POTENTIAL OF BRAN FROM TWO VARIETIES OF RICE (ORYZA) SPP FOR BIOETHANOL PRODUCTION

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BY

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A DISSERTATION SUBMITTED TO THE DEPARTMENT OF BIOCHEMISTRY, FACULTY OF BASIC MEDICAL SCIENCES, BAYERO UNIVERSITY, KANO, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE DEGREE IN BIOTECHNOLOGY

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DECLARATION

I declare that this work is the product of my own research efforts undertaken under the supervision of Dr. Abdullahi A. Imam and has not been presented and will not be presented elsewhere for the award of Degree or Certificate. All sources have been duly acknowledged.

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CERTIFICATION

This is to certify that the research work for this Dissertation and subsequent preparation of the dissertation by Hadiza Musa (SPS/15/MBC/00076) was carried out under my supervision in the Department of Biochemistry, Bayero University, Kano.

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APPROVAL PAGE

This research work has been examined and approved for the award of Master of Science (M.Sc.) degree in Biotechnology of Bayero University, Kano, for its literary contribution to knowledge.

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This Dissertation is dedicated to my parents Alh Musa Abullahi and Haj Aisha A. Tanko. I would also like to extend my dedication to my Husband Pharm Othman M. Salihu and my lovely son Abdullah.

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LIST OF ABBREVIATION

AAS	Atomic Assumption Spectromerty
AFEX	Ammonia Fiber Explosion
AOAC	Association of Analytical Chemist
CPB	Consolidated Bioprocessing
FAO	Food and Agricultural Organization
FPU	Filter Paper Cellulase Unit
SSF	Simultaneous Sacchraification and Fermentation
SSCF	Simultaneous Saccharification and Co-Fermentation (SSCF)
SHF	Separate Hydrolysis and Fermentation
NNPC	Nigerian National Petroleum Corporation
RPM	Revolution per Minute
UV-VIS	Ultraviolet Visible/ Visible Spectroscopy

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ABSTRACT

Bioethanol is an alcohol obtained from fermentation of sugars. Lignocellulosic biomass is regarded as the future feedstock for bioethanol production. Rice bran is a lignocellulosic feedstock with abundant carbohydrate for bioconversion into bioethanol. This study was designed to evaluate the potential of two varieties of rice bran (*Sipi and Wita*) to produce bioethanol. Analysis of the proximate composition was carried out using standard analytical method. Mineral element such as Fe, Ca, Mg, Mn P and Zn were determined using Atomic Absorption Spectrophotometer while Na and K were analyzed with flame photometer. Vitamin B complexes were analyzed with vitamin analyzer. Sodium hydroxide pretreatment was carried out at different concentrations (0.5%, 1%, 2% and 3%) and residence time of (15, 30, 60, and 90min). Simultaneous saccharification and fermentation of cellulosic biomass was carried out for 72hrs with *Saccharomyces cerevisae* and *Mucor indicus*. The result showed that rice bran is a good source of vitamin B complexes and minerals. Proximate composition of rice bran showed high carbohydrate content. Highest reducing sugar was obtained at 90min pretreatment with 2% NaoH. Simultaneous Saccharification and Fermentation of *Wita* variety *with S.cerevisiae* produced highest ethanol concentration of 1.36% while *Mucor indicus* produced 0.75% bioethanol. From the result of these findings, it can be concluded that rice bran could be considered as the future lignocellulosic feedstock for the production of bioethanol.

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUNG OF STUDY

Worldwide interest is increasing in alternative sources of energy due to inevitable depletion of energy supply. The increase in the prices of fossil-based fuels, strict government regulations on exhaust emissions and future depletion of worldwide petroleum reserves, encourage studies searching for alternative fuels. Nejadkoorki *et al*., (2008) stated that emissions of carbondioxide from road traffic worldwide between 1990 and 2020 will increase by 92%. Carbon dioxide (CO_2) is the major greenhouse gas that traps the earth's heat and contributes to climate change (Fogarty and McCally, 2010). Biofuel is used as an alternative for fossil-based fuel due to increased concern about environmental protection. Biomass energy can play an important role in reducing green house gas emissions. Ethanol production process only uses energy from renewable sources. Hence, no net carbon dioxide is added to the atmosphere, making ethanol an environmentally bene-ficial energy source (Bull *et al.,* 1992).

Environmental concerns and the desire to be less dependent on imported fossil fuel have intensified worldwide efforts for production of ethanol from starch- and sugar-producing crops (Tiwari *et al.,* 2015). According to Jagatee *et al.,* (2015), the Utilization of starchy biomass for the production of biofuel offers a renewable alternative. Bioethanol has emerged as the most suitable renewable alternatives to fossil fuel as their quality constituents match diesel and petrol (Tiwari *et al.,* 2013). It contains 35% oxygen which helps to complete combustion of the fuel, hence with no emissions and reduces particulate (Saini *et al*., 2015). It has advantages over gasoline in internal combustion engine, due its higher octane number,

higher flame speeds, broader flammability limits and higher heat of vaporization which give it a higher compression ratio and shorter burning time (Balat and Balat, 2009).

Lignocelluloses as agricultural, industrial and forest residuals account for the majority of the total biomass present in the world and are regarded as the largest known renewable carbohydrate. This has placed attention on the utilization of fermentable sugars from lignocellulose source for biofuel production (Jorgensen *et al.*, 2007). Bioconversion of the cellulosic components into fermentable sugars is necessary for the production of industrially important products. A variety of microorganisms including bacteria, fungi and yeast may have the ability to degrade the cellulosic biomass to glucose monomers.

According to the theory of fermentation, if starting material is carbohydrate (polysaccharide), it is converted in to simple sugars (monosaccharide) such as glucose. Those sugars are then converted to alcohol and carbon dioxide (Tiwari *et al.*, 2011). Today, raw materials used in the manufacture of bioethanol by fermentation are classified as sugars, starch and cellulosic materials. Lignocellulosic biomass is anticipated to serve as a new feedstock for the production of a reproducible liquid fuel, bioethanol, without significant impacts on food production (Alvira *et al.*, 2010; Banerjee *et al.*, 2010).

Lignocellulosic materials such as Jatropa oil cake (Tiwari *et al.,* 2012), De-oiled rice bran (Beliya *et al.,* 2013), fruit wastes, rice bran have been utilized as bioethanol production materials. Cellulosic ethanol offers promise, because cellulose fibers, a major and universal component in plant cell walls, can be used to produce ethanol and the recent developments and commercialization may allay some of these concerns (Demirbas, 2005).

1.2 STATEMENT OF THE RESEARCH PROBLEM

Energy plays vital role in the economic development, security as well as poverty eradication. Future economic growth depend on long term availability of energy from sources that are accessible, affordable, renewable and environmentally friendly (Ramachandra and Boucar 2011). Nigeria is blessed with abundant renewable energy sources, there is need to harness these resources and chart a new energy future for the country.

Used of fossil based fuel resulted to health hazards due to exposure to carbon emissions caused by constant use of generators in different households and business enterprises.

Fuel wood is used by over 70% of Nigerians living in the rural areas, also over 50million tones of fuel wood is consumed annually and this rate exceed replenishment rate. Sourcing of fuel wood is the major cause of erosion and desertification (Report of the inter-ministerial committee on combating deforestation and desertification, 2000).

Rice is the most widely grown cereal in Nigeria, Kano is one of the rice producing state in the north west, Rice bran maybe easily available at low cost and it is an economical good choice for bioethanol production.

1.3 JUSTIFICATION

Bioethanol from biomass is considered the most promising alternative to fossil based fuel, and it is well known that a low cost feedstock is a very important factor in establishing a cost-effective technology (Rabelo *et al.,* 2009). Biofuel are sustainable, biodegradable and have a high combustion efficiency. Application of agro-industrial residues in bioprocesses not only provides alternative substrates but also helps solved their disposal problem there by making the environment clean. Energy plantations may create employment opportunities in rural areas and also contribute to social aspect of sustainability. The use of rice bran as an alternative feedstock for bioethanol will add value and extent the production of rice.

1.4 AIM AND OBJECTIVES

The aim of the present work is to evaluate the potential of rice bran as a sustainable feedstock for bioethanol production.

The specific objectives of the research are:

1. To determine the proximate, vitamins and mineral elemental analysis of *Sipi* and *Wita* rice bran.
2. To delignify *Sipi* and *Wita* rice bran with Sodium Hydroxide
3. To perform Simultaneous saccharification and fermentatin of *Sipi* and *Wita* rice bran with *Saccharomyces cerevisiae* and *Mucor indicus.*

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Biofuel overview

With the inevitable depletion of the world's fossil-based energy supply, there has been an increased interest worldwide towards alternative sources of energy (Aristidou and Penttila, 2000). Biomass energy is used as a means of providing modern energy to the billions who lack it. It would supplement solar, wind, and other intermittent energy sources in the renewable energy mix of the future. One of the most immediate and important applications of biomass energy systems could be in the production of ethanol from biomass. Biomass is an interesting energy source due to several reasons. Bioenergy can contribute to sustainable development; economic, environment and social domains (Monique *et al.,* 2003). Often Resources are locally available, and conversion into secondary energy carriers is feasible with low capital investments. Furthermore, since energy plantations may also create employment opportunities in rural areas, it may also contribute to the social aspect of sustainability. With the advances of biotechnological innovations, mainly in the area of enzyme and fermentation technology, many new approaches have opened for their utilization. Nearly all fuel ethanol is produced by fermentation of corn glucose in the US or sucrose in Brazil (MacDonald *et al*. 2001). It is possible because, during the last two decades, technology for ethanol production from non edible source has been developed to the extent at which large-scale production will be a reality in the next few years. Agronomic residues such as corn stover, sugarcane bagasse, wheat or rice straw, forestry, and paper mill discards, the paper portion of municipal waste and dedicated energy crops collectively termed "biomass" can be converted into fuel ethanol.

2.2 Biofuels

They are derived from organic matter in form of solid, liquid or gaseous fuels. They are generally divided into primary and secondary biofuels. Primary biofuels such as fuel wood are used in an unprocessed form primarily for heating in cooking or electricity production, secondary biofuels such as bioethanol and biodiesel are produced by processing biomass and are used for transportation and various industrial processes. The secondary biofuels can be categorized into three generations: first, second and third generation biofuels on the basis of different parameters, such as the type of feedstock, processing technology or their level of development (Nigam and Singh, 2010).

2.2.1 First Generation Biofuel

First generation biofuels mainly constitute the ethanol produced from food-based crops (Antizar-Ladislao and Turrion-Gomez, 2008). They mainly correspond to ethanol-based fuels obtained from the fermentation of sugars (corn, beet, sugar cane, etc.), biogas emitted from raw material or landfills (Naik *et al.*, 2010) and vegetable oil-based fuels (raw oil, biodiesel and renewable diesel produced from catalytic hydrodeoxygenation) (Knothe, 2010) from oleaginous plants (colza, palm, canola, etc.) However, the fact that food resources could be used to produce biofuels shows economic and environmental limitations. The most common concern related to the first generation biofuels is that with increase in production capacities, likely there will be competition with agriculture for land used for food production. For example, in some European countries such as France, the arable lands available for cultivation of oleaginous plants used for 1st generation biofuel production will not be able to support the demand for biofuel production by the year 2015, except by saturating the land in fallow, which would create soil impoverishment problems (Bordet *et al.,* 2006). The increased pressure on arable land currently used for food production can lead to severe food insecurity, particularly for the developing world where already more than 800 million people

suffer from hunger and malnutrition. Also, the intensive use of land with high fertilizer, and pesticide applications and water can cause environmental pollution (Schenk *et al.*, 2008).

2.2.2 Second Generation Biofuel

Second generation biofuels are the cellulosic-based biofuels obtained from non-food crops materials (wood, leaves, straw, etc.) i.e. second generation biofuel do not compete with food production. Sources include agronomic residues, forest harvesting residues or wood processing waste such as leaves, straw or wood chips as well as the non-edible components of corn or sugarcane. Conversion of cellulosic biomass into fermentable sugars require cost effective technology, which involves pretreatment with specialize enzymes. Second generation biofuels cannot be produced economically on a large scale (Brennal and Owende, 2010). These biofuels include bioalcohols, bio-oil, 2,5-dimethylfuran (BioDMF), biohydrogen, Fischer-Tropsch diesel, wood diesel (Román-Leshkov *et al*., 2007 ; Fatih-Demirbas, 2009).

2.2.3 Third Generation Biofuel

Third generation biofuels are derived from microalgae and are considered to be the possible and reliable alternative energy resource that devoid the major drawbacks associated with first and second generation biofuels (Chisti, 2007 ; Li *et al.,* 2008 and Nigam and Singh, 2010). Microalgae are able to produce 15–300 times more oil for biodiesel production than traditional crops on an area basis. For each ton of microalgal biomass produced, some authors estimate that 1.8 tons of CO_2 would be consumed (80% reduction) (Chisti, 2007).

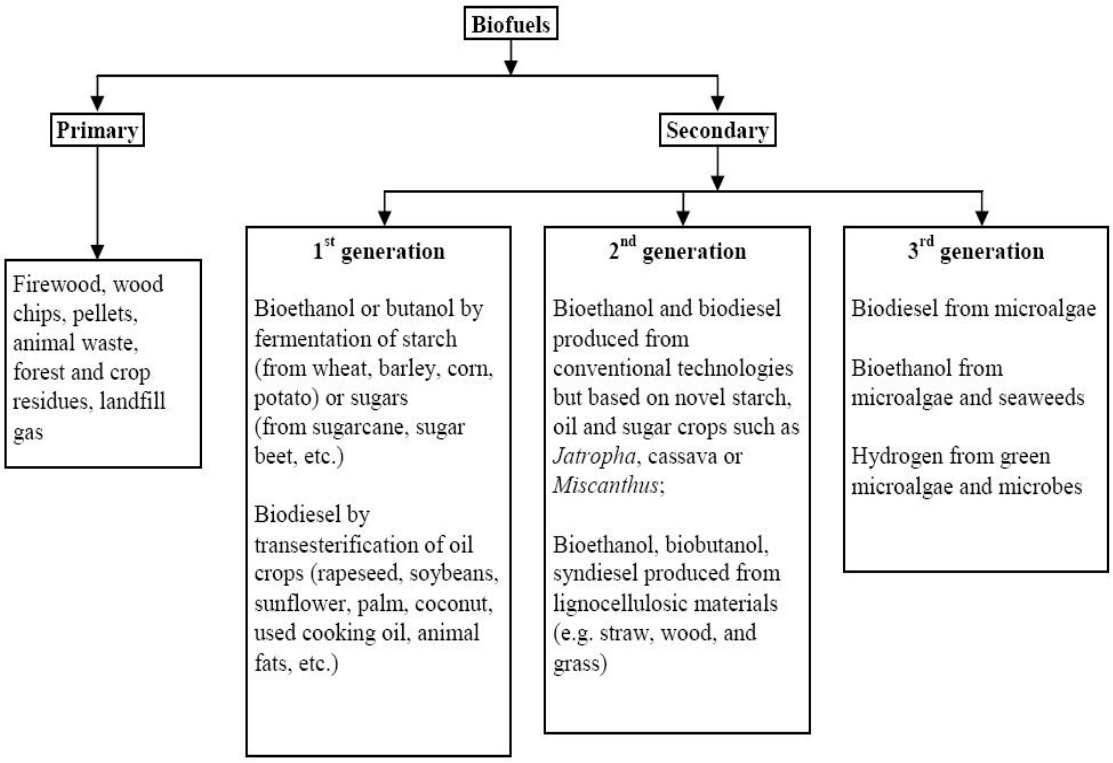


Figure 2.1; Classification of Biofuel (Source: Nigam and Singh, 2010).

2.3 Uses of Bioethanol

Bioethanol is used mostly in transportation as a biofuel, as it is blended at 5% with petrol and 10% in some countries. It has a high octane rating, which improves engine performance and used as an oxygenating additive. Ethanol is used as "E-Diesel", (formulate a blend with diesel fuel) and also in small craft it serve as a replacement for leaded aviation gasoline. Commonly used as a solvent in chemical industries because, it dissolves several organic compounds that are insoluble in water. It is also used in cosmetics, perfumes, colognes, mouthwash, lotions, soaps, shampoo, etc. Ethanol formed intermediates in the synthesis of various organic chemicals and other chemicals such as ethyl esters, ethyl acetate, extractants and antifreeze. For the purpose of human consumption ethyl alcohol is produced always by fermentation of some suitable material to form beer, wine, or distilled spirits of various kinds (Clark, 2003).

2.4 Rice bran

Rice bran is the cuticle existing between the rice and husk of the paddy and consists of embryo and endosperm of the *Oryza sativa*. It constitutes 8% of the weight of the whole grain and contains most of the nutrients (Bhosale and Vijayalakshmi, 2015). It's among the lignocellulosic feedstock used for bioethanol production.

2.5 Feedstock for biofuel production

Edible feed stocks such as corn-starch and sugarcane molasses are used commercially for bioethanol production. In Brazil bioethanol is produced at very low cost by fermentation of sugarcane. While in the united state, corn is the dominant biomass feedstock for production of ethanol. Straw and other agricultural wastes are the preferred biomass in the European countries (Raposo *et al*., 2009). Currently Molasses is a co-product in sugarcane production. The yield of molasses from crushed sugarcane ranges from 4-4.5% (Indian Sugar Mills Association). Alcohol is originally manufactured with a water content of 5-7% so it must first be dehydrated to create anhydrous ethanol, which is 99.5% ethanol (Kumar and Agrawal, 2003).

According to statistics by All India Distillers Association for a year period covering 65 distilleries in different parts of the country, the average efficiency in production of molasses based ethanol is 85% and the amount of fermentable sugars in molasses is about 42% with a yield of 222 L of ethanol per ton of molasses (All India Distillers Association , 2006). Brazil is the largest producer of bioethanol with a potential of 6641 million liter (Balat and Balat, 2008).

2.5.1 Sugarbeet

Sugar beet (B.vulgaris) also known as white beet originally from Europe is used in the production of ethanol. It's a nonfood beet plant whose tuber contains a high concentration of sucrose. it is regarded as one of the main raw materials for biofuel production. Sugar beets

compositions are 75 % water, 18 % sugar, and 7 % insoluble and soluble materials. Most of the sugar beet is in the form of sucrose and consist of other sugars such as maltose, glucose, fructose, however it does not interfere with fermentation and distillation for the production of ethanol (Haankuku *et al*., 2015).

2.5.2 Sweet sorghum

Sweet sorghum belongs to a perennial plant of Andropogoneae tribe and sub-family Panicoidae, Polaes order, Poaceae family, genereo S. Sorghum species (Ratnavathi *et al*. 2010); This plant is a native of tropical grass African countries , Sudan and Ethiopia. Several products can be obtained from sorghum, such as sugar, ethanol, paper, and other chemical compounds (Ratnavathi *et al*., 2010). Depending on the cultivar, chemical composition of the juice obtained from sweet sorghum stalks may result high ethanol yield (Sipos *et al*., 2009).The cultivars have high level of accumulation of fermentable carbohydrates (15–23 %) within the stalk (Sarath *et al,*. 2008; Smith *et al*., 1987). Total fermentable carbohydrates are mainly three sugars; sucrose (70 %), glucose (20 %), and fructose (10 %) variation in percentages depends on environmental conditions and plant variety (Prasad *et al*., 2007). Sweet sorghum is considered the as a viable feedstock option for the production of ethanol in some parts of the world (Davila-Gomez *et al*., 2011). In countries like USA and Brazil, it is already being used in conjunction with sugarcane to increase ethanol production (Pereira *et al*., 2013).

2.5.3 Starchy Crops

Starchy materials such as corn, cassava, wheat, and barley are among biofuels feedstocks (Balat *et al*., 2008). However among the starchy crops, corn is the most employed feedstock used for bioethanol production. Starch-based feedstock are environmental friendly, low cost, and renewable. Starch-based ethanol production is problematic, remains a mature technology capable of immediate contribution to the pressing global environmental and energy security. Limitations of used of starchy crops for bioethanol production include; Biofuels production takes land away from its two primary used: environmental preservation and food production.

2.5.4 Corn

USA's corn production nearly reached 351.3 million metric tons of corn In 2013/2014. In USA, more than one-third corn crop produced is used to feed livestock, 13 % is exported and 40 % is used for ethanol production. While the remainder goes toward food and beverage production (Carter and Miller 2012; EIA 2013). Corn Stover is the residue left in the fields after harvesting corn, has been identified as agricultural feedstock for the lignocelluloses-to ethanol process. The U.S. Department of Agriculture (USDA) has a program devoted to the corn ethanol industry. Scientific research addresses the establishment of new higher-value ethanol co products, the development of microbes that have the capability of converting various biomass substrate into bioethanol, improved enzymatic saccharification processes of corn fibers into sugars, and several methods that will improved corn ethanol process efficiencies (McLoom *et al*., 2000).

2.5.5 Cassava

World cassava production per year is around 281 million tons (Mt). Africa contributes to more than half of global supply. Asia ensures the development of cassava crops for both energy purposes and industrial processes. This continent contributes about one third of world production, Thailand produces 26 Mt and 28 Mt by Indonesia. While in Latin America,

production is around 35 Mt, Brazil is third place in world production, it dominates with around 70 % of regional production (Conab, 2013). Cassava contains 59–70% starch, the starch which is a polysaccharide comprises mainly of glucose monomers that are linked together by glycosidic bonds. It is consist of two types of glucan namely amylose, which is a linear glucose polymer having only a-1,4 glycosidic linkage and amylopectin, it's a branched glucose polymer mainly containing a-1,4 glycosidic linkage in a linear part and a few a-1,6 at a branch structure (Sriroth *et al*., 2012). Cassava is still a small player on the biofuel scenario. In effect, one ton of cassava, contains 30 % starch, and able to produced 280L of 96 % pure ethanol (Sriroth *et al*., 2012).

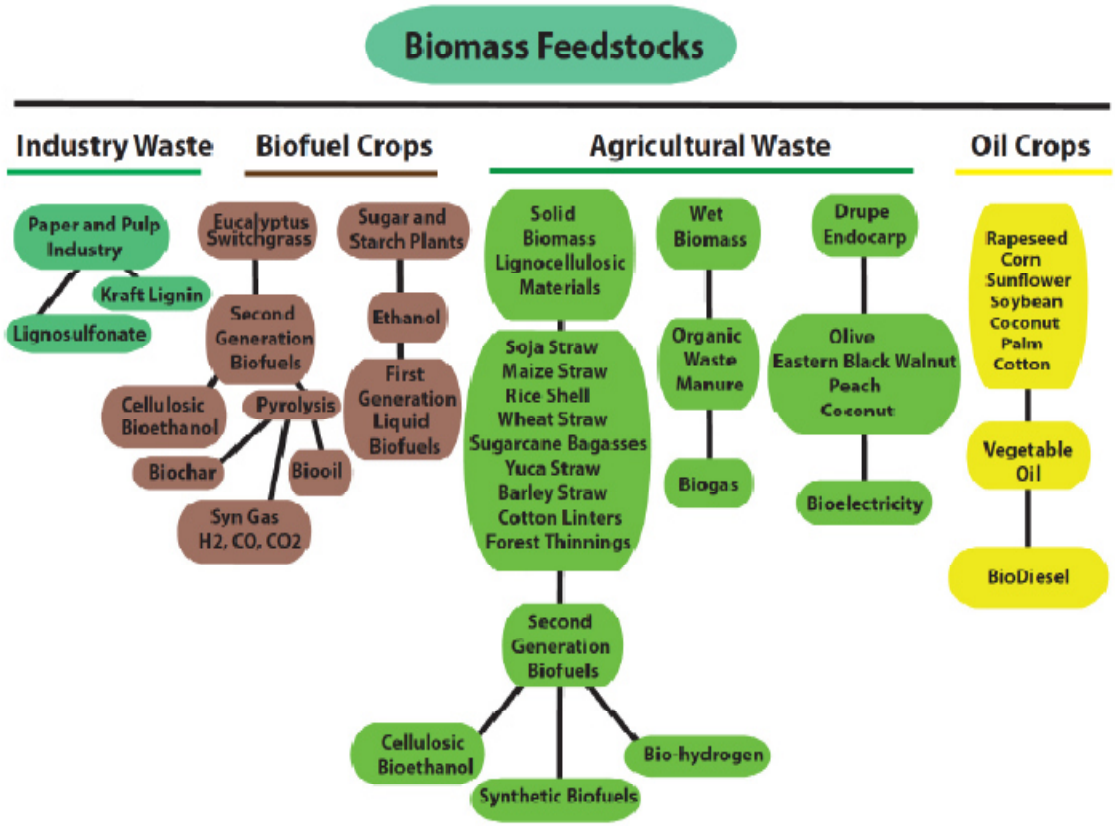


Figure 2.2; Biomass feedstocks and their utilization in the production of biofuels, bioenergy and bioproducts. The figure includes the first- and second-generation feedstocks and their utilization for different bioproducts. (Source: Welker et al., 2015).

2.5.6 Lignocellulosic Biomass

Biofuels produced from lignocellulosic materials are known as Second-generation biofuels. lignocellulosic materials include cereal straw, forest residues, bagasse, and purpose-grown energy crops such as vegetative grasses and short rotation forests (Demirbas, 2009).. Cellulose, hemicellulose, and lignin are the main component of lignocellulosic biomass. Cellulose is a homopolysaccharide it is made up of β-D-pyranose units, linked by β-1, 4-glycosidic bonds. Cellobiose is the smallest repetitive unit and it is formed by two glucose monomers. Cellulose is a long-chain polymers packed together into microfibrils by hydrogen bond and vander Waals force. Hemicellulose and lignin cover the microfibils. Hemicellulose is a mixture of polysaccharides, (pentoses, hexoses and uronic acids). Lignin is the most complex natural polymer which consist of predominant building block of phenylpropane units. P-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol are the most commonly available alcohols (Harmsen *et al*., 2010). Lignocellulosic materials also contain small amounts of pectin, proteins, extractives and ash (Kumar *et al*., 2009). The processes used for obtaining bioethanol from lignocellulosic sources include the following steps: pretreatment to alter the structure of the biomass for the release of cellulose and hemicelluloses fractions; hydrolysis of cellulose and hemicellulose to release sugars (glucose, xylose, galactose, mannose, arabinose) (Sarkar *et al*., 2012).

The biomass composition varies widely according to the nature of feedstock and growing conditions besides climatic condition. The variation in biomass composition is given in Table 2.1. Cellulose, hemicellulose, and lignin Content in various sources of biomass

Table 2.1: The variation in Lignocellulosic biomass composition

Feedstock	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Corn stover	36.4	22.6	16.6
Wheat straw	38.2	24.7	23.4
Ricestraw	34.2	24.5	23.4
Switchgrass	31.0	24.4	17.6
Poplar	49.9	20.4	18.1
Sweetsorghum	33.7	21.6	20.5
Baggas	42	25	20

Source: Wiselogel, *et al*., (1996)

2.6 Biomass and Bioenergy Potentials in Nigeria

Biomass feedstocks can be obtained from two principal different sources which include; conventional agricultural products such as sugar- or starch-rich crops, and oilseeds; and lignocellulosic products and residues (Girard and Fallot, 2006). Lignocellulosic feedstocks are potentially more abundant and cheaper than conventional agricultural feedstocks because their production does not require much resources and quality lands. Also, agricultural and forest residues are currently available from current harvesting activities without the need for additional land cultivation this means it does not require intensive labor. The type of biomass resource available in Nigeria varies with climatic region in the country. For example the savannah zones will generate more crop residues while the rain forest zone will produce the highest quantity of woody biomass (Olaoye, 2011).

2.6.1 Agricultural Crop Residues

Agricultural residues are organic materials produced as byproduct during the harvesting and processing of agricultural crops. Agricultural residues which are produced at the time of harvest are primary based residues while the ones produced along with the product during processing are secondary based residues. Agricultural residues are diverse, differs in bulk density, moisture content, particle size and distribution depending on the mode they are handled. Usually they are fibrous, low in nitrogen and vary with geographical location (Smith, 1989). Some of the field residues are used as fodder for livestock, for erosion control and as fertilizer. Close to 50% of the residue are however burnt on cropland before the start of the next growing season. Processed residues offer high promise as energy source (Cooper and Laing, 2007).

Worldwide production of Agro waste is given in Table 2.2. Asia is the major producer of rice straw and wheat straw, where as corn straw and bargasse are mostly produced in America

Table 2.2: Quantities of Agricultural waste (million tons) reportedly available for bioethanol production.

Agrowaste	Africa	Asia	Europe	America	Oceanic
Rice straw	20.9	667.6	3.9	3.72	1.7
Wheatstraw	5.34	145.20	132.59	62.64	8.57
Cornstraw	0.00	33.90	28.61	140.86	0.24
Bargasse	11.73	74.88	0.01	87.62	6.49

Source: Sarkar *et al*.,(2012).

2.6.2 Aquatic weed

Aquatic plants grow on wet land and do not compet for land that could be use for crops or forest. Use of aquatic plant as feed stock for biofuel production will add value to the land with positive environmental impact (Zhang *et al.,* 2012).

The country has abundance of fast growing weeds in the coastal regions which can be used for energy purposes. There are three common aquatic weed:

- Water hyacinth: Water hyacinth has high productivity, wide tolerance range and competitive ability (Bamgboye, 2012) Bamgboye (1994). It was discovered to be suitable for biogas production.
- Water lettuce: Water lettuce (*Pistia stratiotes* Linn) is a free growing aquatic herb with succulent leaves.
- Brackenfern: It is abundant in the swampy regions of Nigeria. It is a terrestrial weed which can be used to produce biogas. According to Bamgboye (2012) more biogas can be produced using aquatic weeds than when the substrate was terrestrial weeds.

2.6.3 Algae

High oil prices, competing demands between foods as a feedstock for biofuel production, and the world food crisis, have ignited interest in Alga culture for making vegetable oil, biodiesel, bioethanol, and other biofuels, using land that is not suitable for agriculture. Among algal fuels' appealing characteristics is that, they can be grown with minimal impact on freshwater resources (Yang *et al*., 2010).Algae can produce up to 300 times more oil per acre than conventional crops. Algae have a short harvesting cycle of 1 to 10 days; their cultivation permits several harvests in a very short time-frame, a strategy that differs it from yearly crops (Christi, 2007).

2.6.4 Energy crops

Energy crops are grown and harvested specifically for energy purpose. Crops grown in Nigeria include Jatropha, *eucalyptu*s and poplar (*Populus* spp). Currently there is increased interest being shown on the production of Jatropha because it is inedible to animal. Jatropha, known as *Lapalapa* in Yoruba, *Wuluidu* in Igbo and *Bini da zugu* in Hausa, is a multipurpose shrub that grows wildly in Nigeria with no maintenance. Two varieties found in Nigeria are *Jatropha curcas* and *Jatropha glandulifera* (Unilorin, 2012).

2.6.5 Forestry resources

It consist of logging residues (tops, branches) and processed residues from wood industries, (off-cuts, sawdust) and demolition wood. These biomass types vary in composition, quality and volume, depending on the processing steps and soils of origin (Girard and Fallot, 2006).

2.6.6 Urban wastes as a substrate for bioenergy production

These are wastes generated by households and commercial concerns as a result of large population and increased activities in urban areas. Municipal solid wastes increases with increased in human activities such as industrialization and urbanization (Beukering *et al*.,

1999). Most solid wastes end in the waste dumps while the waste water from food industries which contain sugars, starches and other dissolved and solid organic matter which contribute a lot to environmental pollution. Food wastes can be anaerobically digested to produce biogas.

2.6.7 Animal wastes as a substrate for bioenergy production

Animal wastes are mainly the droppings of livestock animals. The main constituents of this waste include organic material, moisture and ash. When decomposed under aerobic conditions, CO_2 and stabilized organic materials are produced while under anaerobic condition CH_4, CO_2 and stabilized organic material are produced. The quantity of manure produced precisely depends on the amount of feed consumed, feed quality and the weight of the animal (Duku *et al*., 2011). Manure generated can be converted into biogas by anaerobic digestion

2.7 Nigerian Biofuels Policy and Incentives

The Nigerian Biofuels Policy and Incentives was drafted in 2007 by the national oil company (NNPC) it was the first of it kind established in Nigeria with the view of integrating agricultural activities with oil and gas exploration and production since the discovery of commercial quantities of oil in 1956. The policy addresses the key government plans regarding the production of bioethanol and biodiesel across the country from the research and development

phase to large scale production and investment stages. The federal government of Nigeria in line with its program (Automotive Biomass for Nigeria) mandated NNPC to draft the policy in August 2005, so that to reduced the nation's overdependence on oil and gas economy and the environmental threats associated with the fossil fuels exploitation. The mandate requires that the policy is designed to allow the future usage of biofuels in the country, to ensure

significant impact on gasoline, diesel and other petroleum products quality enhancement (Galadima *et al*., 2011).

2.7.1 Objectives and the Anticipated Benefits of the Policy

The main objective of the policy is to establish and biodiesel and ethanol industry, which will solely depend on local agricultural products as feed stocks, so that to improve the quality of the fossil fuels for use in automotive industries and other sectors. It seeks to provide an appropriate link between the agriculture and energy sector (NNPC, 2007). It also aims to create an avenue for integrated national development covering all sectors of the economy. The specific anticipated benefits of the policy include the following:

- Environmental pollution due to fossil fuels will be reduced. Biofuels could drastically reduce tailpipe emissions and the destruction of ozone layer. It can also be a desirable replacements to toxic octane associated with fossil based fuel respectively.
- Country's sources of revenue will be diversifying as additional taxes could be generated from commercial activities attributed to the industry.
- Creation of sustainable job opportunities for citizens and the empowerment of rural communities.
- An agricultural benefit is improved by advancing farming techniques and agricultural research.
- The policy ensures that projected energy demand in the country is addressed sustainably.
- The biomass composition varies widely according the nature of feedstock and growing conditions besides climatic condition.

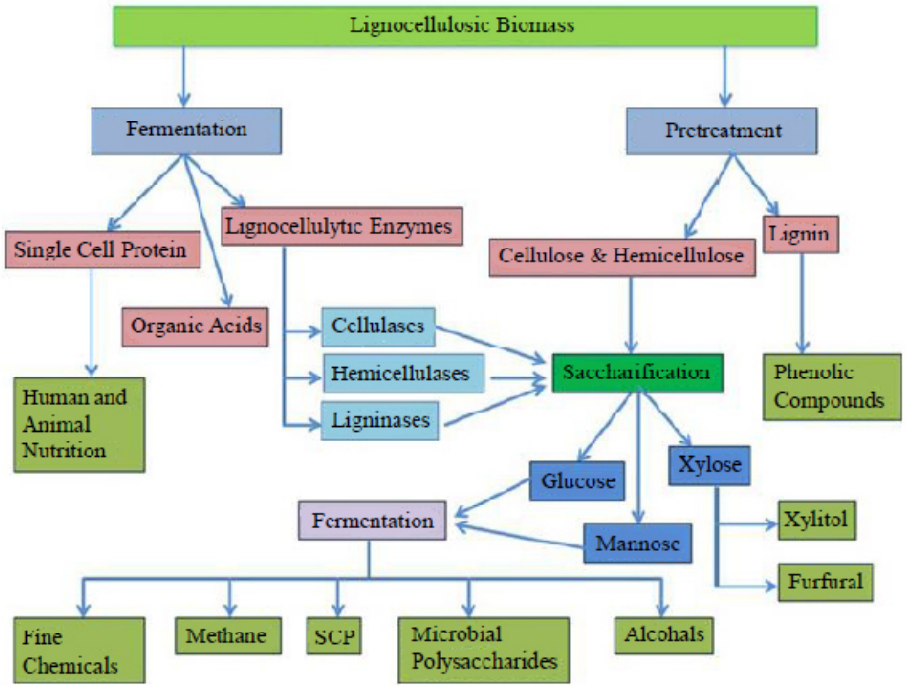


Figure 2.3: Bioconversion of lignocellulosic biomass into value added products (Source: Mussatto and Teixeira, 2010)

2.8 Bioethanol production

The technology of ethanol production from biomass feedstocks involved several steps and varies based on the type of feedstock used. For the production of bioethanol from cellulosic biomass, four major unit operations are required: pretreatment, hydrolysis, fermentation and separation (Taherzadeh and karimi, 2007).

2.8.1 Pretreatment

Pretreatment is considered as one of the most expensive processing step in biomass to fermentable sugar conversion with cost as high as 10,845 Naira/gallon ethanol produced (Moiser *et al*., 2005). Pretreatment is needed to alter the biomass structure and size as well as its submicroscopic chemical composition and structure so that hydrolysis of carbohydrate in to monomeric sugars can be achieved more rapidly and with greater yields (Sun and Cheng, 2002). Pretreatment affects the biomass structure by solubilizing hemicellulose, increasing

the surface area, reducing it crystallinity and increasing the pore volume of the substrate. Technoeconomic analysis have been recently made to assess the cost and performance of pretreatment methods (Eggerman and Elander, 2005). The cost of pretreatment process will be lowered through extensive research & development approaches. Pretreatment cost of cellulosic biomass is a major challenge of cellulosic ethanol technology research and development. Generally lignocellulosic biomass is recalcitrant to enzymatic digestion or hydrolysis. Therefore, different types of thermochemical pretreatment methods have been developed to improve digestibility of the biomass (Wyman *et al*., 2005).

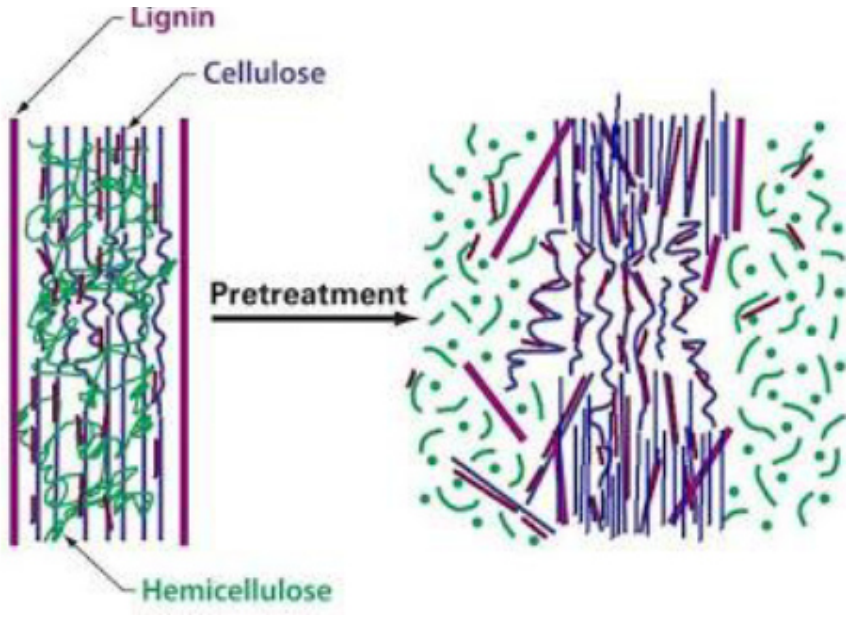


Figure 2.4; Role of pretreatment in the conversion of biomass to fuel. (Source: Hsu *et al*., 1980)

2.8.1 Physical pretreatment: physical pretreatment of lignocellulosic biomass include the mechanical comminution and pyrolysis.

2.8.1.2 Mechanical comminution: To reduce cellulose crystallinity, Waste materials can be comminuted by a combination of chipping, grinding and milling. The size of the materials is reduced to 0.2–2 mm after grinding or milling and 10–30 mm after chipping. Vibratory ball

milling is said to be more effective in breaking down the cellulose crystallinity of aspen chips and spruce and improving the digestibility of the biomass compared to ordinary ball milling (Millet *et al.,* 1976). Mechanical comminution of agricultural materials requirement depends on the final particle size and the characteristics of biomass (Cadoche and Lopez, 1989).

2.8.1.3 Pyrolysis

Pyrolysis is used for pretreatment of lignocellulosic materials. Materials are subjected to higher temperatures greater than 300^{o}C cellulose rapidly decomposes to produce gaseous products and residual charcoal (Shafizadeh and Bradbury, 1979). The decomposition of biomass material is much slower and at low temprerature less volatile products are formed. Mild acid hydrolysis (1 NH_2SO_4, 97^{0}C 2.5hour) of the residues that has been subjected to pyrolsis pretreatment has resulted to 80-85% conversion of cellulose to reducing sugars with more than 50% glucose (Fan *et al.,* 1987). In this method lignocellulosic material undergo explosive decomposition which causes hemicellulose degradation, lignin transformation and thus increases cellulose hydrolysis potential (Fan *et al.,* 1987).

2.8.1.4 Physico-chemical pretreatment: Physicochemical pretreatment include steam explosion, Ammonia fiber explosion and carbondioxide explosion.

2.8.1.5 Steam-explosion

Steam explosion is one of the methods used for pretreatment of lignocellulosic biomass (McMillan, 1994). In steam-explosion method, chipped biomass is treated with high-pressure saturated steam, and then the pressure is reduced, which in turn makes the residue undergo an explosive decompression. This method is usually initiated at a temperature range between 160–260^{0}C with corresponding pressure of 0.69–4.83 mpa for few minutes before exposing the material to atmospheric pressure. The process causes degradation of hemicellulose and transformation of lignin due to high temperature, thereby increasing the potential of cellulose hydrolysis. 99% efficiency of enzymatic hydrolysis has been achieved in 24 hours, for poplar

chips that undergoes steam explosion pretreatment (Grous *et al.,* 1986). The factors that affect steam explosion pretreatment include time, temperature, chip size and moisture content (Duff and Murray, 1996). Addition of sulphuric acid or carbondioxide in steam explosion method improves enzymatic hydrolysis, decreases inhibitory compounds production, and lead to hemicellulose complete removal (Morjanoff and Gray, 1987).

2.8.1.6 Ammonia fiber explosion (AFEX)

Its type of physico-chemical pretreatment which involved exposing lignocellulosic materials to liquid ammonia at high pressure and temperature for a period of time, and then the pressure is swiftly reduced. The concept of AFEX is similar to steam explosion. In AFEX, the dosage of liquid ammonia is 1–2 kg ammonia/kg dry biomass, temperature of 90□, and period of 30 min. This type of pretreatment improved the saccharification rates of various herbaceous crops and grasses significantly. It can be used for the pretreatment of many lignocellulosic materials such as wheat straw, alfafa, wheat chaff (Mes-Hartree *et al.,*1988), barley straw, corn stover, rice straw (Vlasenko *et al*., 1997), municipal solid waste, softwood newspaper, kenaf newspaper (Holtzapple *et al*., 1992a), and bagasse. The AFEX does not solubilize hemicellulose compared to acid pretreatment and acid-catalyzed steam explosion. The composition of the materials after AFEX pretreatment was essentially the same as the original materials. Ammonia must be recycled after the pretreatment in other to reduce the cost and protect the environment. Ammonia pretreatment does not produce inhibitors for the downstream micrbial processes, so washing is not necessary (Mes-Hartree *et al.,* 1988).

2.8.1.7 Carbobdioxide explosion

Its Similar to steam and ammonia explosion pretreatment. It was hypothesized that this type of pretreatment would form carbonic acid and also increases the hydrolysis rate. Dale and Moreira (1982) used CO_2 explosion method to pretreat alfalfa (4 kg CO2=kg fiber at the

pressure of 5.62 MPa) and 75% of theoretical glucose release was obtained during 24h of the enzymatic hydrolysis.

2.8.2 Chemical pretreatment: Chemical pretreatment include ozonolysis, acid hydrolysis, alkaline hydrolysis and organosolv.

2.8.2.1 Ozonolysis

Ozone is used to breakdown the structure of hemicelluloses and ligninin of various lignocellulosic materials such as wheat straw (Ben-Ghedalia and Miron, 1981), bagasse, green hay, peanut, pine (Neely, 1984), and poplar sawdust . The degradation by ozone is limited to lignin and slightly attacked hemicellulose, without affecting cellulose. In ozone pretreatment, the rate of enzymatic hydrolysis is increased by a factor of 5 and 60% removal of the lignin from wheatstraw (Vidal and Molinier, 1988). The advantages of Ozonolysis include the following:

- Effective removal of lignin.
- It does not produce toxic residues or inhibitors for the downstream processes
- The reactions are usually carried out at room temperature and pressure (Vidal and Molinier, 1988).

2.8.2.2 Acid pretreatment

Acid pretreatment is considered as one of the most important techniques aimed for high yield of sugars from lignocellulosic biomass. Acid pretreatment either dilute or concentrated improved the hydrolysis of cellulose (Balat and Balat, 2008). It usually attacked polysaccharide especially hemicelluloses.

Concentrated acids such as H_2SO_4 and HCl have been used to pretreat lignocellulosic materials. These acids are powerful agents for hydrolysis of cellulose, they are toxic, corrosive and also require reactors that are resistant to corrosion. However, to make the

process economically feasible, concentrated acid must be recovered after hydrolysis (Sivers and Zacchi, 1995).

Dilute acid hydrolysis has been developed successfully for the pretreatment of lignocellulosic materials. At moderate temperature, low yield is obtained with direct saccharification due to decomposition of sugar. It uses moderate conditions to achieve high xylan to xylose conversion yields. High xylan to xylose conversion yields is essential to achieve favorable overall process economics, because xylan accounts for up to one third of the total carbohydrate in most lignocellulosic feedstock (Hinman *et al*., 1992). Dilute acid pretreatment can improved the hydrolysis of cellulose, its cost is higher compared to other physico-chemical pretreatment.

2.8.2.3 Alkaline pretreatment

Bases can be used for pretreatment of lignocellulosic materials and the effect of this method of pretreatment depends on the lignin content of lignocellulosic materials (Fan *et al*., 1987; McMillan, 1994). The mechanism of alkaline hydrolysis is base on the saponification of intermolecular ester bonds crosslinking hemicelluloses, xylan and other components such as lignin. lignocellulosic materials porosity increases with the removal of the crosslinks (Tarkow and Feist, 1969). Dilute NaOH treatment of lignocellulosic materials caused swelling, leading to an increase in internal surface area, decreases the degree of polymerization, decreases crystallinity, degradation of the lignin structure and separation of structural linkages between carbohydrate and lignin (Fan *et al*., 1987). Sodium hydroxide-treated hardwood digestibility increases from 14% to 55% and lignin content decreases from 24% to 20%. However, no effect of dilute sodium hydroxide pretreatment was observed for softwoods with lignin content greater than 26% (Millet *et al.,* 1976). Glucose yield of corn stalk was observed to be 20% in untreated samples and 43% after treatment with electron beam irradiation at the dose of 500 kGy and 2% NaOH, but the glucose yields of cassava bark was 3.5% and 2.5% for

peanut. Oxidative delignification Lignin biodegradation in the presence of hydrogen peroxide is catalyzed by the peroxidase enzyme (Azzam, 1989). The pretreatment of sugarcane bagasse with hydrogen peroxide efficiently improved its susceptibility to enzymatic hydrolysis. About 50% lignin and most hemicelluloses were solubilized by 2% H_2O_2 at temperature of 30□ and residence time of 8 h, also 95% glucose release from cellulose was achieved in the subsequent saccharification by cellulose at 45 C for 24 h (Azzam, 1989).

Lime Ca $(OH)_2$ pretreatment is another type of alkaline pretreatment which was proven to be useful method for reducing lignin content of lignocellulosic biomass without significant loss of carbohydrate and also increased biodigestibility. The biomass is pretreated with calcium hydroxide and water under different conditions of temperature and pressure. This method has short pretreatment time and can be applied with or without oxygen. Despite all this advantages, lime pretreatment has some limitation which includes: formation of inhibitors and it is less effective when applied to soft wood (Sierra et al., 2009).

Potassium hydroxide is an effective alkaline reagent for pretreatment (Sun et al., 2014). In contrast with NaOH, KOH could be suitable solution to address the issue of high chemical recovery cost. Because the spent liquor of KOH pretreatment contains potassium, which could be used for the production of potash fertilizer to put nutrients back to soil for clean and sustainable production (Jahan et al., 2016). Potassium hydroxide has a high potential feasibility.

2.8.2.4 Organosolv process

Organosolv process involves the mixing of aqueous organic solvent mixture with inorganic acid catalysts (HCl or H_2SO_4) in various portions and adding them to the biomass. The mixture is heated to dissolve ligning and some of the hemicellulose, leaving the cellulose cake. Methanol, ethanol, acetone, ethylene glycol, triethylene glycol and tetrahydrofurfuryl alcohol are the organic solvents used in organosolv process (Thring *et al*., 1990). Organic

acids such as oxalic, acetylsalicylic and salicylic acid are used as catalysts in the organosolv process (Sarkanen, 1980). At high temperatures above 185 C, the addition of catalyst was not necessary for satisfactory delignification (Sarkanen, 1980). Usually, with the addition of acid, a high yield of xylose is obtained. To reduced the cost of the process, there is need to drained the solvent from the reactor, evaporate, condense and recycle. Advantages of this method include:

- Isolation of lignin
- Various organic solvent can be used for delignification

2.8.3 Biological pretreatment

Biological pretreatment process involved the use 0f microorganisms such as brown-, white- and soft-rot fungi to degrade lignin and hemicellulose in biomass materials (Schurz, 1978). Soft rots attack both cellulose and lignin while Brown rots mainly attack cellulose. A White-rot fungus is considered as the most effective basidiomycetes for biological pretreatment of lignocellulosic materials (Fan *et al*., 1987). According to the study carried out by Hatakka (1983), wheat straw was pretreated by 19 white-rot fungi and found that 35% of the straw was converted to reducing sugars by *Pleurotus ostreatus* within five weeks. A cellulase-less mutant of *Sporotrichum pulverulentum* was developed for the degradation of lignin in wood chips, in order to prevent the loss of cellulose, (Sun and Cheng, 2002). During secondary metabolism of white-rot fungus *P. chrysosporium* in response to carbon or nitrogen limitation, it produces lignin-degrading enzymes which include lignin peroxidases and manganese-dependent peroxidases, (Boominathan and Reddy, 1992). Other enzymes that degrade lignin include polyphenol oxidases, laccases, H_2O_2 producingenzymes and quinone-reducing enzymes (Blanchette, 1991). Biological pretreatment has so many advantages which include low energy requirement and mild environmental conditions. But, the rate of hydrolysis in most biological pretreatment processes is very low.

2.9. Hydrolysis

After pretreatment there are two processes to hydrolyze the feed stocks into monomeric sugar for fermentation into ethanol. The hydrolysis methods most commonly used are enzymatic and acid hydrolysis (dilute and concentrated). To improve the efficiency of enzymatic hydrolysis, the lignin-hemicellulose network has to be loosened for the better amenability of cellulases to residual carbohydrate fraction for sugar recovery. Dilute acid treatment is employed for the degradation of hemicellulose leaving lignin and cellulose network in the substrate (chandel *et al*., 2007).

2.9.1 Enzymatic hydrolysis

Tucker *et al*., (2003) stated that Pretreated lignocellulosics biomass can be saccharified enzymatically to obtain fermentable sugars. Bacteria and fungi are excellent source of cellulases, hemicellulases which could be applied for the hydrolysis of pretreated lignocellulosics. The enzymatic cocktail is a mixture of various hydrolytic enzymes which consist of cellulases, xylanases, hemicellulases and mannanases. In the last decade, new cellulases and hemicellulases have been isolated from bacterial and fungal sources, and also much efforts have been made for the improved production of enzymetic titers (Aro *et al*., 2005). Group of microorganisms such as *Clostridium*, *Cellulomonas, Tricho-derma*, *Penicillium*, *Neurospora*, *Fusarium*, *Aspergillus* etc. shows a high cellulolytic and hemicellulolytic activity, which are able to ferment monosaccharides. Genetic engineering is used to produce super strains, which have the capablility of hydrolysing cellulose and xylan along with fermentation of glucose and xylose to ethanol (Aristidou and Penttila, 2000). According to Lynd *et al*., (2002) cellulose utilization by microorganisms involves a substantial series of fundamental phenomena without those associated with enzymatic hydrolysis of cellulose.

2.9.2 Fermentation

The sugar obtained after cellulosic hydrolysis is used for ethanol fermentation. The ability to ferment pentoses along with hexoses is not widespread among microorganisms (Toivolla *et al*., 1984), *S. cereviseae* is capable of converting only hexose sugars to ethanol. The most promising yeasts that have the ability to use both C5 and C6 sugars are *Pichia stipitis*, *Candida shehatae* and *Pachysolan tannophilus*. Lin (2006) stated that ethanol production from sugars derived from starch and sucrose has been commercially dominated by the yeast *S. cereviseae* (Lin, 2006). Thermotolerant yeast could be more suitable for ethanol production at industrial level. In high temperature process energy savings can be achieved through a reduction in cooling costs. Considering this approach, Sree et al. (1999) developed solid state fermentation system for ethanol production from sweet sorghum and potato employing a thermotolerant *S. cereviseae* strain (VS3). Researchers are now focusing on developing recombinant yeast, which can greatly improve the ethanol production yield by metabolizing all form of sugars, and reduce the cost of operation. Researchers have made efforts by following two approaches. The first approach has been to genetically modify the yeast and other natural ethanologens additional pentose metabolic pathways. The second approach is to improve ethanol yields by genetic engineering in microorganisms that have the ability to ferment both hexoses and pentoses (Jeffries and Jin, 2000).

2.9.2.1 Separate hydrolysis and fermentation (SHF)

Separate enzymatic hydrolysis and fermentation (SHF), temperature and PH can be optimized but also regarding the design of the equipment including stirring. Cellulase enzymes usually has it maximum activity at 50ºC or higher while most fermenting microorganisms, such as .*S. cerevisiae*, do not tolerate higher temperature above 37ºC. Usually Conventional ethanol fermentation is carried out at temperature below 35 ºC. However, enzymes are deactivated faster at a higher temperature than at lower temperatures. Although the enzymatic hydrolysis

is faster at 50 ºC, there is also possibility that the sugar yields after 48 or 72 hours hydrolysis is higher at 40 ºC, or even lower temperatures, due to the deactivation of enzyme (Tengborg *et al.*, 2001a).

2.9.2.2 Simultaneous Saccharification and Fermentation (SSF)

In SSF, sugars formed by enzymatic hydrolysis are converted by yeast immediately after they are released. As a result there is only low concentration of sugars in the broth which alleviates the end-product inhibition of the cellulase enzyme and also reduced the risk for infections. It also has the capability of the yeast to partially detoxify the slurry (Tengborg *et al.*,2001b). These two effects, result to an increased in the productivity of enzymatic hydrolysis also compared to enzymatic hydrolysis performed at higher temperatures. At lower total reactor volume, higher overall ethanol productivity is achieved. Several studies have shown that, ethanol yield is higher in SSF than SHF both for Agricultural residues and softwood, (Söderström *et al.*, 2005).The main limitation with SSF is that the yeast after SSF is difficult to recover because it's mixed with the residual solid. In spite of this limitation SSF is still considered SSF to be better option than SHF for all raw materials that have been investigated so far. The use of SSF is also cost-effective, because it reduces the number of reactors (Wingren *et al.*, 2003).

2.9.2.3 Simultaneous Saccharification and Co-Fermentation (SSCF)

Microorganisms used for bioethanol production is unable to utilize all the sugar release from hydrolysis. For example, the wild-type strain of *S. cerevisiae* cannot utilize pentose sugars, and this represents reduction in bioethanol yield and also biomass waste. To overcome this limitation, recombinant yeast or cocktails of cellulosic enzyme are introduced during fermentation to convert both hexoses and pentoses (Wyman, 1996). It can therefore be considered as an improvement to SSF. Both hydrolysis and fermentation are carried out in the same vessel in SSCF; hence it has similar characteristics as SSF, which

include low cost, short processing time, less inhibitory effect and reduced risk of contamination (Chandel *et al*., 2007). Jin *et al.,* (2010) proposed and studied. A two-step SSCF, Fermentation time was divided into two equal parts and similar conditions were applied as in the process. In this method, 4% of total cellulases were used during the first half of the fermentation process, and then the remaining cellulases were applied in the second half of the fermentation. Improved xylose consumption, significantly increased bioethanol yield

2.9.2.4 Consolidated Bioprocessing (CBP)

Consolidated Bioprocessing simultaneously combines hydrolysis of biomass, utilization of liberated sugars and fermentation in same bioreactor (Xu *et al.,* 2010). According to Lynd *et al*.,(2005). Consolidated bioprocessing is more cost effective than SSCF and is considered energy efficient due to the reduction of processes. However, among all the consolidated bioprocessing potential microbes, thermophilic bacteria, such as *Clostridium thermocellum*, are believed to be feasible, because they possess both cellulolytic and ethanologenic characteristics under high temperature conditions (Georgieva *et al.*, 2008). Cellulosome is s Complexes of cellulolytic enzymes contained *in C. thermocellum* and are responsible for degradation of cellulose and sugar release.

2.9.3 Ethanologenic microorganisms

Microorganism produces ethanol by fermentation of sugar. For efficient ethanol production there is need for competent microorganisms that are able to ferment both pentoses and hexoses. and also at the same time, capable of tolerating alcohol and sugar stress conditions that accur during fermentations. Basically microorganisms take part in two major applications during fermentation process; they are able convert fermentable sugars into ethanol and on the other hand produce enzymes that catalyze conversion of complex carbohydrates into simpler sugars (Thatoi *et al*., 2014). Variety of microorganisms such as *Zymomonas mobilis*,

Saccharomyces cerevisiae, *Saccharomyces uvarum*, *Candida tropicalis*, *Candida shehatae*, *Pichiastipitis*, *Clostridium* sp., have been considered as ethanologenic microbes. The most promising candidates for industrial alcohol production are yeast *S. cerevisiae* and facultative bacterium *Z. mobiles*. So far The best xylulose fermenting yeasts identified are species of *Brettanomyces*sp., *Candida*, *Hansenula*and *Torulospora*. Also in addition to ethanol production, Pentose utilising yeast is capable of converting xylose to xylitol. Nevertheless, an important yeast, *P. stipites evidently* do not produces xylitol during sugar fermentation (Behera et al., 2014). Among many exploited microorganisms for ethanol production, *Saccharomyces cerevisiae genus* still remains as the superior species. Also *Zymomonasmobilis* is one of the most thoroughly investigated species within the past three decades because it possesses some characteristics similar to its counterpart *S. cerevisiae*.

2.9.3.1 *Saccharomyces cerevisiae:*

Saccharomyces cerevisiae has been used as the as the major ethanol producing microorganism all over the world. The sugars metabolized by *S. cerevisiae* include glucose, fructose, mannose, galactose, sucrose, maltose, and maltotriose. Ethanol production by this organism is carried out *via* the glycolytic pathway (also known as the Embden-Myerhof-Parnas or EMP pathway) (Thomas *et al*., 1996). However, the yeast cells suffer from various stresses under anaerobic ethanol fermentation some are environmentalstress, while there are others generated by the yeast cells themselves, e.g. ethanol accumulation, and also strong inhibition on growth of yeast cell and fermentation of ethanol.

2.9.3.2 *Zymomonas mobilis*

It is an anaerobic and gram-negative bacterium which produces ethanol from glucose *via* the Entner-Doudoriff (ED) pathway (Conway, 1992) in junction with the PDC and ADH enzymes. This organism catabolizes only three sugars, D-glucose, D-fructose and sucrose, as its sole carbon and energy sources. Its growth on sucrose is usually associated with

extracellular formation of the fructose oligomers (levan) and sorbitol with a significant reduction in its ethanol yield, and this makes it unsuitable for alcohol production using molasses. however, it is inappropriate for ethanol production using starch materials, because it ferment only glucose in the hydrolysate of starch materials but cannot utilize other sugars like the species of *S. cerevisiae*,. *Zymomonas* is the only microorganism that metabolizes glucose anaerobically using the ED pathway as opposed to the EM or glycolytic pathway. The ED pathway yields only half as much ATP per mole of glucose as the EM pathway. As a consequence, *Zymomonas* produces less biomass than yeast, and more carbon is funneled to fermentation products. Also, as a consequence of the low ATP yield, *Zymomonas* maintains a high glucose flux through the ED pathway. All the enzymes involved in fermentation are expressed constitutively, and fermentation enzymes comprise as much as 50% of the cells' total protein. Despite its advantages as an ethanologen, *Z.mobilis*is not well suited for all of the biomass resources conversion because it ferments only glucose, fructose, and sucrose. Moreover, *Z. mobilis* on synthetic media containing glucose, fructose or sucrose, the specific rates of sugar uptake and ethanol production are at a maximum when utilizing the glucose medium (Conway, 1992).

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1 MATERIALS

3.1.1 All the materials used in this research are listed in Appendix i

3.1.2 All Reagent and chemicals used are listed in Appendix ii

3.1.3 Sample collection and preparation

Rice bran was obtained from Kura local Government area, Kano state. Two local varieties were obtained, known as ***Wita*** and ***Sipi.*** Rice brans were air dried for a week, and then milled to reduce its size.

3.1.3.1 Sample identification

Sample was identified at Bayero University Kano, Department of Plant Biology with accession number BUKHAN 0289.

3.2 METHODS

3.2.1 Proximate analysis of *Sipi* and *Wita* rice bran

The analysis of proximate composition of both samples was carried out as follows:

Determination of Moisture Content in *Sipi* and *Wita* rice bran

Moisture content was determined as described by AOAC (2005) Using oven-dry method. It's the loss in weight due to evaporation from sample at a temperature of 100 ± 2. The weight loss in each case represents the amount of moisture present in the sample. Moisture (%) = {(Weight of original sample – weight of dried sample)} × 100/ (Weight of original sample)

Determination of Crude Fiber content in *Sipi* and *Wita* rice bran

The bulk of roughage in food is referred to as the fiber and is called crude fiber. Crude fiber was determined using the standard method described by AOAC (2005). Milled sample was defatted with ethanol acetone mixture.

Crude Fiber (%) = (Weight of residue – weight of Ash) × 100 / Weight of sample**.**

Determination of Crude Fat content in *Sipi* and *Wita* rice bran

Crude fat was determined by the Soxhlet extraction technique method described by (AOAC, 2005). Fat content of the dried samples can be easily extracted into organic solvent (petroleum ether) at temperature of 40-60□. It was then reflux for 6 hours. Percentage of fat content was calculated using the formula below.

Crude Fat (%) = Weight of fat in sample × 100/ Weight of dry sample.

Determination of Crude Protein content in *Sipi* and *Wita* rice bran

The crude protein content was determined following the micro Kjeldahl method (AOAC 2005). Percentage of nitrogen (N) was calculated using the equation below.

Nitrogen (%) = {(S-B) × N × 0.014 × D× 100} / (weight of sample × V)

Where D = Dilution factor, T = Titration value = (S-B), W = weight of sample, 0.014 = Constant value. Crude protein was obtained by multiplying the corresponding total nitrogen content by a conventional factor of 6.25. \ Protein (%) = % of N × 6.25.

Determination of Ash content in *Sipi* and *Wita* rice bran

Ash content was determined by combusting the samples in a muffle furnace at 600□ for 8 hours (AOAC, 2005).

Ash content (%) = Weight of Ash × 100/ Weight of sample.

Determination of Carbohydrate content in *Sipi* and *Wita*

The carbohydrate content was estimated by the difference method. It was calculated by subtracting the sum of percentage of moisture, fat, protein and ash contents from 100% (AOAC, 2005).

Carbohydrate (%) = 100 – (moisture% + Fat % + Protein % + Ash %)

3.2.2 Determination of Mineral element in *Sipi* and *Wita*

The minerals contents of both rice bran varieties which include Iron, Magnesium, Zinc, Calcium, phosphorous and manganese were determined after acid digestion by using Atomic Absorption Spectrophotometer (AACC, 2000).

Sodium and potassium was analyzed using flame photometer

3.2.3 Vitamin content determination

Vitamins were analyzed using vitamin analyzer (Young Lin Bldg.899-6, Hogye, Anyang, 431-836, Korea).

3.2.4 Compositional analysis of biomass

The lignin, cellulose and hemicelluloses content of both rice bran verities were analyzed using Chesson method as described by (Hernawan *et al*., 2017). To one g (a) of dry sample, 150 ml water was added and refluxed at 100 °C in water bath for 1 hour. It was then filtered, and the residue was washed with 300 ml hot water. The residue was then dried in an oven then weighed (b). To the dried Residue, 150 ml of 1N H_2SO_4 was added and then refluxed in a water bath at 100° C for 1 hour. The residue was filtered and washed with 300 ml of hot water; it was dried and weight (c). 10 ml of 72% H_2SO_4 was added to the dried residue and soaked at room temperature for 4 hours and then filtered. 150 ml of 1 N H_2SO_4 was added again to the residue and refluxed in a water bath for 1 hour, It was filtered, washed with 400 ml H_2O, then dried in an oven at temperature of 105ºC and weighed (d), The residue was ashed in the furnace and weighed (e).

Lignin content = [(d-e)/a] x 100%;

Cellulose content = [(c-d)/a] x 100%;

Hemicellulose content = [(b-c)/a] x 100%.

3.2.5 Removal of extractives from *Sipi* and *Wita* rice bran

The dried biomass was extracted with acetone using soxhlet exractor set up as described by Ayeni *et al.,* (2013) to obtain an extractive free biomass for bioethanol production.

3.2.6 Pretreatment of biomass

Biomass was pretreated with NaOH according to the method described by (Xu *et al*., 2011) with slight modification. Biomass was subjected to NaOH delignification in an auto clave. Pretreatment was carried out with different concentration of NaOH (0.5, 1.0, 2.0, and 3.0 w/v) and residence time of (15, 30, 60 and 90 min). 4g of biomass sample and 40ml NaOH solution of desired concentration was mixed in a conical flask. The pretreated biomass was then recovered by filtration (vacuum filtration) and washed with 400ml deionized water to remove excess alkali.

Table 3.1: pretreatment of biomass at different concentration of sodium hydroxide and time

Pretreatment condition time (min)	NaOH concerntration (%)
15	0.5
	1.0
	2.0
	3.0
30	0.5
	1.0
	2.0
	3.0
60	0.5
	1.0
	2.0
	3.0
90	0.5
	1.0
	2.0
	3.0

3.2.7 Filter Paper Cellulase Unit Assay

The activity of cellulase enzyme obtained from (Sigma Aldrich Novozyme) was determined in terms of filter paper units, A linear glucose standard curve using the absolute amounts of glucose standards (mg/ml) was plotted against absorbance at 540 nm. This graph was used to determine the concentration of reducing sugars in the sample tubes, which had to be incubated with cellulase enzyme solutions of varying dilutions at 50 °C for 60 min. The value of 2.0 mg of reducing sugar as glucose from 50 mg of filter paper in 60 min was used for calculating filter paper cellulase units (FPU) (Ghose, 1987).

3.2.8 Hydrolysis and Fermentation

3.2.8.1 Simultaneous saccharification and fermentation with S. cerevisae

In Simultaneous saccharification and fermentation method, enzymatic hydrolysis and glucose fermentation proceed concurrently in one vessel. The process was conducted according to the method described by (Omidvar *et al*., 2016) with slight modifications. 1.0 g of pretreated rice bran, 20 ml nutrient solution containing: g/L (yeast extract, 5.0; NH_42SO_4, 7.5; $MgSO_4 \cdot 7H_2O$, 0.75; KH_2PO_4, 3.5; $CaCl_2 \cdot 2H_2O$, 1.0) and 0.05 M sodium citrate buffer (pH 4.8) were added to a 100 ml flask. The suspension was then autoclaved for 20 min at 121°C. After cooling to room temperature, the solutions were inoculated with 0.03g of dried *S. cervisia*e and then supplemented with 25 Filter paper unit cellulase per gram of dried substrate. Finally, the flask was sealed and incubated at 35°C and 120 rpm for 72 hours. Liquid samples were taken and analyzed by UV visible spectrophotometer after three days fermentation.

3.2.8.2 Simultaneous saccharification and fermentation with Fungi (*Mucor indicus*).

3.8.2.3 Fungal spore preparation

The fungal spore was prepared based on the method described by (Abo. State, *et al*., 2014) with some modifications. Fungal isolates were inoculated onto 100 ml Potato Dextrose Agar

medium in 250 ml Erlenmeyer flasks. The inoculated media was incubated at room temperature for 5days, and then the spores were collected by adding 30 ml sterile saline containing 1.0% Tween-80. The spore suspension was collected in new sterile flask as stock for inoculation.

Simultaaneous Sacharification and fermentation for ethanol production with fungi was conducted as described by (Omidvar *et al*., 2016) with slight modifications. 1.0 g of pretreated rice bran and 20 ml nutrient solution containing: g/L (yeast extract, 5.0; NH_42SO_4, 7.5; $MgSO_4 \cdot 7H_2O$, 0.75; KH_2PO_4, 3.5; $CaCl_2 \cdot 2H_2O$, 1.0) and 0.05 M sodium citrate buffer (pH 4.8) were added to a 100 ml flask. The suspension was then autoclaved for 20 min at 121°C. After cooling to room temperature, the solutions were inoculated with 100 µL *Mucor indicus* spore suspension, and then supplemented with 25 FPU Cellulase per gram of dried substrate. Finally, the flask was sealed and incubated at 35°C and 120 rpm for 72 hours. Liquid samples were taken and analyzed by UV visible spectrophotometer after three days fermentation.

3.2.9. Determination of Bioethanol Concentration

Determination of concentration of bioethanol produced was carried out using the method described by (Rabah *et al*., 2011). 1ml of standard ethanol was diluted with 100 ml of distilled water to give a concentration of 1 %. From this stock solution 0, 0.2, 0.4, 0.6 and 0.8 % of the ethanol was prepared by diluting it with distilled water. To each of the varying ethanol concentrations 2mls of chromium reagent was added and allowed to stand for an hour for colour development. The absorbance of each concentration was measured at 588 nm using UV-VIS spectrophotometer and the readings used to developed standard ethanol curve. Then 5mls of each bioethanol samples were put in test tubes and treated with 2mls of the chromium reagent. The mixture was allowed to stand for an hour and the absorbance was measured as for standard curve.

3.2.9.3 Determination of Reducing Sugar

For reducing sugar determination, an aliquot of 0.25ml of sample was placed in test tube, then 0.25ml distilled water and 1ml dinitrosalicyclic acid solution were added into the sample. The mixture was heated at 90°C for 10 min in a water bath. The sample was cooled to a room temperature and the absorbance of the sample was recorded at 550 nm was. The concentration of reducing sugar was calculated by substituting the absorbance of the sample into the linear equation obtained from the standard curve (Wasinton *et al*., 2014).

3.2.9.3 Qualitative estimation of Bioethanol

Qualitative estimation was done by Jones reagent [K2Cr2O7+H2SO4] test. 2ml of $K_2Cr_2O_7$ (2%), 1 ml of conc. H_2SO_4 was added to 1ml of fermented sample, It was allowed to stand for few minute for colour development (Bowden *et al*., 1946).

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 RESULTS

4.1.1 Proximate composition

The results obtained for proximate content of both rice bran is presented in Table 4.1. With regard to the total moisture contents, crude fiber, carbohydrate and ash contents there were a significant difference ($p<0.05$) between the two samples. The results showed 66.91% carbohydrate content in *Sipi* rice bran while 63.74% was recorded in *Wita* and 9.36% was recorded in *Sipi*. High fiber content 10% was observed in *Wita* rice bran while 6% was observed in *Sipi*.

Table: 4.1 proximate composition of *Wita* and *Sipi* rice bran

Parameters (%)	***Wita***	***Sipi***
Moisture content	8.73 ± 0.10^{a}	9.62 ± 0.11^{b}
Fat content	8.45 ± 0.18^{c}	9.36 ± 0.34^{c}
Crude fiber	10.23 ± 0.21^{c}	6.00 ± 0.08^{d}
Protein	4.20 ± 0.41^{d}	4.20 ± 0.41^{d}
Carbohydrate	63.74 ± 0.64^{e}	66.91 ± 0.39^{d}
Ash content	4.10 ± 12^{f}	3.41 ± 13^{e}

Data are expressed as mean ± SD of triplicate measurement.
Means with different superscript along rows differ significantly ($p<0.05$).

4.1.2 Mineral elemental content

Mineral element detected in both samples is presented in Table 4.2, All mineral element analyzed for both samples significantly varied ($p<0.05$) with the exception of magnesium. From the finding, Mg concentration was less in both samples. Fe, Mn, P and Zn concentrations were observed to be much in *Sipi* variety While Ca and K concentrations were observed to be higher in the *Wita* variety.

Table 4.2 mineral composition of rice bran

Parameter(mg/kg)	*Wita*	*Sipi*
Fe	23.15 ± 026^{a}	33.21 ± 0.18^{b}
Ca	33.23 ± 0.20^{c}	10.9 ± 0.05^{d}
Mg	4.43 ± 0.03^{d}	4.66 ± 0.29^{d}
Mn	76.44 ± 038^{e}	144.94 ± 0.05^{d}
Na	333.17 ± 0.15^{e}	555.19 ± 0.27^{f}
P	28.24 ± 0.01^{f}	45.06 ± 0.11^{d}
K	161.30 ± 0.10^{c}	138.83 ± 0.05^{b}
Zn	48.24 ± 0.01^{b}	62.01 ± 0.02^{e}

Data are expressed as mean ± SD of triplicate measurement
Means with different superscript along rows differ significantly ($p<0.05$).

4.1.3 Vitamin B content

Vitamins contents of both rice bran are presented in Table 4.3.The B vitamins evaluated include**:** Vitamin B1, B2, B3, B5 and B6. All the B vitamin analyzed was found to be higher in *Wita* rice bran. Differences observed in both samples were statistically significant ($p>0.05$) for all the B vitamins with the exception of vitamin B6.

Table 4.3 Vitamin contents of *Sipi* and *Wita* rice bran

Sample	VitB1 (mg/100 ml)	VitB2 (mg/100g)	Vit B5 (µg/ml)	VitB6 (mg/100 ml)	VitB3 (µg/ ml)
Wita	0.014 ± 0.00^{a}	0.0056 ± 0.00^{c}	123.62 ± 0.84^{d}	1.23 ± 0.8^{e}	0.0015 ± 0.08^{c}
Sipi	0.011 ± 0.00^{b}	0.0039 ± 0.00^{d}	86.24 ± 1.2^{e}	1.21 ± 0.02^{e}	0.00057 ± 0.00^{e}

Data are expressed as mean ± SD of triplicate measurement
Means with different superscript along column differ significantly ($p<0.05$).

4.14 Compositional analysis

The composition of both samples are presented in Table 4.4.*Wita* rice bran has cellulose content of 40%, 23% hemicelluloses and 16% lignin. As well *Sipi* rice bran contains 35% cellulose, 27% hemicelluloses and 13% lignin. Cellulose and lignin content were observed to be higher in *Wita* variety than *Sipi* variety. Higher hemicellulose content was recorded in *Sipi* rice bran.

Table 4.4 composition of *Sipi* and *Wita* rice bran

Lignocellulosic	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Extractives (%)
Wita	40	23	16	21
Sipi	35	27	13	25

4.1.5 Reducing sugar produced during simultaneous saccharification and fermentation of *Wita* and *Sipi* rice brans with *S.cerevisiae*

The result in figure 4.1 Shows Reducing sugar produced during simultaneous saccharification and fermentation of *wita* rice bran with *S.cerevisiae.* Maximum reducing sugar yield of 7.79mg/ml was obtained at 90 minutes pretreatment time with 2.0% NaOH. Lowest yield of 1.05mg/ml was recorded at 15 min pretreatment time with 1% NaOH.

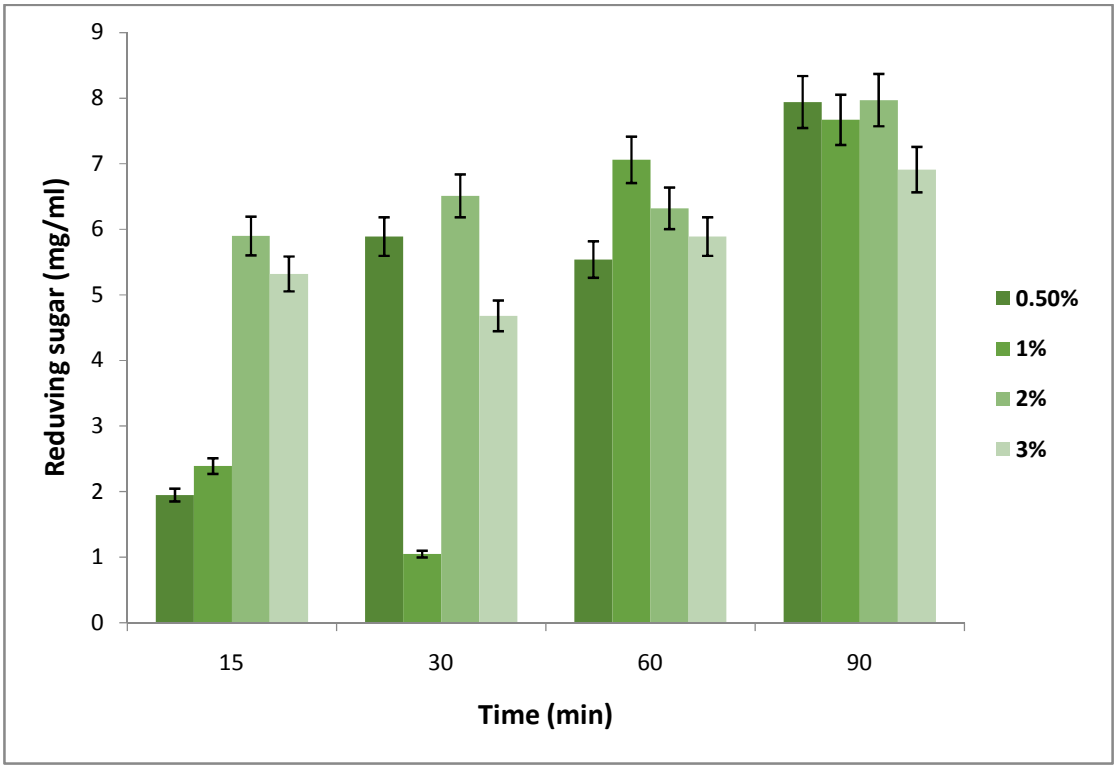


Figure 4.1: Reducing sugar produced from simultaneous saccharification and fermentation of *Wita* rice bran using *S.cerevisiae* at different pretreatment time (15min, 30min, 60min and 90min) and NaOH concerntration (0.5%, 1%, 2% and 3%).

Reducing sugar obtained during simultaneous saccharification and fermentation of *Sipi* rice bran with *S.cerevisiae* are presented in figure 4.2. From the result, it can be seen that highest concentration of reducing sugar (6.63mg/ml) was obtained at 30min pretreatment time with 3% NaOH, this shows that high delignification rate was achieved with 3% NaOH and 30 minutes residence time. Lowest reducing sugar concentration of 3mg/ml was obtained at 15min pretreatment time with 0.5% NaOH.

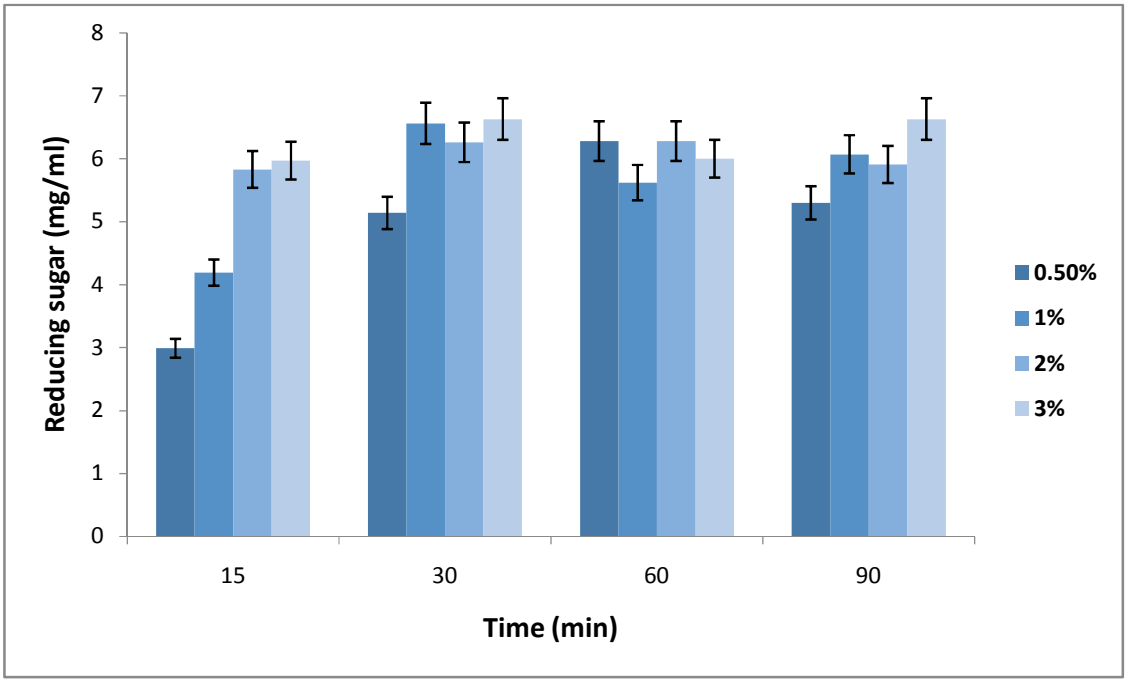


Figure 4.2: Reducing sugar produced from simultaneous saccharification and fermentation of *Sipi* rice bran using *S.cerevisiae* at different pretreatment time (15min, 30min,60min and 90min) and NaOH concentration (0.5%, 1%, 2% and 3%).

4.1.6 Reducing sugar produced during simultaneous saccharification and fermentation of *Wita* and *Sipi* rice brans with *Mucor indicus*.

Result presented in figure 4:3 shows Maximum reducing sugar of 7.27mg/ml at 60 minutes with 2% NaOH and this clearly shows that delignification rate is much at this pretreatment condition. From the result, lowest reducing sugar concentration of 4.13mg/ml was obtained with 1% NaOH pretreatment for 30 minutes.

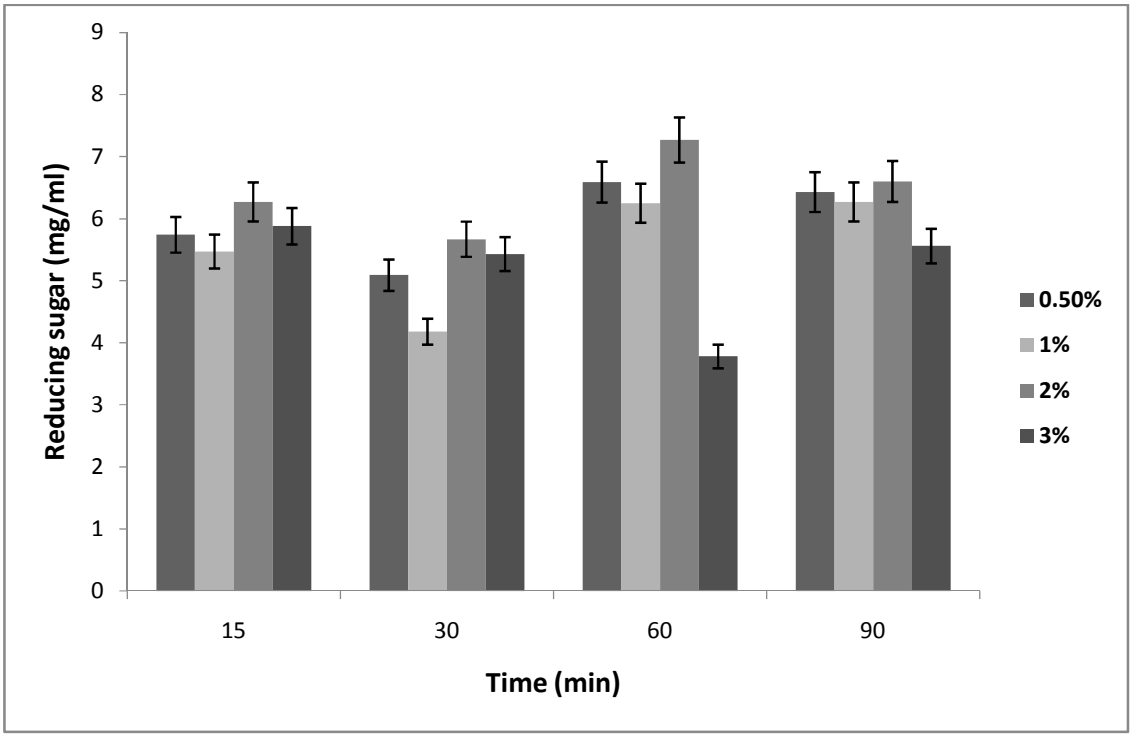


Figure 4.3 Reducing sugar produced from simultaneous saccharification and fermentation of *Wita* rice bran with Fungi at different pretreatment time (15min, 30min,60min and 90min) and NaOH concerntration (0.5%, 1%, 2% and 3%).

From the result presented in figure 4.4, maximum reducing sugar yield (6.93mg/ml) was recorded after alkaline pretreatement of substrate (*Sipi* rice bran) for 6o minute with 2% NaOH and Simultaneous Saccharification and Fermentation. Lowest reducing sugar (3.84mg/ml) was obtained at 30min pretreatment time with 0.5% NaOH.

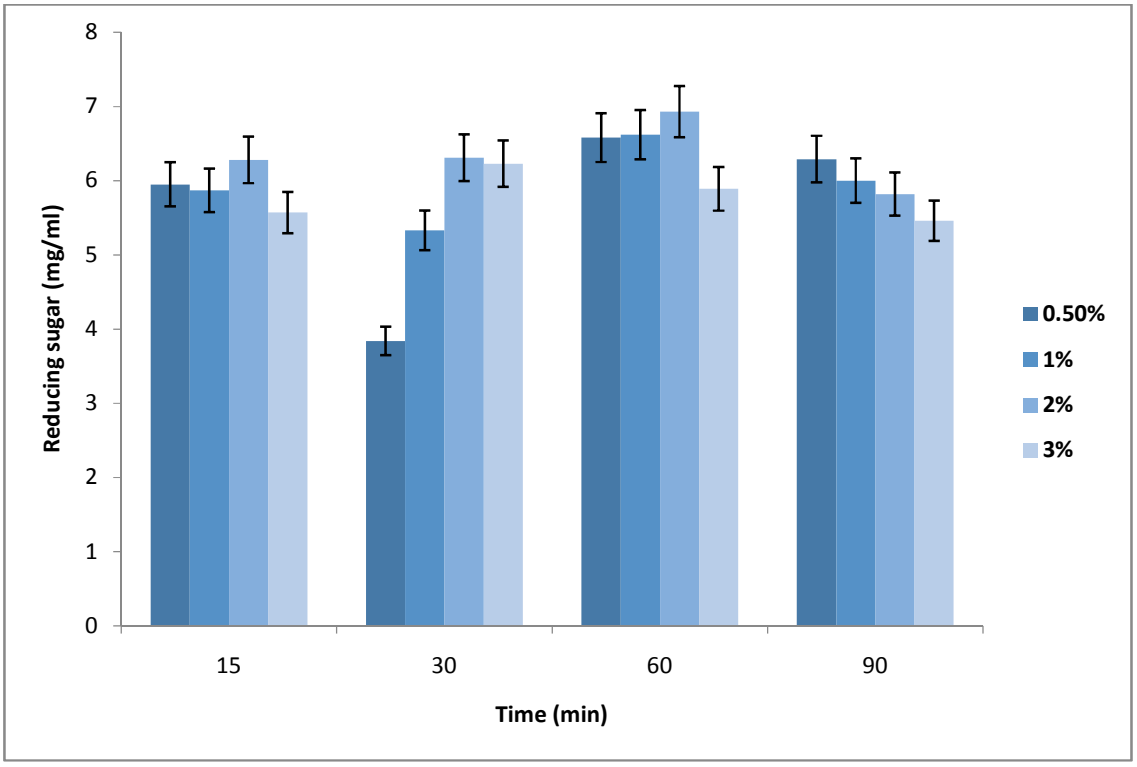


Figure 4.4: Reducing sugar produced from simultaneous saccharification and fermentation of *Sipi* rice bran using *Mucor indicus* at different pretreatment time (15min, 30min, 60min and 90min) and NaOH concerntration (0.5%, 1%, 2% and 3%).

4.1.7 Bioethanol produced from *Wita* and *Sipi* rice bran by simultaneous saccharification and fermentation process with *S.cerevisiae*

The result in figure 4.5 shows ethanol produced by SSF process using *Saccharomyces cerevisiae*. *Wita* rice bran pretreated with 2% NaOH for 90 minutes produced highest ethanol concentration (1.36%) after fermentation. Lowest ethanol concentration of o.15% was recorded after fermentation of substrate pretreated for 15 minutes with 1% NaOH.

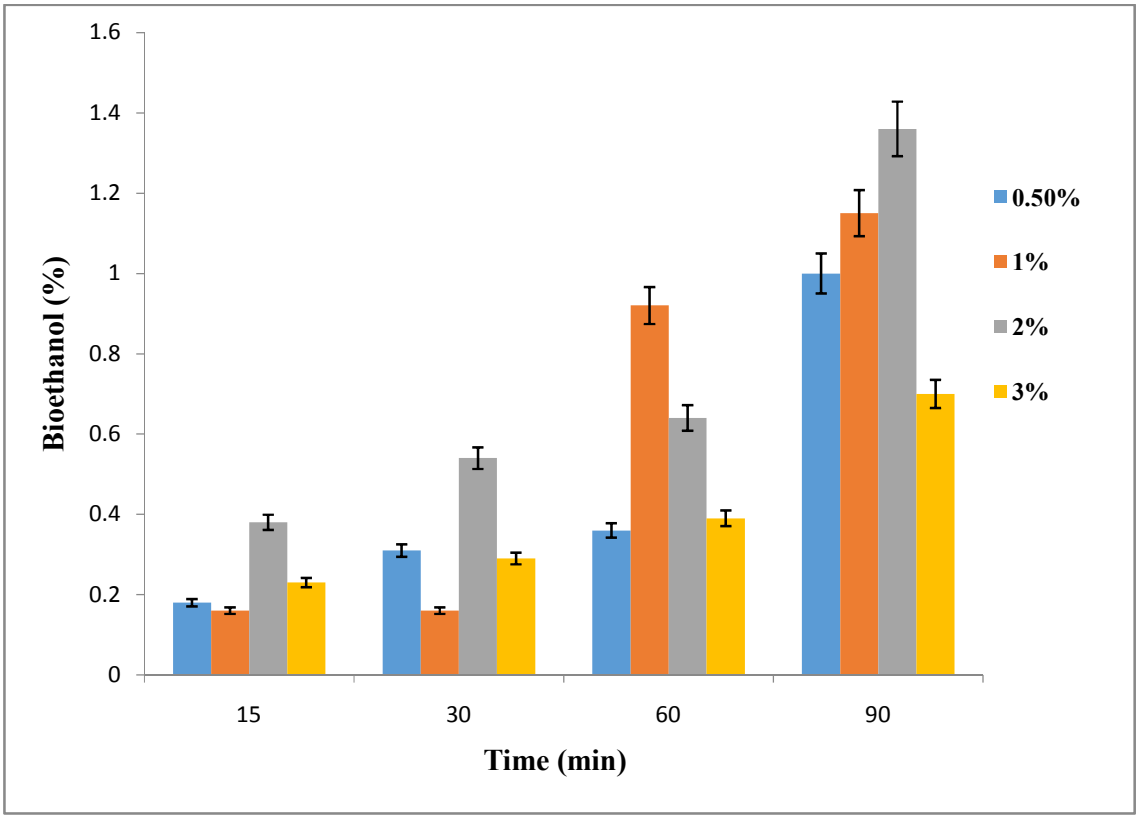


Figure 4.5: Bioethanol produced from simultaneous saccharification and fermentation of *Wita* rice bran using *S. cerevisiae* at different pretreatment time (15min, 30min,60min and 90min) and NaOH concentrations (0.5%, 1%, 2% and 3%).

The result in figure 4.6 shows substrate pretreated with 1% NaOH for 30 minutes produced 0.76% maximum ethanol concentration after fermentation with *Saccharomyces cerevisiae*. *S.cerevisie* was able to utilize sugars and convert it to ethanol. Lowest bioethanol concentration recorded was 0.21% after fermentation of substrate pretreated with 0.5% NaOH for 15 minutes.

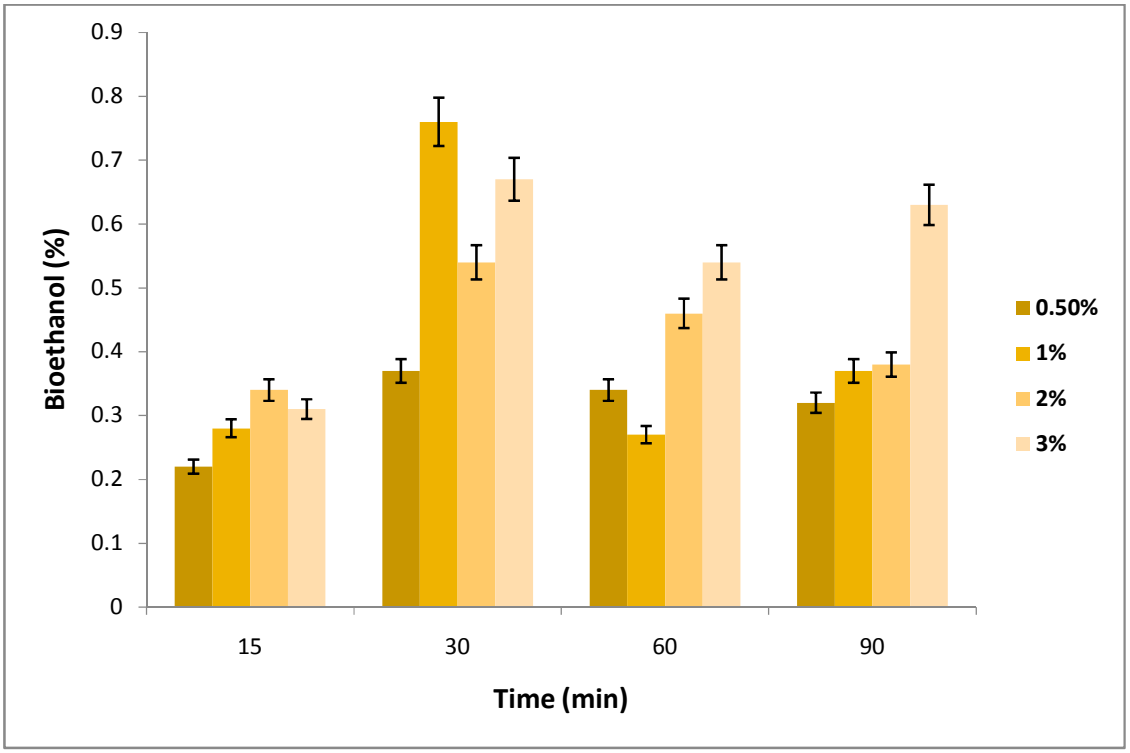


Figure 4.6: Bioethanol produced from simultaneous Saccharification and fermentation of *Sipi* rice bran pretreated with NaOH at different concentrations (0.5%, 1%, 2% and 3%) and pretreatment time (15min, 30min, 60min and 90min) Using *S. cerevisiae*.

4.1.8: Bioethanol produced from *Wita* and *Sipi* rice brans by Simultaneous Saccharification and Fermentation process with *Mucor indicus*.

Result presented in figure 4.7 shows maximum bioethanol concerntration, (0.69%) was obtained after SSF of pretreated substrate with 2% NaOH for 60 minutes. Lowest concentration recorded was 0.23% after SSF of substrate pretreated with 1% NaOH for 30 minutes.

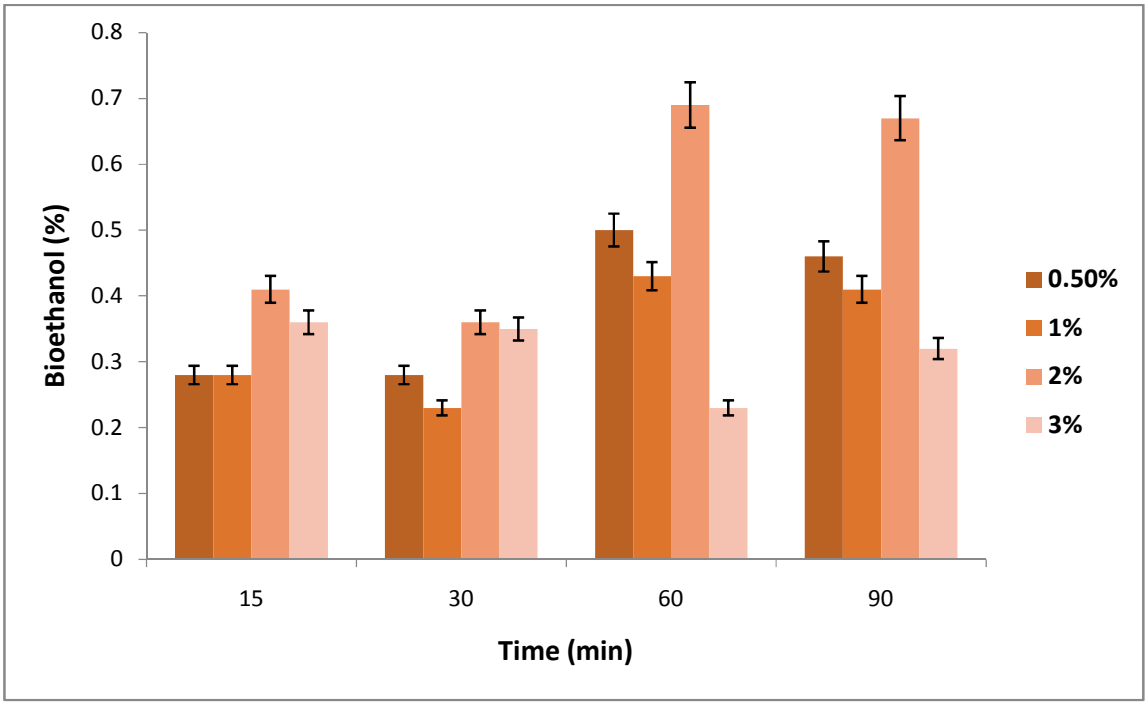


Figure 4.7: Bioethanol produced from simultaneous saccharification and fermentation of *Wita* rice bran pretreated with NaOH at different concentrations (0.5%, 1%, 2% and 3%) and time (15min, 30min, 60min and 90min) using *Mucor indicus*.

From the result presented in figure 4.8, highest concentration of bioethanol (0.75%) was recorded after fermentation of substrate pretreated with 2% NaOH for 60 minutes. Lowest concentration of bioethanol was recorded after SSF of biomass (*Sipi* rice bran) pretreated with 0.5% NaOH for 30 minutes.

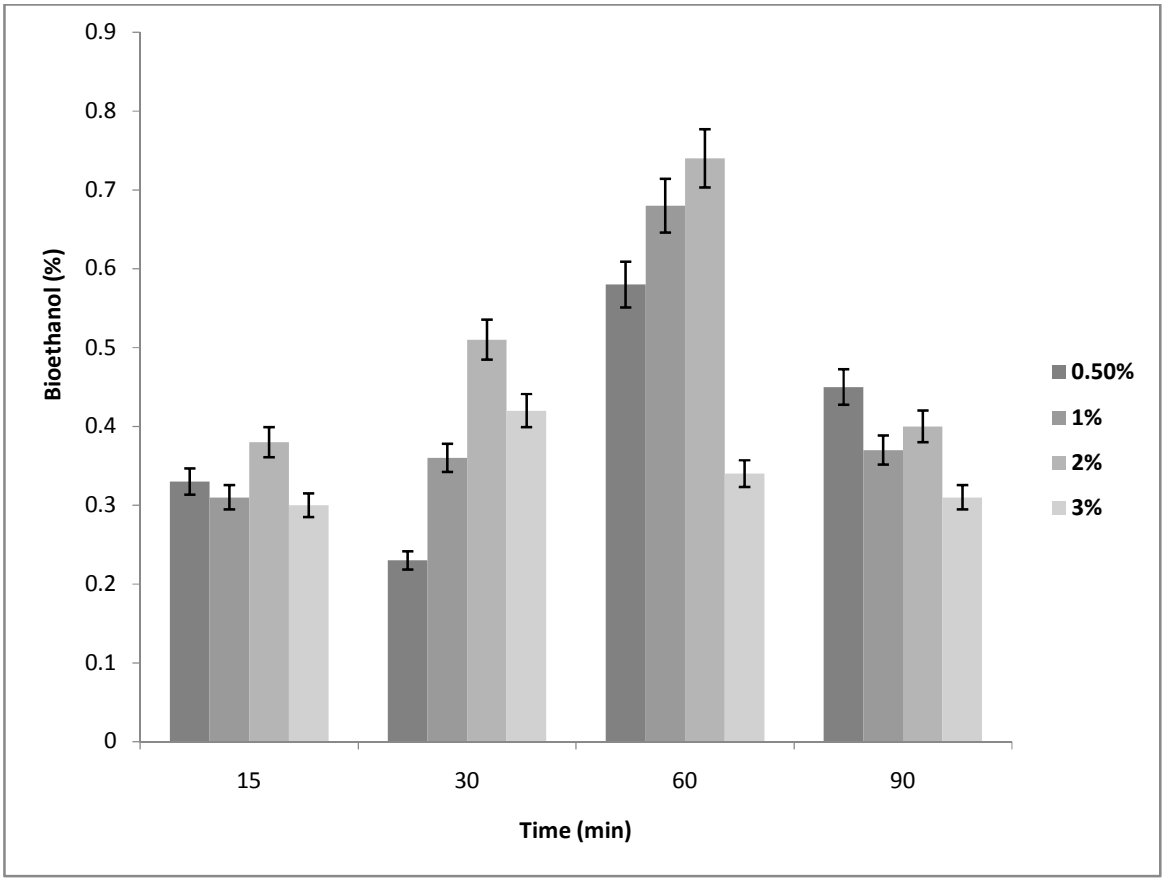


Figure 4.8: Bioethanol produced from simultaneous saccharification and fermentation of *Sipi* rice bran pretreated with NaOH at different time (15min, 30min, 60min and 90min) and concerntrations (0.5%, 1%, 2% and 3%) using *Mucor indicus*.

4.2 DISCUSSION

4.2.1 Proximate composition of rice bran

The results of proximate compositions of *Wita* and *Sipi* rice bran are presented in table 4.1. The differences in composition of bran may be as a result of unequal removal of bran layers which is due to friction action in the machine and irregular geometrical shape of the caryopsis (Resurrection *et al*., 1979). Moisture content is one of the major requirements that determined the quality of dried product. The requirement must be attained as it may affect the product stability and makes it vulnerable to microorganism (Arlina *et al*., 2012). The results obtained from the present study for both samples were in close consistence with the value obtained for rice bran by (Mohammed *et al*., 2014), it shows that rice bran may not be easily attacked by microorganism and may stay for a long time without getting spoiled. The ash content in all types of bran is as a result of high mineral content. . Ash content obtained in this study is lower compared to findings of (Rosniyana *et al*., 2007), (Mohammed *et al*., 2014) and (Amissah *et al*., 2003). The results obtained from the Present finding might have impact on the ethanol yield, because ash content serve as an indicator for lignin content and and higher ash content affect biethanol yield (Ebringerova *et al*., 2005). The major carbohydrate present in rice bran are cellulose, hemicelluloses and starch (Juliano and belchet 1985). According to Mohammed *et al*, (2014) lower carbohydrate content in rice bran might be due to absence of starch content in the bran. The mean value of carbohydrate obtained in the present finding was in agreement with the result of Juliano and Belchet (1985). High carbohydrate content obtain in this study indicates the potential of rice bran as a good source of sugar for bioconversion of ethanol. When wider plant spacing is used because probably more nitrogen is available per plant protein content will be higher, also cultural practice and management have major influence on the content of protein of rice grain (De. Datta *et al*., 1972).The result obtained for protein content in the present finding is closely similar to the value obtained

from rice bran by (Juliano and Betchel 1985). Rice bran fiber has a laxative effect; it increases feacal output and stool frequencies. Bhattacharya (1988) reported that crude fiber to be in the range of 6-14% in his studies of rice bran and the results of the present study is in line with his findings.

Proper assessment of ethanol output from any type of biomass does not depend on the composition of fiber. It actually involves conversion in to ethanol by means of specific assay (Capecchi *et al*., 2017). Rice is considered as an important crop in the world, rice bran is an important source of fat because it has considerable amounts of essential fatty acid (Wang, 1986). Rice bran has higher fat content than rice grain, because most of the fat in the rice are removed during milling as it remains in the bran (Grist 1975).

4.2.2 Minerals and vitamins content in rice bran

Mineral content is strongly influenced by soil structure condition and fertilization (Dos Santos Conceição Faria *et al*., 2012). According to Juliano (2003) minerals are more concentrated in the outer layer of rice grain which is distributed mainly between the bran (72%) and endosorenum (25%). Yeast requires some mineral elements (e.g Ca, Mg, Fe, Zn, K, Na etc) for growth and ethanol fermentation (Alexander, 2010). Mineral element such as K and Ca content were observed to be much in *Wita* variety while Na, Mn, P, Fe and Zn contents were found to be much higher in *Sipi* variety. Na was observed to be the major mineral element in both rice varieties which is up to (333.17mg/kg) in *Wita* and (555.10mg/kg) in *Sipi*. The differences observed in mineral element were statistically significant ($P<0.05$). Walker and Stewart (2016) reported that Magnesium and Zinc may also be used in yeast fermentations to certify steady yeast activity. In the present study high level of trace element was recorded in both *Sipi* and *Wita* rice bran and this is advantageous as ethanol production is related to yeast growth. Rice bran is a very good source of vitamins, minerals, essential fatty acids, dietary fiber and more than 100 antioxidant nutrient which

promote good health and prevent disease (Malekean *et al*., 2000). According to Juliano and Betchel (1985), Rice contains little or no vitamin A, C or D. more than 80% of vitamin in rice is found in the bran layer. Rice bran is a very rich source of vitamins particularly thiamin, niacin and pyridoxine. Present study shows that, *Wita* rice bran has higher vitamin B1,B2, B3 and B5 content when compared with *Sipi* variety, the differences observed was statistically significant ($p<0.05$).

4.2.3 Compositional analysis of rice bran

Lignocellulosic biomass basically comprises cellulose; hemicelluloses and lignin (Hayn e*t al*., 993).The composition of lignocellulosic biomass vary from one plant species to another. For instance leaves and wheat straw have more hemicelluloses where as hardwood has cellulose in greater amount (Sun and Cheng, 2002). Rice bran is an available Agricultural waste with abundant carbohydrate content and other nutrient which support yeast growth (Arasi and Sashi 2010). The result obtained for cellulose, hemicellulose and lignin content in the present Study for both *Sipi* and *Wita* rice bran is in close consistence with the finding of Pratab and kumar (2014) on studies of lignocellulosic composition of rice bran. High carbohydrate content makes lignocellulosic biomass as a potential source for bioethanol production.

4.2.4 Total reducing sugar produced from *Wita* and *Sipi* rice bran at different pretreatment condition with NaOH and SSF with *S.cerevisiae.*

Alkaline hydrolysis can be used for pretreatment of lignocellulosic materials and the effect of this pretreatment depends on the lignin content of the materials (McMillan, 1994). The mechanism of alkaline hydrolysis is believed to be saponification of intermolecular ester bonds crosslinking xylan hemicellulose and other components. Lignocellulosic materials porosity increases with the removal of crosslinks (Tarkow and Feist, 1969). Dilute NaOH treatment of lignocellulosic materials caused swelling, which lead to an increase in the

internal surface area, a decrease in crystallinity, separation of structural linkage between lignin and carbohydrates, and disruption of the structure of lignin (Fan *et al*., 1987). The effectivity of alkaline pretreatment depends on pretreatment condition, physical structure and also composition of substrate (Cheng *et al*., 2013). Reducing sugars are described generally as any sugar that in basic solution has a ketone or aldehyde group which allows the sugar to act as a reducing agent (Kunz *et al*., 2011). Reducing sugar include all monosaccharide (glucose, galactose, fructose, ribose, xylose and glyceraldehydes). Along with some disaccharide, oligosaccharide and polysaccharide. Total reducing sugar release after SSF with *saccharomyces cerevisiae* at different pretreatment condition with NaOH (0.5%, 1%, 2% and 3%). For *Wita* rice bran Highest reducing sugar release was recorded at residence time of 90mins pretreatment and 2% sodium hydroxide concerntration in the present study and the result obtained is in agreement with the finding of Alvarez *et al* .,(2013). Lowest reducing sugar was recorded at residence time of 15mins and 0.5% concerntration of sodium hydroxide. Highest reducing sugar released was recorded at pretreatment time of 60min and 2% sodium hydroxide concentration *Sipi* rice bran. According to Alvarez *et al*., (2013) for chemical pretreatment, higher yield are achieved when higher residence time and alkaline concentration were used. Also kataria and Ghosh (2014) reported best pretreatment condition for reducing sugar release at higher residence time with sodium hydroxide. Also according to Alvarez *et al*., (2013), pretreated biomass is flushed to prevent toxic agents which inhibit enzymatic hydrolysis.

4.2.5 Total reducing sugar produced from *Wita* and *Sipi* rice bran at different pretreatment condition with NaOH and SSF with *Mucor indicus*

Total Reducing sugar yield gives the estimation of the amount sugar that can be liberated during hydrolysis and it is the most important yield because it involved the process step to produce fermentable sugar. High reducing sugar was recorded at 60min residence time of

pretreatment and 2% sodium hydroxide for *Wita* rice bran and lowest yield was recorded at 30 min residence time with 1% sodium hydroxide. For *Sipi* rice bran highest reducing sugar was recorded at 60 minutes residence time of pretreatment with 2% sodium hydroxide and lowest yield was obtained with 0.5% sodium hydroxide pretreatment at 30 minutes residence time. High yield of reducing sugar suggests that the feedstock is suitable for bioethanol production.

4.2.6 Bioethanol Produced from *Sipi* and *Wita* after SSF with *Saccharomyces cerevisae*

Saccharomyces cerevisiae is one of the bioethanol producing organisms employed in industrial processes. When *S.cerevisae* is supplied with appropriate environment and required nutrient, it could ferment increased amount of sugar in the medium (Harisma *et al*., 2012). According to Arasi and Sashi (2010) most preferred candidate in most studies for industrial production of ethanol is S.cerevisiae. *Wita* rice bran produces higher bioethanol concentration of 1.36% after preatreatment with 2% NaOH concentration and 90minutes residence time, followed by SSF. This confirmed that the higher sugar content in the bran the more ethanol is produced, since higher yield of reducing sugar was achieved after this pretreatment condition and72hrs SSF. The result recorded in the present study is closely similar to the result of study on bioethanol production from rice bran using *Saccharomyces cerevisae* by Arasi and Sashi (2010). Maximum ethanol concentration recorded for *Sipi* rice bran after 72hrs SSF was 0.76% which is lower compared to *Wita*, which may be due to high amount of reducing sugar release by *Wita* rice bran. Yamba *et al*., (2007) observed that sugar content was directly proportional to the bioethanol produced from sweet sorghum.

4.2.7 Bioethanol Produced from *Sipi* and *Wita* after SSF with *Mucor indicus*

Simultaneous saccharification and fermentation of *Sipi* rice bran with *Mucor indicus* produces maximum ethanol concerntration of 0.75%. while *Wita* rice bran produces higher bioethanol concentration of 0.69%. *Mucor indicus* is capable of fermenting both hexose and

pentose sugar. Ethanol and glucosamine yield produced by *Mucor* highly depends on the medium composition (Sues *et al*., 2005).

CHAPTER FIVE

5.0 SUMMARY, CONCLUSION AND RECOMMENDATION

5.1 Summary

Two varieties of rice bran (*Sipi and Wita*) were employed for bioethanol production. *Wita* was found to produce maximum bioethanol and releases maximum reducing sugar. Both samples were found to be good source of minerals, major minerals observed include Na, Ca, P and Mg. large portion of rice bran is made up of carbohydrate and in turn contains great amount of cellulose. Rice bran was found to be good source of B vitamins.

5.2 Conclusion

Based on the present study, high level of mineral element such as calcium (Ca), Magnesium (Mg), Iron (Fe), Zinc (Zn), Potasium (K) and Sodium (Na) were observed in both *sipi* and *wita.* Cellulose content up to 40% was recorded in *wita* variety while the *Sipi* variety has 35% cellulose. Maximum reducing sugar yield of 7.7mg/ml was produced by *Wita* variety at 90 minutes pretreatment time with 2% NaOH. Simultaneous saccharification and fermentation of *Wita* rice bran with *S. cerevisae* produces high amount of bioethanol (1.36%). Therefore, this indicates that rice bran has the pontential to produce bioethanol. Hence, *Sacharomyces cerevisae* can be regarded as promising organism for efficient bioethanol production.

5.3 Recommendations

Based on the finding in the present study, the following recommendations are hereby made:

1. Genetically engineered organism that are capable of hydrolyzing cellulose and fermenting sugars should be employed to achieve greater fermentation performance of rice bran and maximum bioethanol yield
2. Further studies should explore various pretreatment processes in order to choose the one that best fit the structure of rice bran.
3. Rice bran should be combine with other feedstock, to achieve greater yield of bioethanol

REFRENCES

AACC (2000). Approved Method of American Association of Cereal Chemists. Am. Assoc. Cereal Chem. Inc., St. Paul., Minnesota, USA.

Abo-State, M.A., *et al*. (2014) Bioethanol Production from Rice Straw Enzymatically Saccharified by Fungal Isolates, *Trichoderma viride* F94 and *Aspergillus terreus* F98. *Soft*, 3, 19-29. http://dx.doi.org/10.4236/soft.2014.32003 G.

Alexander, K. M., (2010). Ethanol production from sugarcane in India: viability, constraints and implications, M Sc thesis, Department of Natural Resources and Environmental Sciences, University of Illinois, USA.

All India Distillers Association (2006). http://www.aidaindia.org/public/AboutAida.php. Website accessed.

Alvarez C.E.S., Miranda, J.L., Castro M.R. et al., (2013). Alkaline pretreatment of Mexican pine residues for bioethanol production. *African Journal of Biotechnology*. Vol. 12(31), pp. 4956-4965.

Alvira P., Tomas Pejo E., Ballesteros M. and Nergrp M.J. (2010). Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: a review. *Bioresource Technology* **101(13)**, 4851-4861.

Amissah, J.G.N., Ellis, W.O., Oduro, i., Manful, J.T. (2003). Nutrient composition of bran from new rice varieties under study in Ghana. *Food Control*, v.14, n.1, p.21-24.

Antizar-Ladislao, B. and Turrion-Gomez, J. (2008). Second-generation biofuels and local bioenergy systems. *Biofuels, Bioproducts and Biorefining*, **2**(5): 455-469.

AOAC (2005). Official Methods of Analysis.17th ed. Associatation of Official Analytical Chemists, Arlington, Virginia, USA.

Arasi, P.S. and Sashi V. (2010). Ethanol production by Saccharomyces cerevisiae from rice bran hydrolysates of Bacillus pimilus and Pseudomonas aueruginosa. Asian Journal of Bio Science Vol. 5 Issue 1 : 84-86.

Aristidou, A. and Penttilä, M. (2000). Metabolic engineering applications to renewable resource utilization. Curr. Opin. Biotechnol. 11: 187–198.

Arlina P. P., Afifah E. and Sukanta (2012). Proximate analysis of rice bran as alternative comestible. Proceeding The Fourth Gruber-Soedigdo Lecture.

Ayeni A.O., Hymore F.K., Mudliar S.N,.Deshmukh , S.C., Satpute D.B, J.A. Omoleyeand Pandey a,R.A. (2013). Hydrogen peroxide and lime based oxidative pretreatment of wood waste to enhance enzymatic hydrolysis for a biorefinery: Process parameters optimization using response surface methodology. Contents lists available at SciVerseScienceDirectFueljournal homepage: www.elsevier.com/locate/fuel

Azzam, M. (1989). Pretreatment of cane bagasse with alkaline hydrogen peroxide for enzymatic hydrolysis of cellulose and ethanol fermentation. *J. En*V*iron. Sci. Health B 24* (4), 421–433.

Balat M. and Balat H. (2008). Progress in bioethanol processing, Prog Energy Combust Sci, 34:551-73.

Balat, M. and Balat, H. (2009) Recent trends in global production and utilization of bio-ethanol fuel. *Applied Energy* 86(11): 2273-2282.

Balat, M., Balat, H. and Cahide O.Z. (2008). Progress in bioethanol processing. Prog Energy Combust 34:551–573.

Bamgboye, A.I. (1994). Pretreatment measures to accelerate and maximizing water hyacinth (*Erchhornia crassipes*) biodigestibilty for biogas production.Unpublished PhD Thesis: University of Ibadan.

Bamgboye, I.A. (2012). The potential of producing fuel from biomass in Nigeria. In Jekayinfa SO (Ed). Building a non-oil export based economy for Nigeria: the potential of value -added products from agricultural residues. Cuvillier Verlag Gottingen. pp. 35-41

Banerjee, S., Mudlia,r S., Sen R., Giri B., Satpute D., Chakrabarti T. and Pandey R.A. (2010). Commercializing lignocellulosic bioethanol: technology bottlenecks and possible remedies. *Biofuels, Bioproducts and Biorefining* **4(1)**, 77-93.

Behera, S., Arora, R., Sharma, N.K. and Kumar, S. (2014). Fermentation of glucose and xylose sugar for the production of ethanol and xylitol by the newly isolated NIRE-GX1 yeast. In: Recent Advances in Bio-energy Research (Ed. Sachin Kumar, A.K. Sarma, SK. Tyagi and Y.K. Yadav), SSS-National Institute of Renewable Energy, Kapurthala, Punjab, India, Vol III, Chapter 16, pp.175-182.

Beliya, E., Tiwari S., Jadhav, S.K., and Tiwari, K. L.(2013) De-oiled rice bran as a source of bioethanol.Energy Exploration and Exploitation 31(7): 771-782.

Ben-Ghedalia, D., and Miron, J. (1981). The effect of combined chemical and enzyme treatment on the saccharification and in vitro digestion rate of wheat straw. *Biotechnol. Bioeng. 23*, 823–831.

Beukering P., Sehker, M., Gerlagh, R. and Kumar, V. (1999). Analyzing urban solid waste in developing countries: a perspective on Banglore, India. Warmer Bull. 67: 8-10.

Bhattacharya, K. R. (1988). Rice Bran: Regional Extension Service Centre (Rice Milling) Scientific Series No. 7. Department of Food, Government of India, CFTRI, Mysore 570013.

Bhosale S and Vijayalakshmi (2015). Processing and nutritional composition of rice bran.. *Nutrition food science journal*., vol.3(1), 74-80.

Blanchette, R.A. (1991). Delignification by wood-decay fungi. Annu. Rev. Phytopathol. 29, 381–398.

Boominathan, K. and Reddy, C.A. (1992). CAMP-mediated differential regulation of lignin peroxidase and manganese-dependent peroxidases production in the white-rot basidiomycete Phanerochaete chrysosporium. *Proc. Natl. Acad. Sci. U.S.A.89* (12), 5586–5590.

Bordet, J., Michez, J. and Gilot, A. (2006). Mise en oeuvre du plan biocarburant au regard de la protection de la ressource en eau. Ministère de l'agriculture, de l'alimentation, de la

pêche, de la ruralité et de l'aménagement du territoire, (pp. 1-39). Retrieved from http://agriculture.gouv.fr/IMG/pdf/plan_biocarburants.pdf

Bowden, K., Heliron, I. M., Jones, E. R. H., and Weedon., B. C. L. (1946) Researches on Acetylenic compounds Part 1. The preparation of acetylenic ketones and oxidation of acetylenic carbinols and glycols. *Journal of the Chemical Societ*y: 39-45.

Brennan, L. and Owende, P. (2010). Biofuels from microalgae-A review of technologies for production, processing, and extractions of biofuels and co-products. *Renewable and Sustainable Energy Reviews*, **14**: 557-577.

Bull, S.R., Riley CJ., Tyson K.S and Costella, R. (1992). Total fuel cycle and emission analysis of biomass to ethanol . In: Energy from Biomass and wastes vol. XVI (Klass DL ed.) Institute of gas technology , Chicago , IL. pp. 1-14.

Cadoche, L. and Lopez, G.D (1989). Assessment of size reduction as a preliminary step in the production of ethanol from lignocellulosic wastes. Biol. Wastes 30, 153–157.

Capecchi, L., Nissen, L., Modesto,M., et al., (2017).Crop Factors Influencing Ethanol Production from Sorghum Juice and Bagasse. Energies 2017, 10, 940; doi:10.3390/en10070940.k

Carter, C. and Miller, H.I (2012).Corn for food, not fuel. http://www.nytimes.com. Corn for food not-fuel.html. Accessed 8 Nov 2015.

Chandel, A.K., Chan, ES., Rudravaram, R., Narasu, M.L., Rao, L.V. and Ravindra, P. (2007). Economics and environmental impact of bioethanol production technologies: an appraisal. *Biotechnology and Molecular Biology Review*, Vol.2, No.1. pp. 14-32, ISSN 1538-2273.

Cheng Y. et al., (2013). Biogas from lignocellulosic biomass, biotechnology biofuels 6-8.

Chisti, Y. (2007). Biodiesel from microalgae. *Biotechnology Advances,* **25**: 294-306.

Clark, J. (2003). The direct hydration of alkenes**.**

Conab(2013).http://www.conab.gov.br/OlalaCMS/uploads/arquivos/13_09_12_17_38_24_5_ma dioca_.Pdf.

Conway, T. (1992). The entner-doudoroff pathway: history, physiology and macular biology. FEMS Microbiol. Rev., 103:1-28.

Cooper, C.J and Laing C.A. (2007). A macro analysis of crop residue and animal wastes as a potential energy source in Africa. J. Energy Southern Africa 18(1):10-19.

Dale, B.. and Moreira, M.J. (1982). A freeze-explosion technique for increasing cellulose hydrolysis. *Biotechnol. Bioeng. Symp. 12*, 31– 43.

Davila-Gomez AC., Chuck-Hernandez AE., Perez-Carrilloa W and Rooney BSO (2011). Serna-Saldivar Evaluation of bioethanol production from five different varieties of sweet and forage sorghums (Sorghum bicolor (L) Moench). Ind Crops Prod 33:611–616.

De. Datta. S.K., Obcemea W. and Jana R.K. (1972). Protein content of rice grain as affected by nitrogen fertilizer and some triazines and substituted areas. *Agron. J*. 64:785-788.

Demirbas, A. (2005). Bioethanolfromcellulosic materials: A renewable motor fuel from biomass. *Energy Sources*27: 327-337.

Demirbas, M.F. (2009). Biorefineries for biofuel upgrading: A critical review. Appl Energy 86.

Dos Santos Conceição Faria **S.A.,** Zaczuk P.B., and Marilene de Vuono Camargo P. **(2012).** Nutritional composition of rice bran submitted to different stabilization procedures. Brazilian Journal of Pharmaceutical Sciences vol. 48, n.

Duff, S.J.B and Murray, W.D. (1996). Bioconversion of forest products industry waste cellulosics to fuel ethano l: a review. Bioresour.Technol. 55, 1–33.

Duku MH., Gu, S. and Hagan E.S. (2011). A comprehensive review of biomass resources and biofuels potential in Ghana. Renewable Sustainable Energy Rev. 15:404-415.

Ebringerova, A Hromadkova, Z., and Heinze, T. (2005). Hemicellulose, Advances in Polymer Science, vol. 186, pp. 1–67.

Eggeman T. and Elander R.T. (2005). Process and economic analysis of pretreatment technologies. Bioresource technology. 96 (18):2019-25.

Environmental Impact Assessment (2013) http://www.eia.gov/todayinenergy/detail. cfm?id=11551. Acessed 8 Nov.

Fan, L.T., Gharpuray, M.M. and Lee, Y.H. (1987). In: Cellulose Hydrolysis Biotechnology Monographs. Springer, Berlin, p.

Fatih-Dermibas, M. (2009). Biorefineries for biofuel upgrading: A critical review. *Applied Energy,* **86**(1): S151-S161.

Fogarty, J and McCally ,M.(2010). Health and safety risks ofcarbon capture and storage. *JAMA* 303 67–69.

Food and Agriculture Organization (2001). Farming systems and poverty: improving farmers' livelihoods in a change world. Rome: Food and agricultural organization.

Galadima, A., Garba, B.M., Ibrahim, M.N., Almustapha, L. and Leke. I.K. (2011). Biofuels Production in Nigeria: The Policy and Public Opinions. Journal of Sustainable Development · DOI: 10.5539/jsd.v4n4p22.

Georgieva T.I., Mikkelsen, M.J. and Ahring, B.K (2008). Ethanol production from wet exploded.

Ghose T.K., (1987). Measurement of cellulose activities. International union of pure and applied chemistry. Applied chemistry division commission on biotechnology.

Girard, P. and Fallot, A. (2006). Review of existing and emerging technologies for the production of biofuels in developing countries. Energy for Sustainable Development. 10(2):92-108.

Grist, D. H. (1975). Nutritional value of rice. In D. H. Grist (Ed.), Rice (pp. 450–456). New York: Longman Inc.

Grous, W.R., Converse, A.O and Grethlein, H.E (1986). Effect of steam explosion pretreatment on pore size and enzymatic hydrolysis of poplar. Enzyme Microb. Technol. 8, 274–280.

Haankuku, C., Epplin F.M. and Kakani V.G. (2015). Industrial sugar beets to biofuel: Field to fuel production system and cost estimates. Biomass Bioenergy 80:267–277

Harismah, K., Da'i, M., Asngad A. and Samlawi (2012). Bioethanol production from rice bran by saccharomyces cerevisiae. The 1th International and The 4th National Congress on Recycling of Organic Waste in Agriculture Isfahan, Iran.

Harmsen, P.F.H., Huijen, W.J.J., Bermúdez López, L.M. and Bakker, R.R.C (2010). Literature Review of Physical and chemical Pretreatment Processes for Lignocellulosic Biomass, in *Wageningen UR, Food & Biobased Research*, (September 2010), pp. 1-54., ISBN 9789085857570.

Hatakka, A.I. (1983). Pretreatment of wheat straw by white-rot fungi for nenzymatic saccharification of cellulose. *Appl. Microbiol. Biotechnol.18*, 350–357.

Hayn, M., Klinger R. and Esterbauer (1993). Isolation and partial characterization of a low molecular weight endogluconase from trichoderma reesie. Helsinki foundation for biotechnical and industrial fermentation research. Pp: 147-151.

Hernawan, R. Maryana, D. Pratiwi, S. K. Wahono, et al., (2017). Bioethanol production from sugarcane bagasse by simultaneous sacarification and fermentation using Saccharomyces cerevisiae. *International Conference on Chemistry, Chemical Process and Engineering (IC3PE)* AIP Conf. Proc. 1823, 020026-1–020026-5; doi: 10.1063/1.4978099.

Hinman, N.D., Schell, D.J., Riley, C.J., Bergeron, P.W. and Walter, P. J. (1992). Preliminary estimate of the cost of ethanol production for SSF technology. *Appl. Biochem. Biotechnol. 34/35*, 639–649.

Holtzapple, M.T., Lundeen, J.E and Sturgis, R.(1992). Pretreatment of lignocellulosic municipal solid waste by ammonia fiber explosion (AFEX). Appl. Biochem. Biotechnol. 34/35, 5–21.

Hsu, T. A., Ladisch, M.R. and Tsao, G. T (1980). Alcohol from cellulose. *Chem. Technol. 10* (5), 315–319.

Jagatee S., Behera S., Krishna P.D., Sahoo S. and Chandra R.M. (2015).Bioprospecting Starchy Feedstocks for Bioethanol Production: A Future Perspective. Journal 0f microbiology research and reviews Vol. 3(3): 24-42, ISSN:2350 1510.

Jahan M.S., Haris F., Rahman M.M., Samaddar P.R. and Sutradhar S. (2016).Bioresources technology., 219, 445-450.

Jeffries TW., and Jin Y.S. (2000). Ethanol and thermotolerance in the bioconversion of xylose by yeasts. Adv Appl Microbiol 47: 221–268s

Jin, M., Lau, MW., Balan, V. and Dale, BE. (2010). Two-step SSCF to convert AFEX-treated switchgrass to ethanol using commercial enzymes and Saccharomyces cerevisiae

424A (LNH-ST). *Bioresource Technology*, Vol. 101, No. 21, pp. 8171–8178, ISSN 09608524.

Jorgensen H., Kristensen J.B. and Felby C., (2007). Enzymatic conversion of lignocellulose into fermentable sugars: challenges and opportunities. *Biofuels, Bioproducts and Biorefining* **1(2)**, 119-134.

Juliano, B.O. and Bechtel, D.B. (1985). The grain and its gross composition. In*: Rice: Chemistry and Technology* p. 17–57. Los Banos, Laguna: IRRI.

Juliano, b.o.(2003). *Rice*: chemistry and quality. Muñoz: Philippine Rice Research Institute, 480 p.

Kataria R. & Ghosh S. (2014) NaOH Pretreatment and Enzymatic Hydrolysis of Saccharum spontaneum for Reducing Sugars Production, Energy Sources, Part A: Recovery, Utilization, and Environmental Effects, 36:9, 1028-1035, DOI: 10.1080/15567036.2010.551268.

Knothe, G. (2005). Viscosity of Biodiesel, In: The biodiesel handbook, Knothe, G., Krahl, J. and Gerpen, J. V., pp. 81-82 AOCS Press, *Campaign, Illinois*, USA

Kumar, P., Barrett, D.M., Delwiche, M.J. and Stroeve, P. (2009). Methods for Pretreatment of Lignocellulosic Biomass for Efficient Hydrolysis and Biofuel Production*, Industrial & engineering chemistry research*, Vol. 48, No.8, (January 2009), pp. 3713-3729.

Kumar, S. and Agrawal, P.K. (2003). Alcohol – The Viable Energy Substitute, All India Seminar on Ethanol and Co-generation.

Kunz, T., Lee, E. J., Schiwek, V., Seewald T. and Methner, F.-J. (2011). Glucose – a Reducing Sugar? Reducing Properties of Sugars in Beverages and Food. Brewing science.

Li, Y., Horsman, M., Wu, N., Lan, C. ad Dubois-Calero N. (2008). Biofuels from microalgae. *Biotechnology Progress*, **24**: 815-820.

Lin T., (2006). Ethanol fermentation from biomas resources: Current state and prospects. ppl. Microbiol. Biotechnol. 69: 627 642.

Lynd, L.R (1996). Overview and evaluation of fuel ethanol productionfrom cellulosic biomass: technology, economics, the environment,and policy. Annu Rev Energy Environ 21:403–465

Lynd, LR., van Zyl, WH., McBride, JE. and Laser, M. (2005). Consolidated bioprocessing of cellulosic biomass: an update. *Current Opinion in Biotechnology*, Vol.16, No.5, (October 2005), pp. 577–583, ISSN 09581669.

Lynd, LR., Weimer, PJ., van Zyl., W.H. and Pretorius I.S (2002). Microbial cellulose utilization: fundamentals and biotechnology. Microbiol. Mol. Biol Rev. 66: 506-577.

MacDonald, T., Yowell, G. and McCormack, M. (2001). Staff report.US ethanol industry production capacity outlook. California energy commission. Available at http://www.energy.ca.gov/reports/2001-08-29_600-01-017.PDF

Malekean, F., Ramu, Rao M., Prinyawiwatkul, W., Wayne, E. Marshall., Windhauser., M. and Ahmedna M.(2000). Lipase and Lipoxygenase Activity,Functionality, and Nutrient Losses in Rice Bran During Storage. Louisiana State University, Baton Rouge .Bulletin Number 870.

McLoom, A., Taylor F., Yee W., Ibsen and Wooley, R. (2000) Determining the cost of producing ethanol from corn starch and lignocellulosic feedstocks. National Renewable Energy Laboratory. http://www.nrel.gov/docs/fy01osti/28893.pdf. Retrieved on July 2016.

McMillan, J.D. (1994). Pretreatment of lignocellulosic biomass. In: Himmel, M.E., Baker, J.O., Overend, R.P. (Eds.), Enzymatic Conversion of Biomass for Fuels Production. American Chemical Society, Washington, DC, pp. 292–324.

Mes-Hartree, M., Dale, B. E. and Craig, W. K.(1988). Comparison of steam and ammonia pretreatment for enzymatic hydrolysis of cellulose. *Appl. Microbiol. Biotechnol. 29*, 462–468.

Millet, M.A., Baker, A.J and Scatter, L.D. (1976). Physical and chemical pretreatment for enhancing cellulose saccharification. Biotech Bioeng. Symp. 6, 125–153.

Mohammed A.S, Ara H., Jabin S.A., Abedin N., Azad A.K, Abul Hossain and Ara U. (2014). Nutritional Composition and Stabilization of Local Variety Rice Bran BRRI-28. International Journal of Science and Technology Volume 3 No. 5.

Monique, H., Faaij, A,, van den Broek, R., Berndes G., Gielen D. and Turkenburg W (2003) Exploration of the ranges of the global potential of biomass for energy. Biomass Bioenergy 25:119– 133.

Morjanoff, P.J and Gray, P.P (1987). Optimization of steam explosion as method for increasing susceptibility of sugarcane bagasse to enzymatic saccharification. Biotechnol. Bioeng. 29, 733–741.

Mosier, N., Wyman, CE., Dale, BD., Elander, RT., Lee, YY., Holtzapple, M. and Ladisch, CM. (2005). Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresource Technology*, Vol.96, No.6, (April 2005), pp. 673–86, ISSN 09608524.

Mussatto, S.I. and Teixeira, J.A. (2010). Lignocellulose as Raw Material in Fermentation Processes. In: Mendez-Vilas, A., Ed., *Current Research*, *Technology and Education*, *Topics in Applied Microbiology and Microbial Biotechnology*, Formatex Research Center, Badajoz, 897-907.

Naik, S., Goud, V., Rout, P. and Dalai, A. (2010). Production of first and second generation biofuels: A comprehensive review. *Renewable and Sustainable Energy Reviews,* **14**(2): 578-597.

Neely, W.C. (1984). Factors affecting the pretreatment of biomass with gaseous ozone. *Biotechnol. Bioeng. 26*, 59–65.

Nejadkoorki, F., Nicholson, K., Lake, I. and Davies, T. (2008) An approach for modeling CO2 emissions from road traffic in urban areas. *Science of the Total Environment* 406: 269–278.

Nigam, P. S. and Singh, A. (2010). Production of liquid biofuels from renewable resources. *Progress in Energy and Combustion Science*. In press. DOI: 10.1016/ j.pecs.2010.01.003.

Nigerian National Petroleum Corporation NNPC, (2007). Nigerian Biofuel Policy and Incentives.

Olaoye, J.O. (2011). An analysis of the environmental impacts of energy crops in Nigeria towards environmental sustainability. In: Ogunlela, AO (Ed). Tillage for Agricultural Productivity and Environmental Sustainability. Proceedings of Nigerian Branch of International Soil Tillage Research Organization. pp. 204-212.

Omidvar M., Karimi K. and Mohammad M (2016). Enhanced ethanol and glucosamine production from rice husk by NAOH pretreatment and fermentation by fungus *Mucorhiemalis.* Biofuel Research Journal 11 (2016) 475-481.

Pereira, A., Parrella, R.A.C., Moreira, J.A.A., May, A., Souza, VF. and Cruz, J. (2013). Avaliação decultivares de sorgo sacarino (Sorguhm bicolocr (l.) Moench) em diferentes densidades de semeadura visando a características importantes na produção de etanol Revista Brasileira de Milho e Sorgo. 12, n.2, 118–127.

Prasad, SA., Singh, N. Jain and Hoshi H.C. (2007). Ethanol production from sweet sorghum syrup for utilization as automotive fuel in India. Energy Fuel 21:2415-2420.

Pratap D.S. and Kumar R.T. (2014). Biofuel from wastes an economic and environmentally feasible resource. Energy Procedia 634 – 641.

Rabah, A. B., Oyeleke, S. B., Manga, S. B. and Hassan, L. G. (2011).Utilization of millet and guinea corn husks for bioethanol production. *African Journal of Microbiology Research* Vol. 5(31), pp. 5721-5724.

Rabelo, S.C., Filho, R.M., and Costa, A.C. 2009. Lime Pretreatment of Sugarcane Bagasse for Bioethanol Production. Appl. BiochemBiotechnol, vol.153, 139–150.

Ramachandra P. and Boucar D (2011). Green energy and technology. Springer, London.heidelberg newyork

Raposo, S., Pardao, J.M., Diaz, I. and Costa, M.E.L. (2009). Kinetic modelling of bioethanol production using agro-industrial by-products, Int. J. of Energy Env., 3(1), 8.

Ratnavathi, CV., Suresh K., Vijay-Kumar, BS., Pallav, IM., Komala V.V. And Seetharam N (2010) Study on genotypic variation for ethanol production from sweet sorghum juice. Biomass Bioenergy 34 (7):947–952.

Report of inter-ministerial committee on combating deforestation and desertification.(2000).

Resurrection, A.P., Juliano, B.O. and Tanaka Y. (1979). Nutrient content and distribution in milling fractions of rice grain. J.sc. food Agric.30:475-81

Román-Leshkov, Y., Barrett, C.J., Liu, Z.Y. and Dumesic, J.A. (2007). Production of dimethylfuran for liquid fuels from biomass-derived carbohydrates. *Nature,* **447** (7147): 982-985. S151–S161.

Rosniyana, A., Hashifah M.A. and Shariffah Norin S.A (2007). The physico-chemical properties and nutritional composition of rice bran produced at different milling degrees of rice. *J. Trop. Agric. and Fd. Sc: 99–105.*

Saini, J.K., Saini, R. and Tewari, L. (2015). Lignocellulosic agriculture wastes as biomass feedstocks for second generation bioethanol production: concepts and recent developments. *3Biotech*.5:337-353.

Sarath G., Mitchell, RB., Sattler, S.E., Funnell, D., Pederson, J.F., Graybosch, RA. and Vogel, KP. (2008) Opportunities and roadblocks in utilizing forages and small grains for liquid fuels. J Ind Microbiol Biotechnol 35:343–354.

Sarkanen, K.V. (1980). Acid-catalyzed delignification of lignocellulosics in organic solvents. *Prog. Biomass Con*V*ers. 2*, 127–144.

Sarkar, N., Ghosh, S.K., Bannerje, S.E, and Aikat, K. (2012) Bioethanol production from agricultural wastes: An overview. Renewable Energy 37:19–27.

Schenk, P., Thomas-Hall, S., Stephens, E., Marx, U., Mussgnug, J., Posten, C., Kruse, O. and Hankamer, B. (2008). Second generation biofuels: high efficiency microalgae for biofuel production, **1**: 20-43.

Schurz, J. (1978). In: Ghose, T.K. (Ed.), Bioconversion of Cellulosic Substances into Energy Chemicals and Microbial Protein Symposium Proceedings, IIT, New Delhi, pp. 37.

Shafizadeh, F. and Bradbury, A.G.W. (1979). Thermal degradation of cellulose in air and nitrogen at low temperatures. *J. Appl. Poly. Sci*. 23, 1431–1442.

Shahid, E.M. and Jamal, Y.(2011). Production of biodiesel:a technicalreview. Renew Sustain Energy Rev (9):4732–45.

Sharma R., Palled V,, Sharma-shivappa R.R. and Osborne J (2013).Potential of potassium hydroxide pretreatment of switch grasss for fermentable sugar production. Application biochem biotechnology. Doi:10.1007/s12010-012-0009-x.

Sierra R, Granda CB, and Holtzapple MT (2009). Lime pretreatment.review. Methods mol Biology.581:115-24. Doi:10.1007/978-1-60761-214-8-9.

Sipos, B., Réczey, J., Somorai, Z., Kádár, Z., Dienes, D. and Réczey K (2009) Sweet sorghum as feedstock for ethanol production: enzymatic hydrolysis of steam-pretreated bagasse. Appl Biochem Biotechnol 153:151–162.

Sivers, M. V. and Zacchi, G.(1995). A techno-economical comparison of three processes for the production of ethanol from pine. *Bioresour. Technol. 51*, 43–52.

Smith, GA., Bagby, M.O., Lewellan, R.T., Doney, DL., Moore, PH., Hills, FJ., Campbell, LG., Hogaboam G.J. And Freeman K. (1987) Evaluation of sweet sorghum for fermentable sugar production potential. Crop Sci 27:788–793.

Smith, OB. (1989). Utilization of crops residues in the nutrition of sheep and goats in the humid tropics of West Africa. In Atta Krah A.N and Reyholds L. Sheep and goat meat

production in the humid tropics of West Africa. Yamoussoukro 21-25 September 1987. FAO Animal Production and Health paper.

Söderström, J.and Galbe, M. Zacchi, G. (2005) Separate versus simultaneous saccharification and fermentationof two-step steam pretreated softwood for ethanol production. *J Wood Chem. Technol.* 25(3):187–202.

Sree NK, Sridhar M, Suresh K, Rao LV, Pandey A (1999). Ethanol production in solid substrate fermentation using thermotolerant yeast. Proc Biochem 34:115-119.

Sriroth, K., Wanlapatit S. and Piyachomkwan, K. (2012). Cassava Bioethanol, Bioethanol. In: Marco Aurelio Pinheiro Lima (ed), ISBN: 978-953-51-0008-9, In Tech, Available from http://www.intechopen.com/books/bioethanol/-cassava-bioethanol.

Sues, A., Millati, R., Edebo L. et al.,(2005). Ethanol production from hexose, pentoses and dilute acid hydrolyzate by *Mucor indicus*. FEMS yeast Res 5(6-7):669-76.

Sun S., Cao X ., Xu F.,Song X., Sun R.C. and Jones G.L (2014)..Biotechnology. biofruels.7, 116.

Sun, Y. and Cheng, J. (2002). Hydrolysis of lignocellulosic materials for ethanol production: a review. *Bioresource Technology*, Vol. 83, No.1, (2002). pp. 1–11, ISSN 09608524.

Taherzadeh J. and Karimin k.(2007). Enzyme based hydrolysis process for ethanol from lignocellulosic materials. Bioresource 2(4), 707-738.

Tarkow, H. and Feist, W.C. (1969). In: A Mechanism for Improving the Digestibility of Lignocellulosic Materials with Dilute Alkali andLiquid NH3 Advance Chemistry Series 95. American Chemical Society, Washington, DC, pp. 197–218.

Tengborg, C., Galbe, M. and Zacchi G (2001bs). Reduced inhibition of enzymatic hydrolysis of steam-pretreated softwood, *Enzyme Microb Technol*. 28, 835-844.

Tengborg, C., Galbe, M. and Zacchi, G. (2001a). Influence of enzyme loading and physical parameters on the enzymatic hydrolysis of steam-pretreated softwood, *Biotechnol Progr.* 17, 110-117.

Thatoi, H., Dash, PK., Mohapatra, S. and Swain, M.R. (2014). Bioethanol production from tubercrops using fermentation technology: a review. *Int. J. Sust.Energy.,DOI:10 .1080/14786451.2014.918616*

Thomas, KC., Hynes SH. and Ingledew, W.M. (1996). Practical and theoretical considerations in theproduction of high concentration of alcohol by fermentation.Process Biochem., 31:321-331.

Thring, R. W., Chorent, E. and Overend, R. (1990). Recovery of a solvolytic lignin: Effects of spent liquor/acid volume ratio, acid concentration and temperature. *Biomass* 289–305.

Tiwari, K.L., Jadhav S.K. and Tiwari, S., 2011. Studies of Bioethanol production from some carbohydrate sources by gram positive bacteria. *Journal of Sustainable Energy & Environment* **2**, 141-144.

Tiwari, S., Jadhav, S. K., and Tiwari, K. L. (2012) Production of bioethanol from "Jatropha Oil Cake.*Researcher* 4(7): 7-10..

Tiwari, S., Jadhav, S.K. and Tiwari, K.L.(2015). Bioethanol production from rice bran with optimization of parameters by Bacillus cereus strain McR-3 'Fermentation of rice bran for fuel ethanol production. Int. J. Environ. Sci. Technol.12:3819–3826DOI 10.1007/s13762-014-0746-1.

Toivola A, Yarrow D, Van-den-bosch E, Van-dijken JP, Sheffers WA (1984). Alcoholic fermentation of D-xylose by yeasts. Appl. Microb. Biotechnol. 47: 1221-1223.

Tucker, MP., Kim, KH., Newman, M.M. and Nguyen, Q.A. (2003). Effects of temperature and moisture on dilute-acid steam explosion pretreatment of corn stover and cellulase enzyme digestibility. Appl Biochem Biotechnol 10: 105–108.

Unilorin, (2012). Jatropha: A goldmine plant. www.new.unilorin. edu.ng/unilorin/index. php/jatropha-plant. Accessed on 30/10/12.

Vidal, P.F. and Molinier, J. (1998). Ozonolysis of ligninsImprovement of in vitro digestibility of poplar sawdust. *Biomass 16*, 1–17.

Vlasenko, E.Y., Ding, H., Labavitch, J.M. and Shoemaker, S.P.(1997). Enzymatic hydrolysis of pretreated rice straw. Bioresour. Technol. 59, 109–119.

Walker G.M. and Stewart G.G (2016). Review Saccharomyces cerevisiae in the Production of Fermented Beverages. Beverages; doi:10.3390/beverages2040030

Wang, W. (1986) Rice bran oil extraction and refining. In: Postharvest prevention of Paddy/rice loss. Council of Agriculture, Executive Yuan, Rep. of China. pp. 326–329.

Wasinton S., Satria H., and Utami N (2014). Production of reducing sugar from cassava solid waste by simultaneous ultrasonication and acid hydrolysis. *indo. j. chem., 233 – 238.*

Watanabe, M., Takahashi, M., Sasano, K., Kashiwamura, T., Ozaki, Y., Tsuiki T., Hidaka H. and Kanemoto, S. (2009). Journal of Bioscience and Bioengineering, Vol. 108 (6) 524 – 526..

Watanabe, Y. (1997). Genomic constitution of Genus Oryza. (Tokyo: Food and Agriculture Policy Research Center).

Wayman, C. (1996). *Handbook of Bioethanol: Production and Utilization*, Taylor & Fracis, ISBN 1-56032- 553-4, Washington, USA.Wheat straw hydrolysate by thermophilic anaerobic bacterium Thermoanaerobacter BG1L1 in a continuous immobilized reactor. *Applied Biochemistry and Biotechnology*, Vol.145, No. 1-3, pp. 99–110, ISSN 02732289.

Welker,M.C., Balasubramanian, V.K., Petti, C. et al.,(2015). *Review:*Engineering Plant Biomass Lignin Content and Composition forBiofuels and Bioproducts. *Energies* 7654-7676; doi:10.3390/en8087654

Wingren, A. Galbe, M. and Zacchi, G. (2003). Techno-economic evaluation of producing ethanol from softwood: comparison of SSF and SHF and identification of bottlenecks. *Biotechnol. Progr.* 19(4):1109–17.

Wiselogel, A. Tyson, S. and Johnson, D. (1996).Biomass Feedstock Resources and Composition," pp. 105–18 in Handbook on Bioethanol: Production and Utilization (Applied Energy Technology Series), ed. C. E. Wyman, Taylor and Francis (1996).

Xu J., Chen Y., Jay J.,Ratna R.,Shivappa S. and Joseph C.(2011). Delignification of switch grass cultivars for bioethanol production. Bioresources *6(1), 707-720.*

Xu, C., Qin, Y., Li, Y., Ji, Y., Huang, J., Song, H. and Xu, J. (2010). Factors influencing cellulosome activity in Consolidated Bioprocessing of cellulosic ethanol. *Bioresource Technology*, Vol.101, No.24, (December 2010), pp. 9560–9, ISSN 09608524.

Yamba, F.D. Wamukwamba, C.K. Matsika, E., and Sangiso, M. (2007). Investigation into the Production and Use of Bioethanol from Sweet Sorghum as an Alternative Fuel. Department of Mechanical Engineering, School of Engineering, University of Zambia, Lusaka.

Yang, J., Xu M., Zhang, X., Hu Q., Sommerfeld M.and Chen Y (2010). Life - cycle analysis on biodiesel production from microalgae: Water footprint and nutrient balance. Bioresour. Technol. doi:10.1016/j.biortech.2010.07.017.

Zhang B. Xiu S. and Shabazi A. (2012) Chapter 7 Aquatic Plants: is it a Viable Source for Biofuel Production? In Advances in Energy Research. Volume 11, (pp. 203-216) Morena J. Acosta (Ed) ISBN:978-1-61942-825-6, Nova Science Publishers, Inc.

APPENDICES

APPENDIX I

- Atomic absorption spectrophotometer (Buck Scientific Vgp 2I0)
- Higher performance liquid chromatography (1260 Infinity Agilent Technologies, USA)
- Muffle furnace
- Measuring cylinder
- Hot plate
- Soxhlet extractor
- Electric oven
- Digital weighing
- Water bath
- Hand held stirrer
- Oven
- Distillation apparatus
- UVVIS Spectrophotometer UV0906MO2
- Autoclaving machine, (Autoclave RAU-530D Markers REXMED Industries CO. Ltd).
- Young lin Vitamin analyse

APPENDIX II

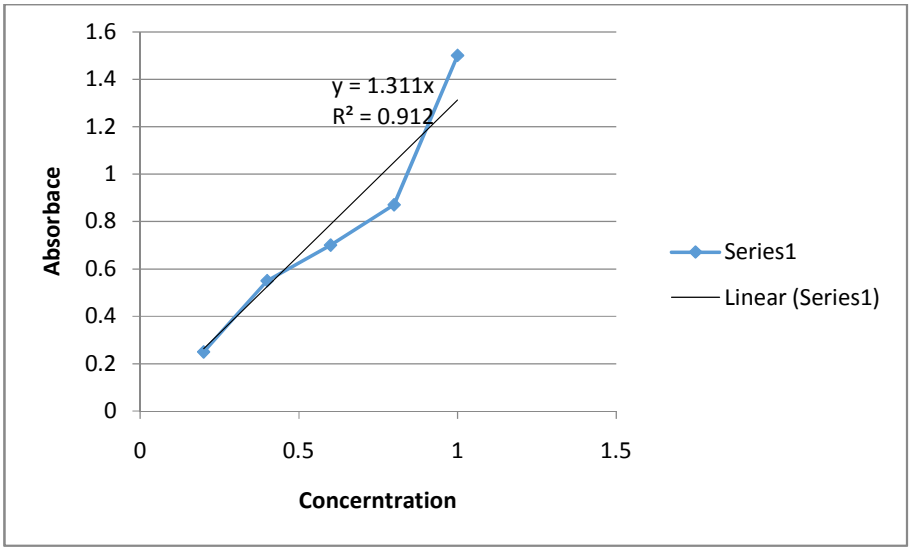
Reagents and chemicals: all reagents used and chemical are of analytical grade

- Dinitro salicyclic acid (DNS reagent).
- Chromium reagent
- Cellulase
- Acetone
- Sulpuric acid
- Sodium Hydoxide
- Yeast (dry instant yeast *Saccharomyces cerevisae*)
- sodium Chloride
- Absolute ethanol

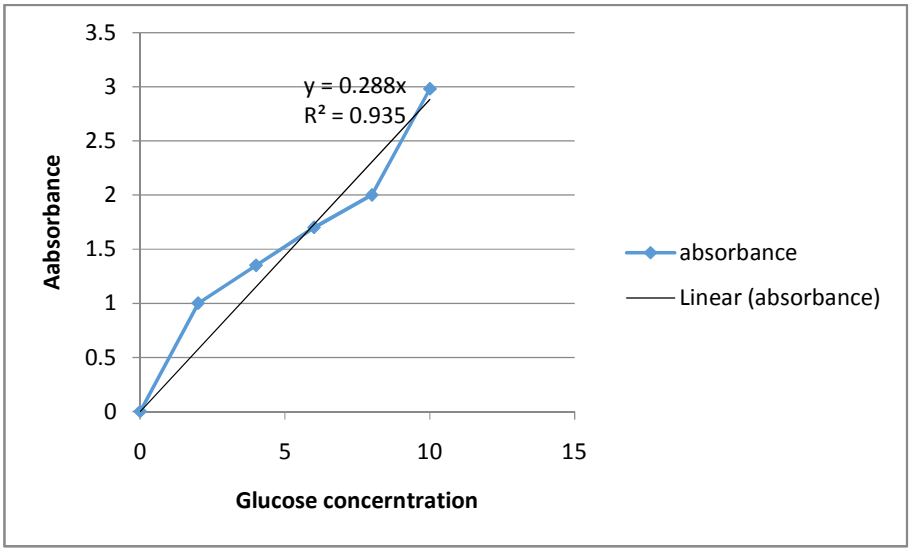
APPENDIX III

Standard curves

Ethanol standard curve

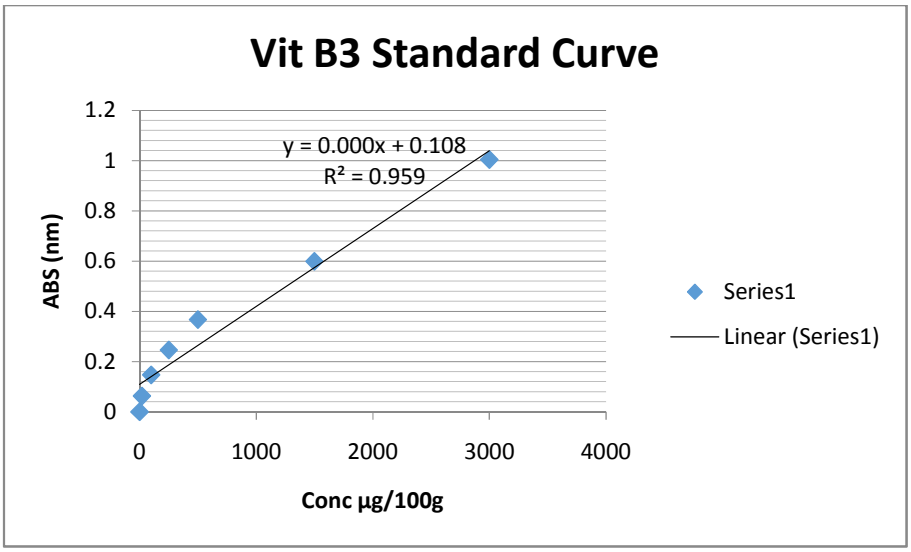


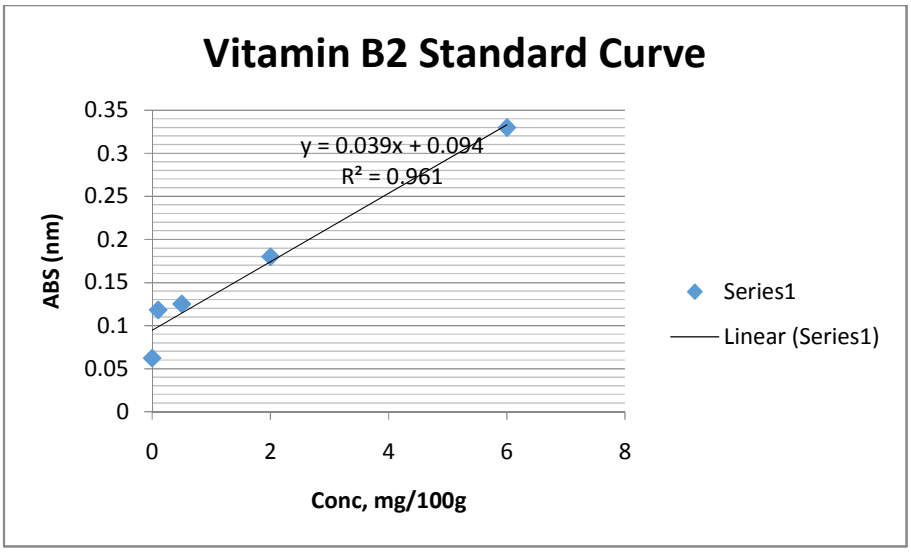
Glucose standard curve

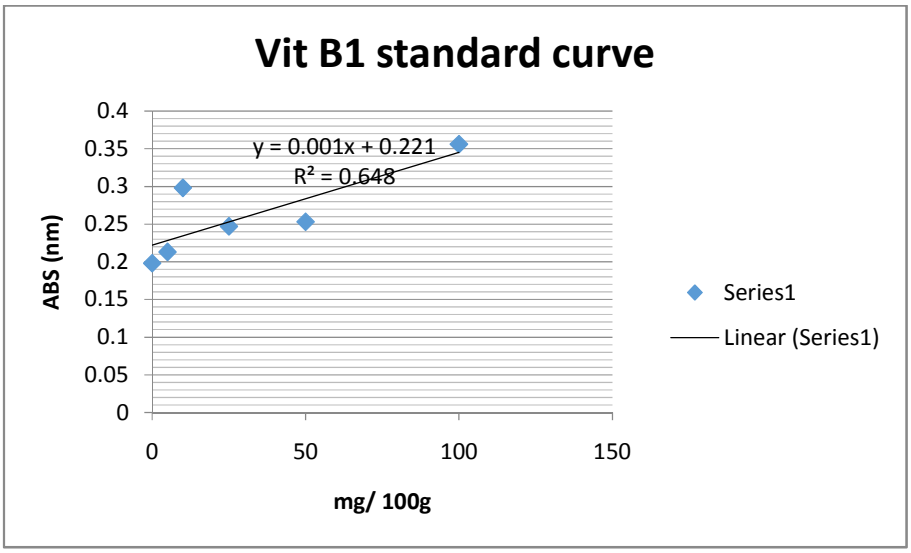


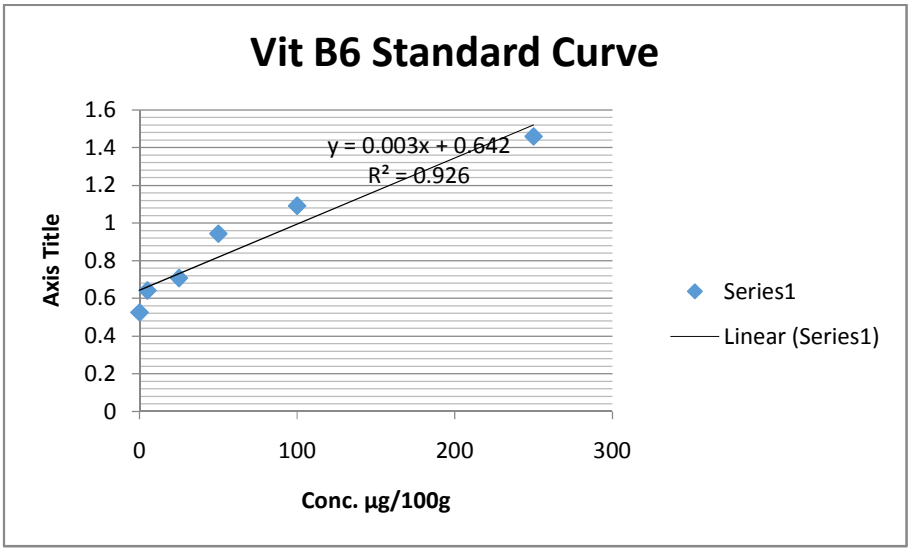
APPENDIX IV

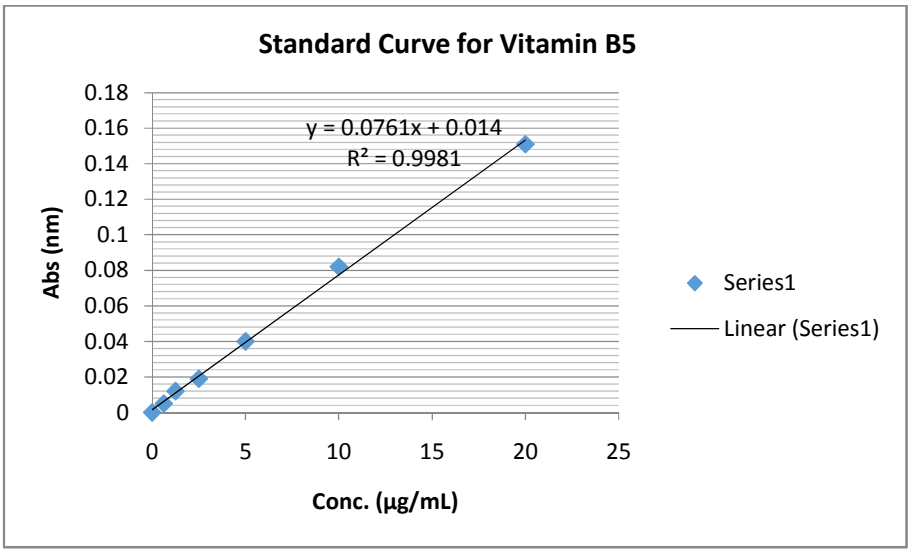
B Vitamins standard curve






Vit B1 standard curve
y = 0.001x + 0.221
R² = 0.648
ABS (nm)
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
50
100
150
mg/ 100g
Series1
Linear (Series1)


Vit B6 Standard Curve
y = 0.003x + 0.642
R² = 0.926
Axis Title
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
100
200
300
Conc. µg/100g
Series1
Linear (Series1)


Standard Curve for Vitamin B5
y = 0.0761x + 0.014
R² = 0.9981
Abs (nm)
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
0
5
10
15
20
25
Conc. (µg/mL)
Series1
Linear (Series1)

Bioethanol produced from *Wita* using *S.cerevisiae*

Naoh conc	15min	30min	60min	90min
0.5	0.17	0.31	0.35	1.04
1	0.15	0.16	0.96	1.17
2	0.38	0.54	0.64	1.36
3	0.23	0.29	0.38	0.73

Bioethanol produced from *Sipi* using *S.cerevisiae*

Naoh conc	15min	30min	60min	90min
0.5	0.21	0.37	0.34	0.33
1	0.27	0.76	0.27	0.37
2	0.32	0.54	0.45	0.39
3	0.31	0.67	0.54	0.62

Bioethanol produced from *Wita* using *Mucor indicus*

Naoh conc	15 min	30 min	6omin	90 min
0.5	0.28	0.284	0.501	0.23
1	0.272	0.228	0.427	0.401
2	0.409	0.358	0.339	0.309
3	0.332	0.328	0.229	0.207

Bioethanol produced from *Sipi* using *Mucor indicus*

Naoh conc	15min	30 min	60 min	90 min
0.5	0.33	0.229	0.59	0.446
1	0.323	0.34	0.678	0.377
2	0.381	0.506	0.38	0.393
3	0.306	0.421	0.324	0.312