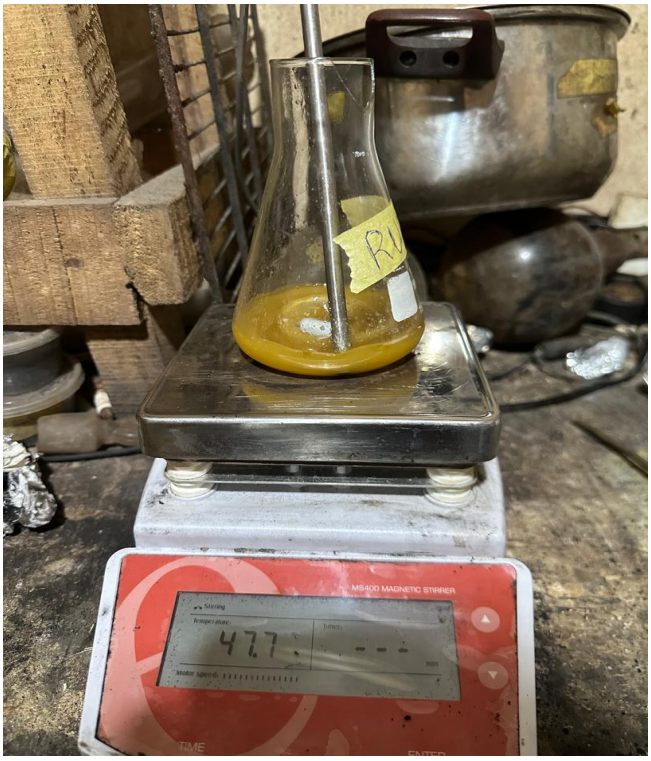


Figure 3.1 Conical flask containing the biomass and acid on a heating/magnetic stirrer

3.2.4 Neutralization

Neutralization was done to adjust the PH of the pretreated samples to a near neutral state suitable for the analysis of sugar using DNS solution. After pretreatment with hydrochloric acid, the mixture was cooled and neutralized with drops of NaOH solution. Then the mixture was filtered.

Figure 3.2 Filtration of the mixture after neutralizing

3.2.5 Determination of Sugar Yield using DNS method

The filtered pretreated solution was tested for sugar yield using DNS method. 4ml of the solution was measured. 1ml of DNS solution was added to the solution and placed on a hotplate for 10 minutes to boil. The sample was cooled and tested for sugar yield using a spectrophotometer.

3.2.6 Design of experiment

A three factor Box-Behnken Design was employed for the experimental design. The responses obtained from the BBD were optimized using response surface methodology. Factors to be optimized were coded at 3 levels which gave range for Acid concentration (1-5%w/w), Temperature (30-100℃), Time (15-90 minutes). The coded and uncoded factors and levels are shown below in the table below. The total sugars yield was chosen as the response for optimization of pretreatment using Response Surface Methodology (RSM). The experimental design carried out made up to 46 runs. Analysis of variance (ANOVA) and response surface plots were generated using Design Expert software. The optimized value of the independent variables for optimum response was determined using numerical optimization. The experimental design without responses is shown in the table below.

Table 3.3 Coded and actual values of factors

Name	Minimum	Maximum	Coded Low	Coded High	Mean
A: Cassava peel (g)	0.0000	10.00	-1 ↔ 0.00	+1 ↔ 10.00	5.00
B: Sawdust (g)	0.0000	10.00	-1 ↔ 0.00	+1 ↔ 10.00	5.00
C: Temperature (°C)	30.00	100.00	-1 ↔ 30.00	+1 ↔ 100.00	65.00
D: Time (minutes)	15.00	90.00	-1 ↔ 15.00	+1 ↔ 90.00	52.50
E: Acid conc.	1.0000	5.00	-1 ↔ 1.00	+1 ↔ 5.00	3.00

Table 3.4 Experimental Design without Responses

Run	Cassava peel (g)	Sawdust (g)	Temperature (°C)	Time (minutes)	Acid conc. (%w/w)
1	5	5	65	90	5
2	5	5	65	15	1
3	5	5	100	52.5	1
4	5	0	65	52.5	5
5	0	5	100	52.5	3
6	0	5	65	52.5	1
7	5	10	30	52.5	3
8	5	5	65	52.5	3
9	5	5	65	90	1
10	5	5	65	52.5	3
11	5	0	65	52.5	1

12	10	5	65	90	3
13	5	5	65	52.5	3
14	10	5	100	52.5	3
15	5	5	30	15	3
16	5	5	65	52.5	3
17	5	5	100	15	3
18	0	5	65	15	3
19	5	10	65	52.5	1
20	0	0	65	52.5	3
21	10	5	65	52.5	1
22	10	5	30	52.5	3
23	5	10	100	52.5	3
24	5	5	100	90	3
25	5	10	65	90	3
26	10	5	65	52.5	5
27	5	5	30	52.5	5
28	5	5	65	15	5
29	0	10	65	52.5	3
30	10	5	65	15	3
31	5	5	65	52.5	3
32	5	0	100	52.5	3
33	5	0	65	90	3

34	0	5	30	52.5	3
35	5	5	30	52.5	1
36	5	0	30	52.5	3
37	5	5	100	52.5	5
38	5	10	65	52.5	5
39	5	5	65	52.5	3
40	10	10	65	52.5	3
41	0	5	65	90	3
42	5	5	30	90	3
43	0	5	65	52.5	5
44	10	0	65	52.5	3
45	5	10	65	15	3
46	5	0	65	15	3

CHAPTER 4

4.0 RESULT AND DISCUSSION

4.1 Result

4.1.1 Box Behnken Design of Experiment

The relationship between the response (sugar yield) and the three independent process variables (temperature, time and acid concentration) were evaluated by using response surface methodology (RSM). A total of 46 runs were developed using the Box-Behnken design, the build information is shown below table 4.1

Table 4.1 Build Information

Design	Info
File Version	13.0.1.0
Study Type	Response Surface
Design Type	Box-Behnken
Design Model	Quadratic
Build Time (ms)	2.00
Subtype	Randomized
Runs	46.00
Blocks	No Blocks

Table 4.2 Analysis of Variance (ANOVA) for Quadratic model

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1.748E+08	20	8.741E+06	37.11	< 0.0001	significant
A-Cassava peel	1.808E+07	1	1.808E+07	76.77	< 0.0001	
B-Sawdust	1.623E+07	1	1.623E+07	68.93	< 0.0001	

C-Temperature	7.091E+06	1	7.091E+06	30.10	< 0.0001
D-Time	6.145E+06	1	6.145E+06	26.09	< 0.0001
E-Acid conc.	5.698E+05	1	5.698E+05	2.42	0.1324
AB	2.209E+05	1	2.209E+05	0.9378	0.3421
AC	5.177E+05	1	5.177E+05	2.20	0.1507
AD	1.618E+05	1	1.618E+05	0.6868	0.4151
AE	38486.59	1	38486.59	0.1634	0.6895
BC	1.353E+06	1	1.353E+06	5.74	0.0244
BD	2.536E+06	1	2.536E+06	10.77	0.0030
BE	5.887E+06	1	5.887E+06	25.00	< 0.0001
CD	1.752E+06	1	1.752E+06	7.44	0.0115
CE	1.673E+06	1	1.673E+06	7.10	0.0133
DE	1.516E+05	1	1.516E+05	0.6438	0.4299
A^2	3.687E+07	1	3.687E+07	156.52	< 0.0001
B^2	3.845E+07	1	3.845E+07	163.24	< 0.0001
C^2	6.492E+07	1	6.492E+07	275.62	< 0.0001
D^2	4.598E+07	1	4.598E+07	195.21	< 0.0001
E^2	4.418E+07	1	4.418E+07	187.59	< 0.0001
Residual	5.888E+06	25	2.355E+05		
Lack of Fit	5.888E+06	20	2.944E+05		
Pure Error	0.0000	5	0.0000		
Cor Total	1.807E+08	45			

Based on the ANOVA results in Table 4.2, the Model F-value of 37.11 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case, A, B, C, D, BC, BD, BE, CD, CE, A^2, B^2, C^2, D^2, E^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (excluding those required to support the hierarchy), model reduction may improve your model.

Table 4.3 Statistical information of Box-Behnken design

R^2	0.9674
Adjusted R^2	0.9414
Predicted R^2	0.8697
Adequate Precision	19.6992
Std,. Dev.	485.31
Mean	2353.21
C.V. %	20.62

Based on Table 4.3, the Predicted R^2 of 0.8697 is in reasonable agreement with the Adjusted R^2 of 0.9414; i.e., the difference is less than 0.2. Adequate Precision measures the signal-to-noise ratio. A ratio greater than 4 is desirable. The ratio of 19.699 indicates an adequate signal. This model can be used to navigate the design space.

4.1.2 RSM Modelling and Analysis

The coded and actual values of the factors A (cassava peel), B (sawdust), C (Temperature), D (Time) and E (Acid concentration) as designed by design expert and their corresponding responses are shown in Table 4.4

Table 4.4 Coefficients in Terms of Coded Factors

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF

Intercept	6327.77	1	198.13	5919.72	6735.83	
A-Cassava peel	1063.03	1	121.33	813.15	1312.91	1.0000
B-Sawdust	1007.30	1	121.33	757.42	1257.18	1.0000
C-Temperature	665.70	1	121.33	415.82	915.58	1.0000
D-Time	619.74	1	121.33	369.86	869.62	1.0000
E-Acid conc.	-188.71	1	121.33	-438.60	61.17	1.0000
AB	-234.98	1	242.66	-734.75	264.78	1.0000
AC	359.74	1	242.66	-140.02	859.50	1.0000
AD	201.10	1	242.66	-298.66	700.86	1.0000
AE	-98.09	1	242.66	-597.85	401.67	1.0000
BC	581.52	1	242.66	81.76	1081.28	1.0000
BD	796.26	1	242.66	296.50	1296.02	1.0000
BE	-1213.19	1	242.66	-1712.95	-713.43	1.0000
CD	661.79	1	242.66	162.03	1161.55	1.0000
CE	-646.79	1	242.66	-1146.55	-147.03	1.0000
DE	-194.70	1	242.66	-694.47	305.06	1.0000
A^2	-2055.27	1	164.28	-2393.61	-1716.93	1.20
B^2	-2098.96	1	164.28	-2437.30	-1760.62	1.20
C^2	-2727.35	1	164.28	-3065.69	-2389.01	1.20
D^2	-2295.27	1	164.28	-2633.61	-1956.93	1.20
E^2	-2250.05	1	164.28	-2588.39	-1911.71	1.20

The coefficient estimate represents the expected change in response per unit change in factor value when all remaining factors are held constant. The intercept in an orthogonal design is the overall

average response of all the runs. The coefficients are adjustments around that average based on the factor settings. When the factors are orthogonal, the VIFs are 1; VIFs greater than 1 indicate multicollinearity. The higher the VIF, the more severe the correlation of factors. As a rough rule, VIFs less than 10 are tolerable.

Table 4.5 Final Equation in Terms of Coded Factors

Sugar yield =

+6327.77	
+1063.03	A
+1007.30	B
+665.70	C
+619.74	D
-188.71	E
-234.98	AB
+359.74	AC
+201.10	AD
-98.09	AE
+581.52	BC
+796.26	BD
-1213.19	BE
+661.79	CD

-646.79 CE

-194.70 DE

-2055.27 A^2

-2098.96 B^2

-2727.35 C^2

-2295.27 D^2

-2250.05 E^2

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1, and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

4.2 Parity Plot of Predicted and Actual Sugar Yield

The relationship between the predicted and actual yield is observed from the plot that the data points are distributed near the straight line. This is an indication that there is high correlation (R^2 value close to unity), which means that the data fit well with the model and convincingly is a good estimate of response for the system in the ranges studied. This further indicates that the model could be employed as the significant model for predicting response over the independent input variables.

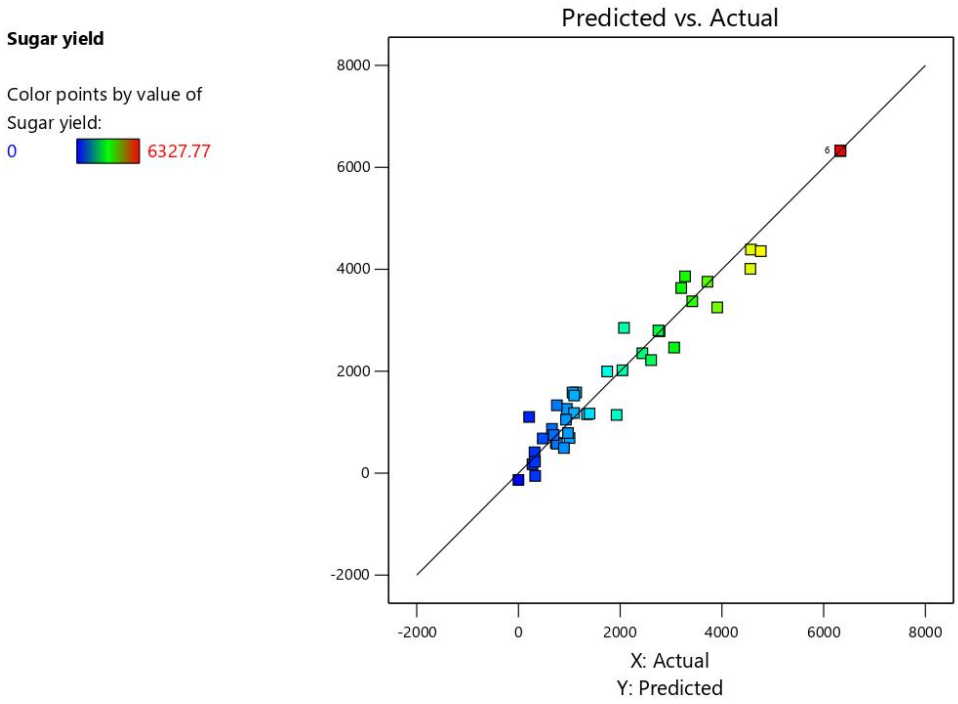


Figure 4.1 Parity plot of predicted against actual sugar yield

4.1.3 Response Surface Plots

Response surface plot shows the visual observation among (Temperature, Time and Acid concentration) for the sugar yield after acid pretreatment. The optimisation of the pretreatment variables for the total sugar yield after pretreatment is achieved using the 3D surface response plot. The data were generated by keeping one of the independent variable at a constant (central) level and varying the other two within their experimental range. The shape of the 3D plots shows there are significant interactions among factors considered in this work. The dome shape of the plots indicates there are shared interaction among factors.

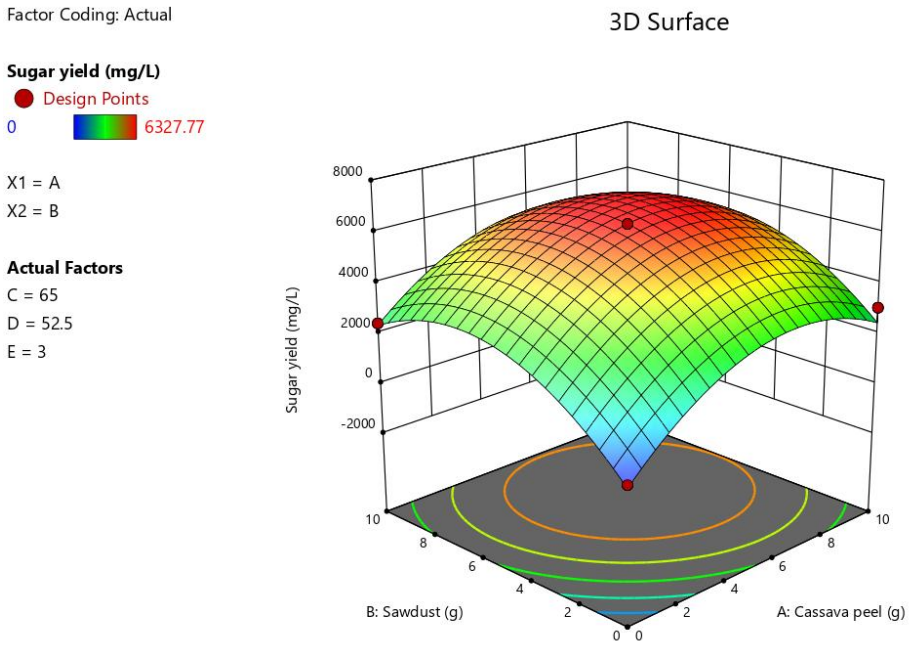


Figure 4.2 shows the response surface plot for sugar yield as a function of biomass (cassava peels and sawdust) amount.

Figure 4.2 shows the effect of the interaction between biomass (cassava peels and sawdust) amount and temperature on sugar yield. The centre point of Figure 4.2 reveals the optimal values of biomass (cassava peels and sawdust) amount that may be combined to obtain optimal sugar yield. This was revealed to be 5g of cassava peels biomass and 5g of sawdust biomass. Any further increase in both the amount of biomass (cassava peels and sawdust) led to no appreciable effect on the sugar yield.

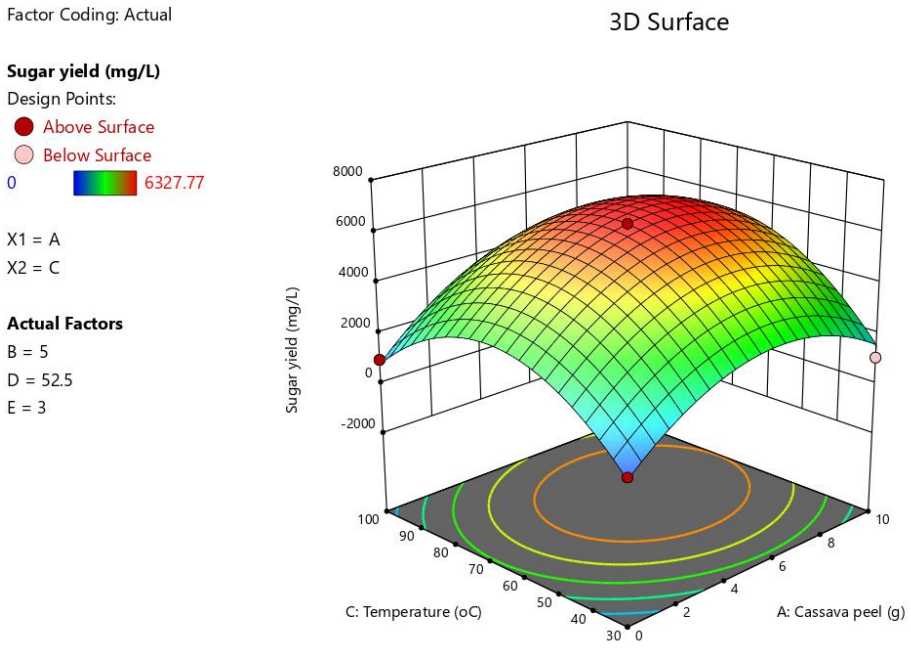


Figure 4.3 Response surface plot showing the effect of temperature and amount of biomass (cassava peels) on sugar yield

Figure 4.3 shows the effect of the interaction between amount of biomass (cassava peels) and temperature on sugar yield. The centre point of Figure 4.3 reveals the optimal values of biomass (cassava peels) amount and temperature that may be combined to obtain optimal sugar yield. This was revealed to be 5g of cassava peels biomass and at temperature of 65^{O}C. Any further increase in the amount of biomass (cassava peels) and temperature led to no appreciable effect on the sugar yield.

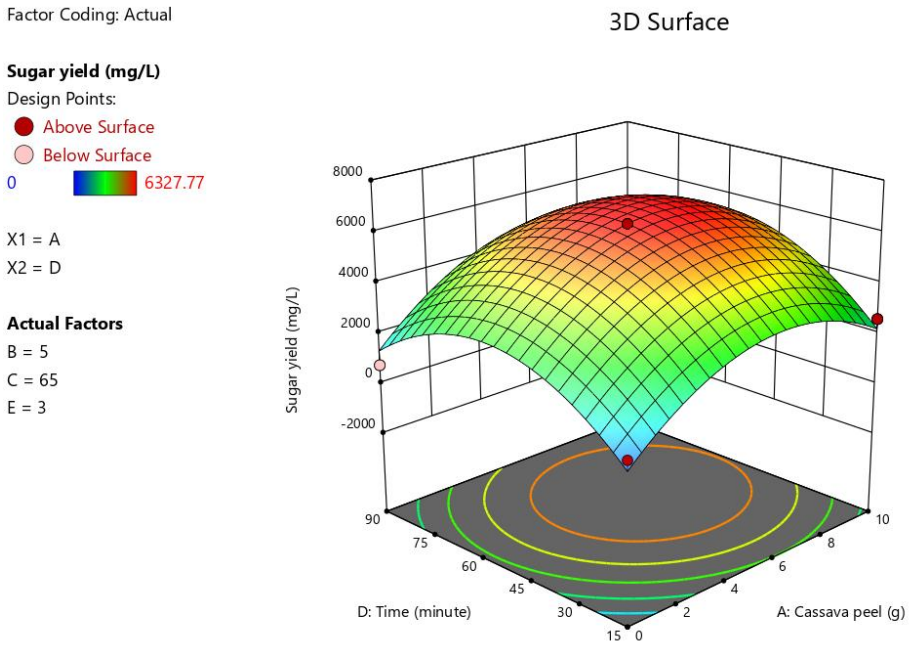


Figure 4.4 Response surface plot showing the effect of time and amount of biomass (cassava peels) on sugar yield

Figure 4.4 shows the effect of the interaction between amount of biomass (cassava peels) and time on sugar yield. The centre point of Figure 4.4 reveals the optimal values of biomass (cassava peels) amount and time that may be combined to obtain optimal sugar yield. This was revealed to be 5g of cassava peels biomass and at 52.5mins. Any further increase in the amount of biomass (cassava peels) and time led to no appreciable effect on the sugar yield

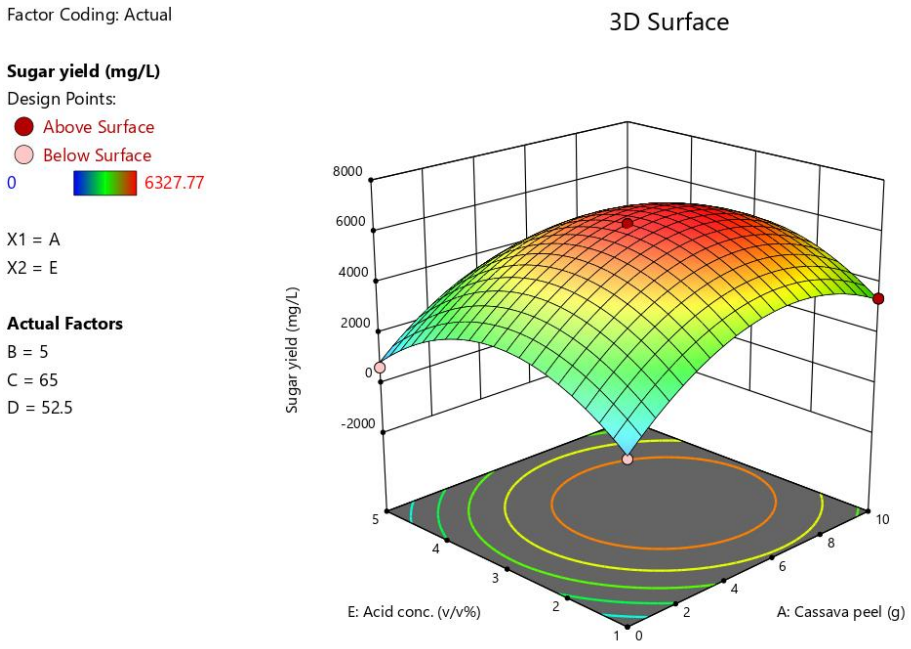


Figure 4.5 Response surface plot showing the effect of acid concentration and amount of biomass (cassava peels) on sugar yield

Figure 4.5 shows the effect of the interaction between amount of biomass (cassava peels) and acid concentration on sugar yield. The centre point of Figure 4.5 reveals the optimal values of biomass (cassava peels) amount and acid concentration that may be combined to obtain optimal sugar yield. This was revealed to be 5g of cassava peels biomass and at a concentration of 3%w/w. Any further increase in the amount of biomass (cassava peels) and time led to no appreciable effect on the sugar yield

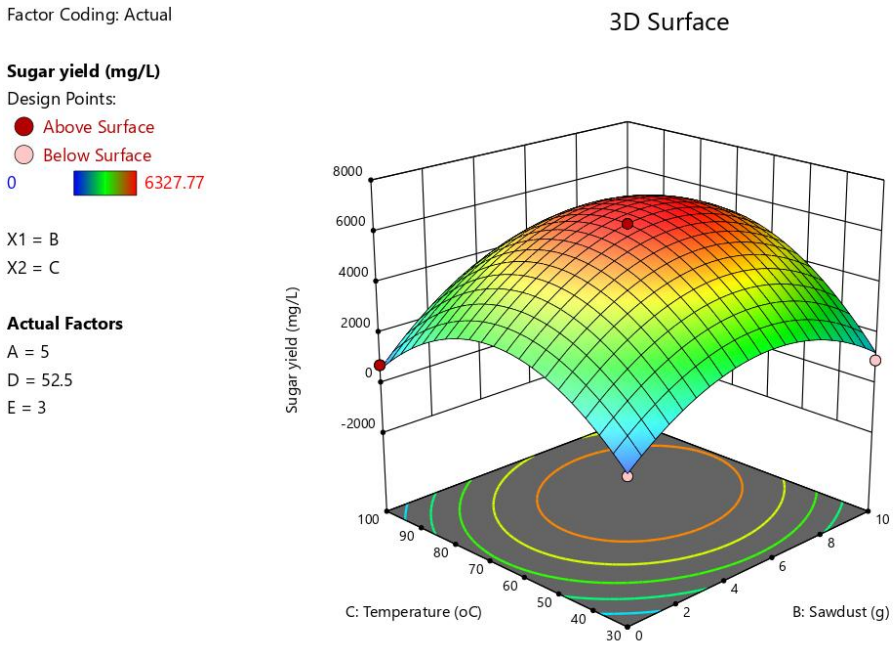


Figure 4.6 Response surface plot showing the effect of temperature and amount of biomass (sawdust) on sugar yield

Figure 4.6 shows the effect of the interaction between amount of biomass (sawdust) and temperature on sugar yield. The centre point of Figure 4.6 reveals the optimal values of biomass (sawdust) amount and temperature that may be combined to obtain optimal sugar yield. This was revealed to be 5g of cassava peels biomass and at temperature of 65^{O}C. Any further increase in the amount of biomass (sawdust) and temperature led to no appreciable effect on the sugar yield.

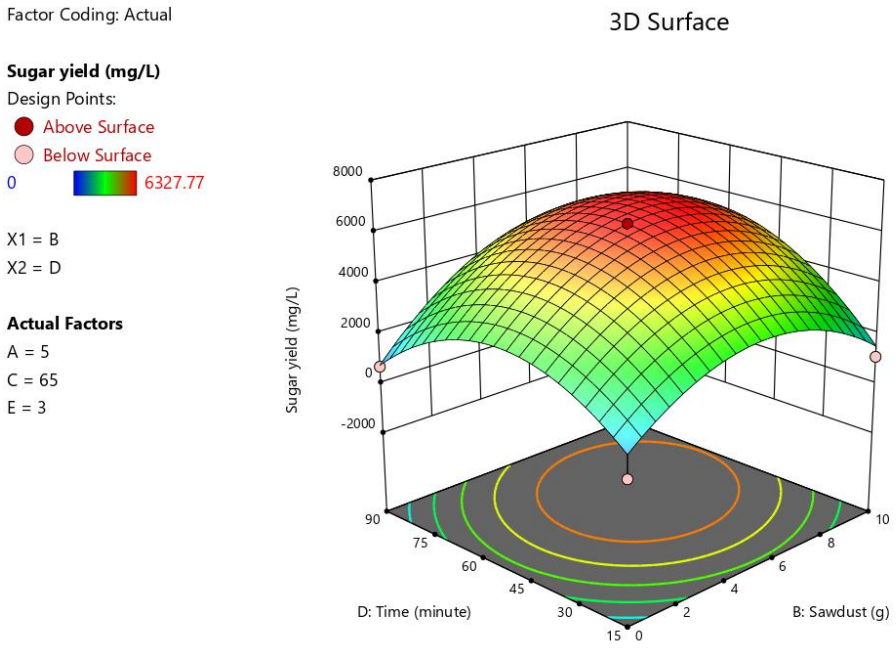


Figure 4.7 Response surface plot showing the effect of time and amount of biomass (sawdust) on sugar yield

Figure 4.7 shows the effect of the interaction between amount of biomass (sawdust) and temperature on sugar yield. The centre point of Figure 4.7 reveals the optimal values of biomass (sawdust) amount and temperature that may be combined to obtain optimal sugar yield. This was revealed to be 5g of cassava peels biomass and at 52.5mins. Any further increase in the amount of biomass (sawdust) and temperature led to no appreciable effect on the sugar yield.

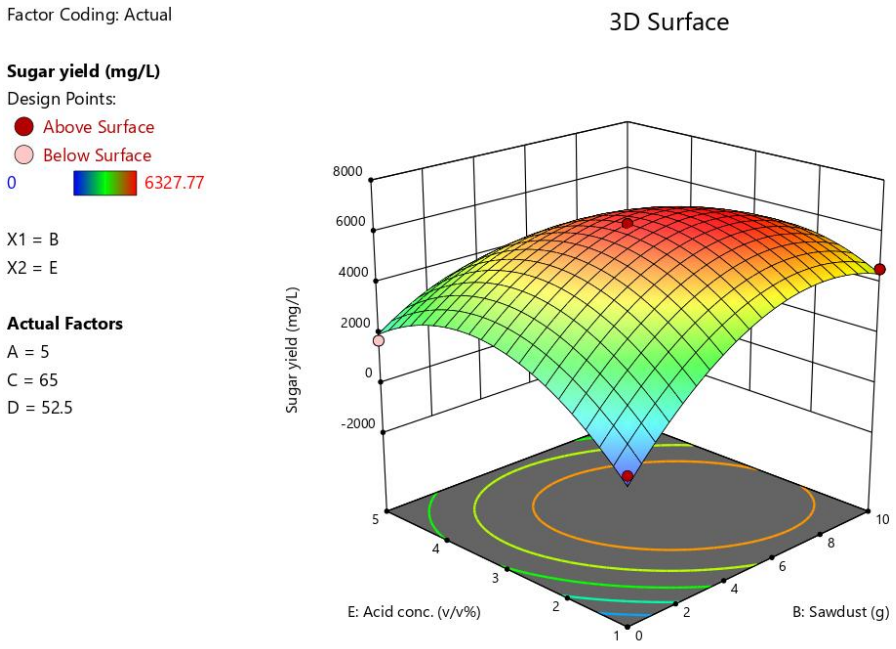


Figure 4.8 Response surface plot showing the effect of acid concentration and amount of biomass (sawdust) on sugar yield

Figure 4.8 shows the effect of the interaction between amount of biomass (sawdust) and acid concentration on sugar yield. The centre point of Figure 4.4 reveals the optimal values of biomass (sawdust) amount and acid concentration that may be combined to obtain optimal sugar yield. This was revealed to be 5g of cassava peels biomass and at a concentration of 3%w/w. Any further increase in the amount of biomass (sawdust) and time led to no appreciable effect on the sugar yield

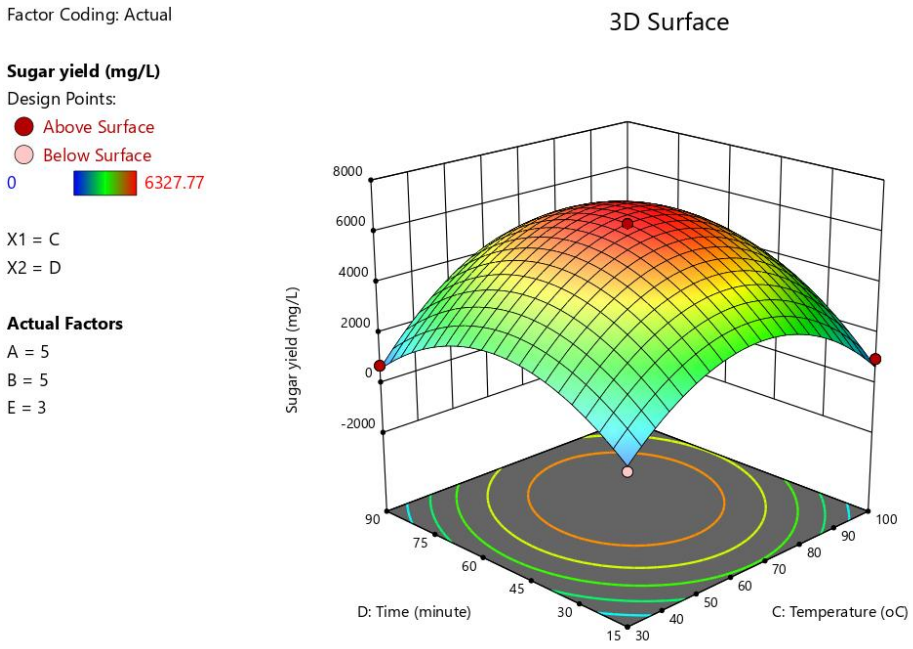


Figure 4.9 Response surface plot showing the effects of temperature and pretreatment time on sugar yield

Figure 4.9 shows the effect of the interaction between pretreatment time and temperature on sugar yield. The centre point of Figure 4.9 reveals the optimal values of pretreatment time and temperature that may be combined to obtain optimal sugar yield. This was revealed to be 52.5 minutes pretreatment time and 65^{O}C temperature. Any further increase in both the pretreatment time and temperature led to no appreciable effect on the sugar yield.

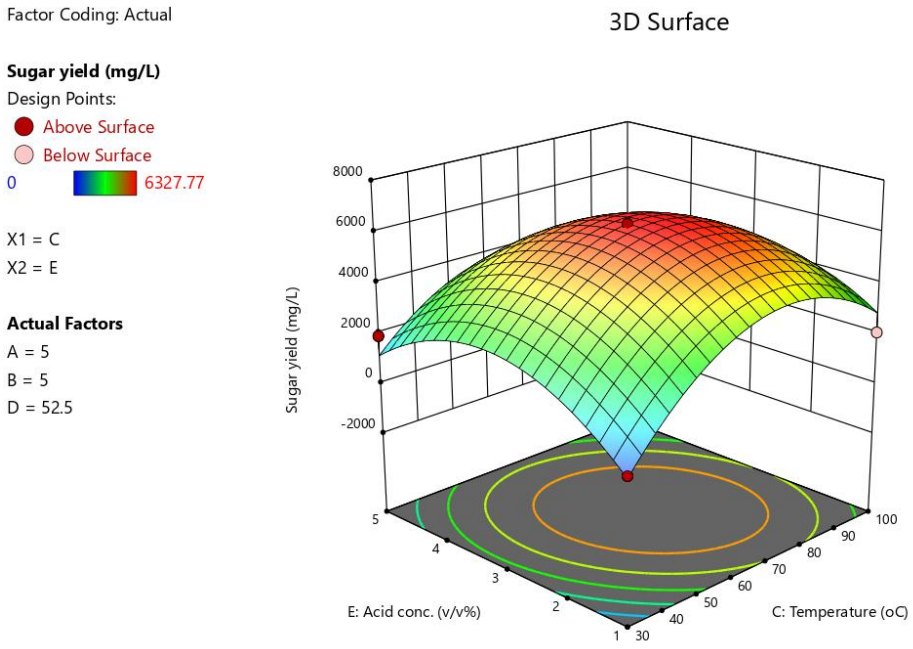


Figure 4.10 Response surface plot showing the effects of temperature and acid concentration on sugar yield

An increase in the acid concentration with temperature resulted in an increase in the sugar yield until an optimum value of about 6327.77mg/L i.e., 65^{O}C temperature and 3% (w/w) acid concentration. Any further increase in the acid concentration was found to be unfavourable for the sugar yield as explained by the decreasing trend observed.

CHAPTER 5

5.0 CONCLUSION AND RECOMENDATION

5.1 Conclusion

From this study, it is observed that cassava peels and sawdust are suitable feedstock for bioethanol production using acid pretreatment method. The results obtained could be scaled up to pilot production of ethanol and reduce disposal of the material into the environment. All three factors significantly influence the sugar yield produced and a quadratic model obtained was significant and able to predict the sugar yield. At the optimum amount of sugar yield of 6327.77mg/L, the optimum values of temperature at 65^{O}C, acid concentration of 3%w/w and time 52.5mins.

5.2 Recommendations

Cassava peels and sawdust have been demonstrated to be a potential feedstock for bioethanol production. The following are therefore recommended for the purpose of scale up or future researches:

- Future studies should be done on how to increase the sugar yield for bioethanol production from cassava peels and sawdust in order to produce more.
- Future studies should be done to determine the effect of various factors on the production of bioethanol from cassava peels and sawdust.

REFERENCE

Adedokun, M. O., Oladoye, A. O., Oluwalana, S. A., & Mendie, I. I. (2010). Socio-economic importance and utilization of Spondias mombin in Nigeria. *Asian Pacific Journal of Tropical Medicine*, *3*(3), 232–234. https://doi.org/10.1016/S1995-7645(10)60015-6

Amenaghawon, A. N., Okieimen, C. O., & Ogbeide, S. E. (2014). Modeling and Statistical Optimization of Dilute Acid Hydrolysis of Eucalyptus Wood Chips using Response Surface Methodology . *The Pacific Journal of Science and Technology*, *15*(1), 245–256.

Amenaghawon, N. A. (2010). Evaluation of ethanol stripping from fermenters. *Imperial College London*, 123. Retrieved from https://workspace.imperial.ac.uk/centreforprocesssystemsengineering/Public/MSc Thesis/2010 MSc Thesis/2010.2 - Andrew Amenaghawon .pdf

Anwar, Z., Gulfraz, M. and Irshad, M. (2014) 'Agro-industrial lignocellulosic biomass a key to unlock the future bio-energy: A brief review', *Journal of Radiation Research and Applied Sciences*, 7(2), pp. 163–173. doi: 10.1016/j.jrras.2014.02.003.

Ariunbaatar, J., Panico, A., Esposito, G., Pirozzi, F., & Lens, P. N. L. (2014). *Pretreatment methods*

Betiku, E., Emeko, H. A. and Solomon, B. O. (2016) 'Fermentation parameter optimization of microbial oxalic acid production from cashew apple juice', *Heliyon*, 2(2). doi: 10.1016/j.heliyon.2016.e00082.

Bezerra, M. A. *et al.* (2008) 'Response surface methodology (RSM) as a tool for optimization in analytical chemistry', *Talanta*, 76(5), pp. 965–977. doi: 10.1016/j.talanta.2008.05.019.

Binod, P. *et al.* (2011) *Hydrolysis of lignocellulosic biomass for bioethanol production*. 1st edn, *Biofuels*. 1st edn. Elsevier Inc. doi: 10.1016/B978-0-12-385099-7.00010-3.

Campbell, M. M., & Sederoff, R. R. (1996). *Variation in Lignin Content and Composition'*.

Čedík, J., Pexa, M., Mařík, J., Hönig, V., Horníčková, Š., & Kubín, K. (2015). *Influence of butanol and FAME blends on operational characteristics of compression ignition engine*. *13*(2), 541–549.

Gupta, V. K. and Tuohy, M. G. (2014) *Biofuel technologies: Recent developments*, *Biofuel Technologies: Recent Developments*. doi: 10.1007/978-3-642-34519-7.

Jin, C., Yao, M., Liu, H., Lee, C. F., & Ji, J. (2011). Progress in the production and application of n-butanol as a biofuel. *Renewable and Sustainable Energy Reviews*, *15*(8), 4080–4106. https://doi.org/10.1016/j.rser.2011.06.001

Kaminski, W., Tomczak, E., & Gorak, a. (2011). Bioutanol - Production and Purifciaton Methods. *Ecological Chemistry and Engineering S-Chemia I Inzynieria Ekologiczna S*, *18*(1), 31–37.

Kautto, J., Realff, M. J. and Ragauskas, A. J. (2013) 'Design and simulation of an organosolv process for bioethanol production', *Biomass Conversion and Biorefinery*, 3(3), pp. 199–212. doi: 10.1007/s13399-013-0074-6.

Kim, J. S., Lee, Y. Y. and Kim, T. H. (2016) 'A review on alkaline pretreatment technology for bioconversion of lignocellulosic biomass', *Bioresource Technology*, 199, pp. 42–48. doi: 10.1016/j.biortech.2015.08.085.

Kumar, A. K., & Sharma, S. (2017). Recent updates on different methods of pretreatment of lignocellulosic feedstocks : a review. *Bioresources and Bioprocessing*. https://doi.org/10.1186/s40643-017-0137-9

L., F., P. Andrade, C. C. and A. Sant, M. H. (2013) 'A Review of Xylanase Production by the Fermentation of Xylan: Classification, Characterization and Applications', *Sustainable Degradation of Lignocellulosic Biomass - Techniques, Applications and Commercialization*. doi: 10.5772/53544.

Lamsal, B. P., Madl, R. and Tsakpunidis, K. (2011) 'Comparison of Feedstock Pretreatment Performance and Its Effect on Soluble Sugar Availability', *Bioenergy Research*, 4(3), pp. 193–200. doi: 10.1007/s12155-010-9112-4.

Li, X., Weng, J., & Chapple, C. (2008). *Improvement of biomass through lignin modification*. 569–581. https://doi.org/10.1111/j.1365-313X.2008.03457.x

Liu, W. *et al.* (2020) 'Comparative study on different pretreatment on enzymatic hydrolysis of corncob residues', *Bioresource Technology*, 295, p. 122244. doi: 10.1016/j.biortech.2019.122244.

Liu, Y. *et al.* (2017) 'Irradiation pretreatment facilitates the achievement of high total sugars concentration from lignocellulose biomass', *Bioresource Technology*, 232, pp. 270–277. doi: 10.1016/j.biortech.2017.01.061.

Liu, S., & Qureshi, N. (2009). *How microbes tolerate ethanol and butanol*. *26*(October), 117–121. https://doi.org/10.1016/j.nbt.2009.06.984

Loow, Y. L. *et al.* (2015) 'Recent Advances in the Application of Inorganic Salt Pretreatment for Transforming Lignocellulosic Biomass into Reducing Sugars', *Journal of Agricultural and Food Chemistry*, 63(38), pp. 8349–8363. doi: 10.1021/acs.jafc.5b01813.

Lopes, D. B. *et al.* (2011) 'Lipase and esterase - to what extent can this classification be applied accurately?', *Ciencia e Tecnologia de Alimentos*, 31(3), pp. 608–613. doi: 10.1590/s0101-20612011000300009.

Mäki-Arvela, P. *et al.* (2011) 'Synthesis of sugars by hydrolysis of hemicelluloses- A review', *Chemical Reviews*, 111(9), pp. 5638–5666. doi: 10.1021/cr2000042.

Muzikova, Z., Simacek, P., Pospisil, M., & Sebor, G. (2014). *Density , Viscosity and Water Phase Stability of 1-Butanol-Gasoline Blends*. *2014*, 1–8.

Nakagame, S., Chandra, R. P. and Saddler, J. N. (2011) 'The influence of lignin on the enzymatic hydrolysis of pretreated biomass substrates', *ACS Symposium Series*, 1067, pp. 145–167. doi: 10.1021/bk-2011-1067.ch006.

Päkkilä, J. M., Hillukkala, T., Myllykoski, L., & Keiski, R. L. (2008). *An anaerobic bioreactor system for biobutanol production*. 17–21.

Pandey, V. P. *et al.* (2017) 'A Comprehensive Review on Function and Application of Plant Peroxidases', *Biochemistry & Analytical Biochemistry*, 06(01). doi: 10.4172/2161-1009.1000308.

Puls, J. (1997). *CHEMISTRY AND BIOCHEMISTRY OF HEMICELLULOSES: RELATIONSHIP*

Raganati, F., Procentese, A., Olivieri, G., Götz, P., Salatino, P., & Marzocchella, A. (2015). Kinetic study of butanol production from various sugars by Clostridium acetobutylicum using a dynamic model. *Biochemical Engineering Journal*, *99*, 156–166. https://doi.org/10.1016/j.bej.2015.03.001

Ranjan, A., Khanna, S., & Moholkar, V. S. (2013). *Feasibility of rice straw as alternate substrate for biobutanol production*. *103*, 32–38.

Satarn, J., Lamamorphanth, W., & Kamwilaisak, K. (2014). Acid Hydrolysis from Corn Stover for Reducing Sugar. *Advanced Materials Research*, *931–932*, 1608–1613. https://doi.org/10.4028/www.scientific.net/amr.931-932.1608

Satimanont, S., Luengnaruemitchai, A. and Wongkasemjit, S. (2012) 'Effect of temperature and time on dilute acid pretreatment of corn cobs', *International Journal …*, 6(0), pp. 333–337. Available at: http://www.idc-online.com/technical_references/pdfs/chemical_engineering/Effect of Temperature.pdf.

Shaaban, M., & Petinrin, J. O. (2014). Renewable energy potentials in Nigeria: Meeting rural energy needs. *Renewable and Sustainable Energy Reviews*, *29*(January), 72–84. https://doi.org/10.1016/j.rser.2013.08.078

Sharma, H. K., Xu, C. and Qin, W. (2019) 'Biological Pretreatment of Lignocellulosic Biomass for Biofuels and Bioproducts: An Overview', *Waste and Biomass Valorization*, 10(2), pp. 235–251. doi: 10.1007/s12649-017-0059-y.

Shi, N. (2009). *Introduction to Biofuel The Green Energy Economy Is the Future*.

Singh, S. *et al.* (2015) 'Comparison of different biomass pretreatment techniques and their impact on chemistry and structure', *Frontiers in Energy Research*, 3(FEB), pp. 1–12. doi: 10.3389/fenrg.2014.00062.

Shuai, L., & Luterbacher, J. (2016). *Organic Solvent Effects in Biomass Conversion Reactions*. 133–155. https://doi.org/10.1002/cssc.201501148

Singhvi, M. S., Chaudhari, S., & Gokhale, D. V. (2014). *RSC Advances Lignocellulose processing : a current challenge*. 8271–8277. https://doi.org/10.1039/c3ra46112b

Travaini, R. *et al.* (2015) *Ozonolysis*, *Pretreatment of Biomass: Processes and Technologies*. doi: 10.1016/B978-0-12-800080-9.00007-4.

Travaini, R. *et al.* (2016) 'Ozonolysis: An advantageous pretreatment for lignocellulosic biomass

revisited', *Bioresource Technology*, 199, pp. 2–12. doi: 10.1016/j.biortech.2015.08.143.

Tu, W. C. and Hallett, J. P. (2019) 'Recent advances in the pretreatment of lignocellulosic biomass', *Current Opinion in Green and Sustainable Chemistry*, 20, pp. 11–17. doi: 10.1016/j.cogsc.2019.07.004.

Vasco-Correa, J., Ge, X. and Li, Y. (2016) 'Biological Pretreatment of Lignocellulosic Biomass', *Biomass Fractionation Technologies for a Lignocellulosic Feedstock Based Biorefinery*, pp. 561–585. doi: 10.1016/B978-0-12-802323-5.00024-4.

Wang, D., Xu, Y., Hu, J., & Zhao, G. (2004). *Fermentation Kinetics of Different Sugars by Apple Wine Yeast Saccharomyces cerevisiae*. *110*(4), 340–346.

Wang, M. *et al.* (2012) 'Cellulolytic enzyme production and enzymatic hydrolysis for second-generation bioethanol production', *Advances in Biochemical Engineering/Biotechnology*, 128(January), pp. 1–24. doi: 10.1007/10_2011_131.

Wang, Y. I., & Blaschek, H. P. (2014). *Fermentative Biobutanol Production : An Old Topic with Remarkable*. 227–260.

Wiens, J., Fargione, J., & Hill, J. (2011). Biofuels and biodiversity. *Ecological Applications*, *21*(4), 1085–1095. https://doi.org/10.1890/09-0673.1

Yang, M. *et al.* (2011) 'Improvement of cellulose conversion caused by the protection of Tween-80 on the adsorbed cellulase', *Biochemical Engineering Journal*, 56(3), pp. 125–129. doi: 10.1016/j.bej.2011.04.009.

Yu, Z. *et al.* (2012) 'A real explosion: The requirement of steam explosion pretreatment', *Bioresource Technology*, 121, pp. 335–341. doi: 10.1016/j.biortech.2012.06.055.

Zhou, Y. *et al.* (2015) 'Non-ionic surfactants do not consistently improve the enzymatic hydrolysis of pure cellulose', *Bioresource Technology*, 182, pp. 136–143. doi: 10.1016/j.biortech.2015.01.137.

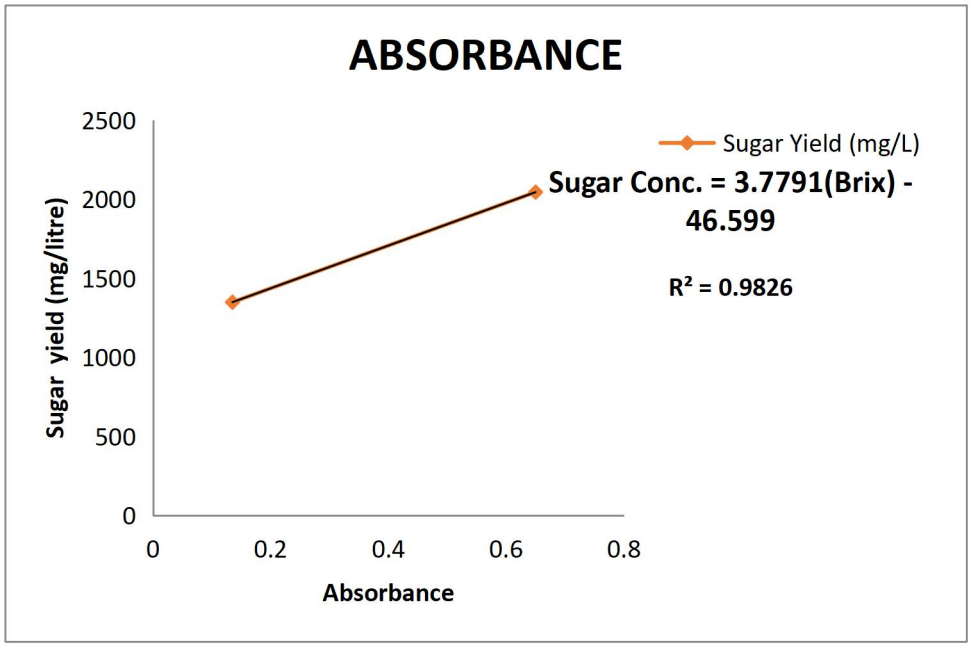
APPENDIX

Time (minute)	Sugar Yield (mg/L)
7.50	2278.91
10.08	2689.23
15.07	3422.65
20.06	4073.20
25.05	4643.65
30.04	5132.60
35.03	5540.06
40.03	5866.02
45.03	6111.88
50.02	6274.86
55.02	6357.73

Run	Absorbance	Sugar Yield (mg/L)
Run 1	0.65	2045.21
Run 2	0.135	1348.91
Run 3	1.418	2075.17
Run 4	0.425	1745.83

Run 5	0.461	974.86
Run 6		933.92
Run 7	0.349	951.06
Run 8	0.406	6327.77
Run 9	0.358	2774.68
Run 10	0.308	6327.77
Run 11	0.28	329.35
Run 12	1.126	3275.42
Run 13	0.919	6327.77
Run 14	0.191	3200.96
Run 15	0.304	478.615
Run 16	0.598	6327.77
Run 17	1.157	1004.53
Run 18	0.098	893.86
Run 19	0.678	4571.65
Run 20		0
Run 21	2.219	3418.42
Run 22	0.56	1063.86
Run 23	1.703	3716.77
Run 24	0.349	3908.38
Run 25		4764.94
Run 26	0.235	2751.52

Run 27	0.623	1930.29
Run 28	0.409	1398.26
Run 29	0.754	2437.81
Run 30	1.025	2608.84
Run 31	0.563	6327.77
Run 32	1.255	756.74
Run 33	0.798	694.75
Run 34	0.079	276.721
Run 35	0.31	325.753
Run 36	0.225	317.12
Run 37	2.133	1092.55
Run 38	1.154	1135.38
Run 39	0.565	6327.77
Run 40	1.331	4559.96
Run 41	0.121	756.03
Run 42	0.626	735.309
Run 43	0.138	659.38
Run 44	1.413	3062.09
Run 45	0.411	1095.54
Run 46	0.733	210.385


ABSORBANCE
Sugar Yield (mg/L)
Sugar Conc. = 3.7791(Brix) - 46.599
R² = 0.9826
Sugar yield (mg/litre)
2500
2000
1500
1000
500
0
0
0.2
0.4
0.6
0.8
Absorbance