ADDIS ABABA UNIVERSITY,

ADDIS ABABA INSTITUTE OF TECHNOLOGY (AAIT)

SCHOOL OF CHEMICAL AND BIO-ENGINEERING

PRODUCTION OF BIO- ETHANOL FROM CORNCOB

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ADDIS ABABA, ETHIOPIA

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ADDIS ABABA INSTITUTE OF TECHNOLOGY (AAIT)
SCHOOL OF CHEMICAL AND BIO-ENGINEERING

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Declaration

I, the under signed, declare that this thesis is my own work and has not been presented for the award of a degree in any university and that all sources of material used for the thesis have been accordingly acknowledged.

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LIST OF ACRONOYMS

AFEX	Ammonia fiber explosion
CCD	Central composite design
CIMMYT	International Maize and Wheat Improvement Center
CSA	Central Statistical Authority
DH and AOGTR Regulator	Department of Health and Ageing office of the Gene Technology
DP	Degree of polymerization
E10	(10% ethanol and 90% gasoline)
ECEA	Ethiopia Commodity Exchange Authority
EIO	Ethanol Industry Outlook
ETBE	Ethyl tertiary butyl ether
FFV	Flexible-fuel vehicles
GHG	Greenhouse gas
HMF	Hydroxymethyl furfural
MSW	Municipal solid waste
MTBE	Methyl tertiary buthyl ether
NUEP	United Nation Environmental Program
RFA	Renewable Fuels Association
Rpm	Rotation per minute
FTIR	Fourier Transform Infrared spectroscopy
SNNP	Southern Nation Nationality and peoples
US	United state
USA	United state of America

ABSTRACT

The objective of this study was production of bio ethanol from corncob which in effects to minimize energy cost and substituting non renewable energy by using renewable resources. It was employed dilute acid hydrolysis, because it is easy and productive process. The experiment was designed by Central Composite Design (CCD) with three factor (Concentration, temperature and time) and triplicate run, the acid concentration was varied from 3 to 5%, hydrolysis temperature was varied from 120 to 140℃, and hydrolysis time was varied from 30 to 90minutes.The maximum experimental observed yield of 48.4 % at the operating process variable at a temperature of 130℃, acid concentration of 3%, and a hydrolysis time of 60 minutes. Acid concentration and hydrolysis time have a statistically significant effect on the yield with p-value of 0.0113 and 0.0237 respectively. However, high acid concentration as well as increasing hydrolysis time causes a decline in the ethanol yield. The statistical analysis also showed that the ethanol yield of (47.19 %) was obtained at optimized variables of 3% acid concentration, 124.3 ℃ temperature, and at a time of 30 minutes. So this reveals that a good agreement with the observed value of ethanol yield (48.4%). From this, it can conclude that the selected model was adequate to fit the data of response variable. Chemical characterization of the bio-ethanol produced was performed by FTIR. The result shows that, the ethanol produced contains O-H, C-O, -CH_2, and CH_3 functional groups which indicate the presence of ethanol in the product.

1. INTRODUCTION

1.1 Background

Energy consumption has increased during the last century due to the world population development and growth. Nowadays, the increasing problem of the CO_2 emissions due to energy consumption, besides to the future petroleum scarcity, has strengthened the interest in alternative, nonpetroleum-based sources of energy (Montoya G, 2014).

One of the potential options to solve the environmental and energetic problems is the use of bio-ethanol. This is a renewable fuel, which avoids the negative environmental impacts generated by petroleum-based fuels. (Montoya G, 2014)

Bio-ethanol can be produced by using different technologies. One of the most important technology, fermentation, produces bio-ethanol by means of biological transformation of natural starch and sugars resources such as energy-rich crops, (first-generation biofuels) and lignocellulosic biomass (second-generation biofuels).

The use of renewable biomass resources to produce liquid biofuels such as bioethanol offers attractive solutions to reducing greenhouse gas (GHG) emissions, decreasing reliance on foreign oils, addressing energy security concerns, strengthening rural and agricultural economies, and increasing sustainability of the world transportations system. Apart from biofuels, many other valuable products for chemical and pharmaceutical industry can be produced from organic byproducts through microbial fermentation. Bio-ethanol feedstocks can be divided into three major groups: (1) sucrose-containing feedstocks (e.g. sugar cane, sugar beet, sweet sorghum and fruits), (2) starchy materials (e.g. corn, milo, wheat, rice, potatoes, cassava, sweet potatoes and barley), and (3) lignocellulosic biomass (e.g. wood, straw, and grasses).

Most current bioethanol production processes (1st generation) utilize more easily degradable biomass feedstocks such as cereals (corn or grain) and sugarcane juice. However, the utilization of these agricultural crops exclusively for energy production is heavily conflicting with food and feed production (Ikechukwu, 2012). Great effort is enforced on advancing a cellulosic bioethanol concept (2^{nd} generation) that utilizers lignocellulosic biomass.

Lignocelluloses wastes (LCW) refer to plant biomass wastes that are composed of cellulose, hemicellulose and lignin. They may be grouped into different categories such as wood residues (including sawdust and paper mill discards), grasses, waste paper, agricultural residues (including straw, stover, peelings, cobs, stalks, nutshells, non food seeds, bagasse, domestic wastes (lignocellulose garbage and sewage), food industry residues, municipal solid wastes and the like (Roig *et* al., 2006 ;Rodríguez *et* al., 2008). Currently, the second generation bio-products such as bioethanol, biodiesel, biohydrogen and methane from lignocellulose biomass are increasingly been produced from wastes rather than from energy crops (jatropha, switchgrass, hybrid poplar and willow) because the latter competes for land and water with food crops that are already in high demand.

Ethiopia, one of the world's centers of genetic diversity in crop germplasm produces more of maize than any other crop (CSA 2010). The area under maize cultivation in 2009/2010 was 1.69 hectares from which 37.8 million quintals of maize were produced which was higher than that of any other cereal crop (Geta, 2013).

Corncob, a waste product of corn contains large amount of sugars that can be further utilized to produce various compounds (Yah *et* al., 2010). The bioconversion of lignocellulosic to biofuel from cheap non-edible materials such as corncob for renewal energy is imperative.
Corncobs contains sufficient amount of cellulosic material, which is the best source of fermentable sugars. Corncob consists of polymers of mainly two types of sugars: glucose and xylose (Hsu, 2008). The use of cobs in cellulosic ethanol production creates an identical alternative to grain produced ethanol and reduces dependence on corn grain (Zych, 2008).
Agricultural residue is gaining much importance in these days because of its abundance, low cost, whole year decentralized availability for the biological production of industrial chemicals such as glucose, furfural, hydroxymethyl furfural, levulenic acid, lactic acid, acetic acid, propionic acid and fuels.

1.2 Statement of the Problem

In recently, due to the environmental concerns about air pollution caused by the combustion of fossil fuels, thus an alternative energy sources need to be renewable, sustainable, efficient, cost-effective, convenient and safe. The use of food crops (like corn, maize) for biofuels production may cause inflation of cost of these crops leading to food insecurity. To alleviate such problems, alternative and non-edible agricultural products must be investigated.

Corncob is an important byproduct corn for every 100 kg of corn grain approximately 18 kg of corncobs are produced (Ruzene *et* al., 2008). However, corncob is still discarded in not very productive ways usually burnt which results in irreparable harm to environment, directly tied to air pollution. While such residues may contain valuable materials such as 32.3-45.6% cellulose, 39.8% hemicelluloses - mostly composed of pentosan and 6.7-13.9% lignin (Zych, 2008).

Bioethanol fermentation from edible, cellulosic feedstocks using enzymatic hydrolysis has been carried out with success; very little research has been done on fermenting bioethanol from non-edible, lignocellulosic material using acid pretreatment with acid hydrolysis on corncobs. Due to the tough crystalline structure, the enzymes currently available require several days to achieve good results. Since long process times tie up reactor vessels for long periods, these vessels have to either be quite large or many of them must be used. Either option is expensive. Currently the cost of enzymes is also too high. Acid is low cost, non-volatileness, easily available, and productive. However bioethanol fermentation from lignocellulosic material can only be achieved by adequately pre-treating the lignocellulosic material. Lignocellulosic material contains cellulose which is surrounded by a matrix of hemicellulose and lignin. Pre-treatment would remove the lignin and make the cellulose and hemicellulose accessible for conversion to sugars.

Both cellulose and pentose converts in to fermentable sugar. However, the primary industrial yeast used in bioethanol production, Saccharomyces cerevisiae converts only hexose sugars such as glucose and is not able to co-ferment glucose and xylose (Ho NWY, Chang SF, 1989). Thus, corncob can be used for the production of second generation biofuel. In addition to environment benefit, ethanol production from corncob can stimulate community based jobs and economic growth.

Therefore, the aim of this work was to investigate ethanol production from corn cob.

1.3. Objectives

1.3.1. General Objective

The general objective of this study was production of Bio-ethanol from corn cob.

1.3.2 Specific Objectives

Specific Objectives of the thesis were the following;

- Characterization of corncob such as proximate analysis (moisture content, volatile constitutes, and ash content), and chemical composition (lignin, cellulose, hemicellulose and extractives).
- To investigate the effects of process variables such as hydrolysis time, concentration of H_2SO_4 and temperature in the hydrolysis process,
- Determine the optimum operating conditions, and
- Characterization of product compositions.

1.4 Significance of the study

This study has great significance in terms of assuring the production of an alternative form of energy from corncobs; which is locally available, abundant and no economical value .This study also highly contributes in the substitute fossil fuel by biofuel. Fossil fuels are quickly being depleted due to extensive and continuing over-utilization. If consumption goes in this rate the fossil fuel reserve will be depleted completely within short period of time. In addition to this, continuous burning of fossil fuel increases emission of green house gasses to the atmosphere and causes global warming. As , a renewable and non-food competitive feedstock raw material is desirable for the production of alternative fuel oil such as bioethanol; corncob is ,therefore, is one of such renewable and non-food competitive raw material.

2. LITERATURE REVIEW

2.1 Introduction

National energy security, energy sustainability and climate changes are the primary reasons to find alternative, renewable and reliable resources to fulfill energy demand. Currently, the world energy demand increases at an annual growth rate of 1.6% up to 45% by 2030.Figure 2.1 depicts the world consumption of marketed energy from different fuel sources and most of our energy demand depends on conventional fossil fuels. However, fossil fuels are nonrenewable energy sources, and will be exhausted in near future. In addition, the consumption of fossil fuels produces different pollutants and causes environmental issues. These issues and depletion of fossil fuel resources have led a rapid expansion of renewable resources. At present, there are different renewable resources, namely wind, tide, hydropower and biomass. These renewable resources can satisfy energy demand in power sector, but transportation sector mainly depends on liquid fuels, which cannot be produced from other renewable sources except biomass. The world energy outlook projected to meet 5% of world demand for transportation fuel by biofuels in 2030 (Alabama, 2012).

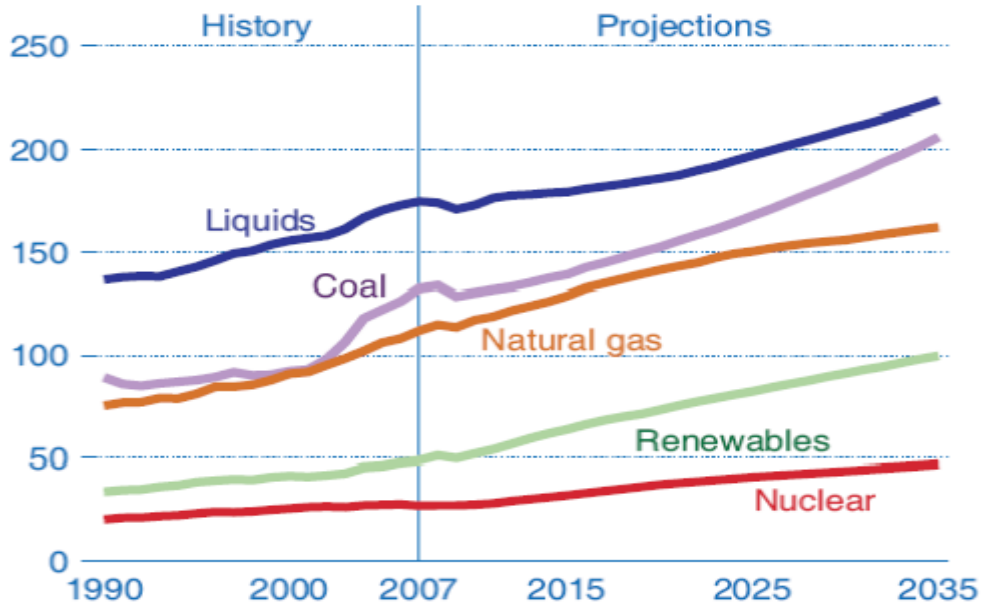


Figure 2.1: world market energy use by fuel type, 1990-2035 (quadrillion Btu)
Source: (Alabama, 2012)

Biofuels can be categorized into three major groups, namely first-generation, second-generation and third-generation types. The main distinction among them is the type of feedstock used in the production process, their current and future availability. First generation

biofuels are currently produced in large commercial quantities in many countries from agricultural crops such as sugarcane, maize, soybean and jatropha through well-established technologies such as hydrolysis, fermentation and trans-esterification. Bioethanol and biodiesel are the two most well-known examples of first-generation biofuels used in the transport sector and account for over 90% of global biofuel usage.

Second-generation biofuels may be produced from lignocellulosic biomass such as agricultural crop residues, forestry and wood processing wastes, organic components of municipal solid waste (MSW) and energy crops such as *Mischantus giganteus*, using either thermochemical or biochemical processes(Duku, 2014).

Cellulosic ethanol or ethyl alcohol (CH_3-CH_2OH) and cellulosic butanol (CH_3-CH_2-CH_2-CH_2OH) are two well-known examples produced from lignocellulosic biomass through biochemical processes. They can be used as alternative sources to petroleum-gasoline for transportation. While the properties of cellulosic ethanol are similar to those of first-generation bioethanol, the relative abundance and wide range of lignocellulosic biomass used as feedstock, which is non-food, give them an advantage (Duku, 2014)

Third-generation biofuels are, however, produced from feedstocks such as micro- and macro-algae, vegetable oils and biodiesel (Duku, 2014).

Bio-ethanol: a distilled colorless liquid fuel obtained from numerous potential feedstock varieties such as sugar beet, wheat, corn, cassava, fruits, bagasse, barley, molasses, skim milk (whey),potatoes, sorghum, switch grass and cellulose biomass such as wood, paper, straw and other cellulose wastes such as grasses, others includes municipal solid wastes. These various waste streams for Ethanol production have their peculiar properties and generally differ.

Ethanol as an alternative fuel, offers a sustainable economy by reducing the use of imported petroleum, emitting neutral CO_2 (g), boost economy providing value added market opportunities for the Agricultural sector (Shell Global, 2001 as cited in Wondale, 2012).

2.2 Bioethanol application and its utilization

Ethanol or ethyl alcohol, has been identified as one of the most interesting synthetic oxygen-containing organic chemical because of its unique combination of properties as a solvent, a beverage, an antifreeze, and more especially due to its versatility as a chemical intermediate for other chemicals. Ethanol is an industrial chemical which has high significant utilization. It

can be used in the transportation sectors as well as in production of pharmaceutical products, dyestuffs, perfumes and numerous products. Ethanol under ordinary condition is a volatile, flammable, clear, colorless chemical compound. The largest bioethanol producers in the world are the USA and Brazil, though they utilize cornstarch and sugarcane juice as the main substrate for bioethanol production, which is globally seen as unsustainable because of energy, food and feed controversy. Bioethanol can be blended with normal gasoline in various forms: low-level blends (E10), high-level blends (E85 or E95). E10 (10% ethanol and 90% gasoline) is the most common ethanol blend in USA, and this can be used in new vehicle engines with non-engines. Most new cars sold in Brazil are flexible-fuel vehicles (FFV) that can run on pure 100% anhydrous ethanol as well as blends with up to 80% of gasoline. In Europe, a large volume of bioethanol is used in blends with gasoline (5% ethanol and 95% gasoline). However, the market potential for bioethanol is not just limited to transport fuel or energy production but has a great potential to supply the existing chemical industry. Ethanol is also used as an oxygenate additive for conventional gasoline, as a replacement for methyl tertiary buthyl ether (MTBE), which is normally mixed with gasoline as additive to improve the octane number. Due to toxic properties associated with MTBE, which is also responsible for groundwater contamination, it is therefore more frequently replaced by ethyl tertiary butyl ether (ETBE) that is normally produced from bioethanol (Ikechukwu, 2012). Ethanol is therefore an excellent additive for preventing engine knock and overheating of the engine valves. Ethanol has higher octane number (96- 113) than conventional gasoline (86-87) and thus, when blended the octane number increases, thereby reducing the need for toxic, octane enhancing additives. It enables combustion engines to run at a higher compression ratio and therefore provides a net performance gain of nearly 15% w/w (Ikechukwu, 2012). As earlier mentioned, the main chemical industries that patronize ethanol industry are: solvents and alcoholic for beverages.

According to (Vincent, 2012), about 73% of produced ethanol worldwide is used as fuel ethanol; while the rest goes to the beverage and industrial sectors (see Figure 2.2).

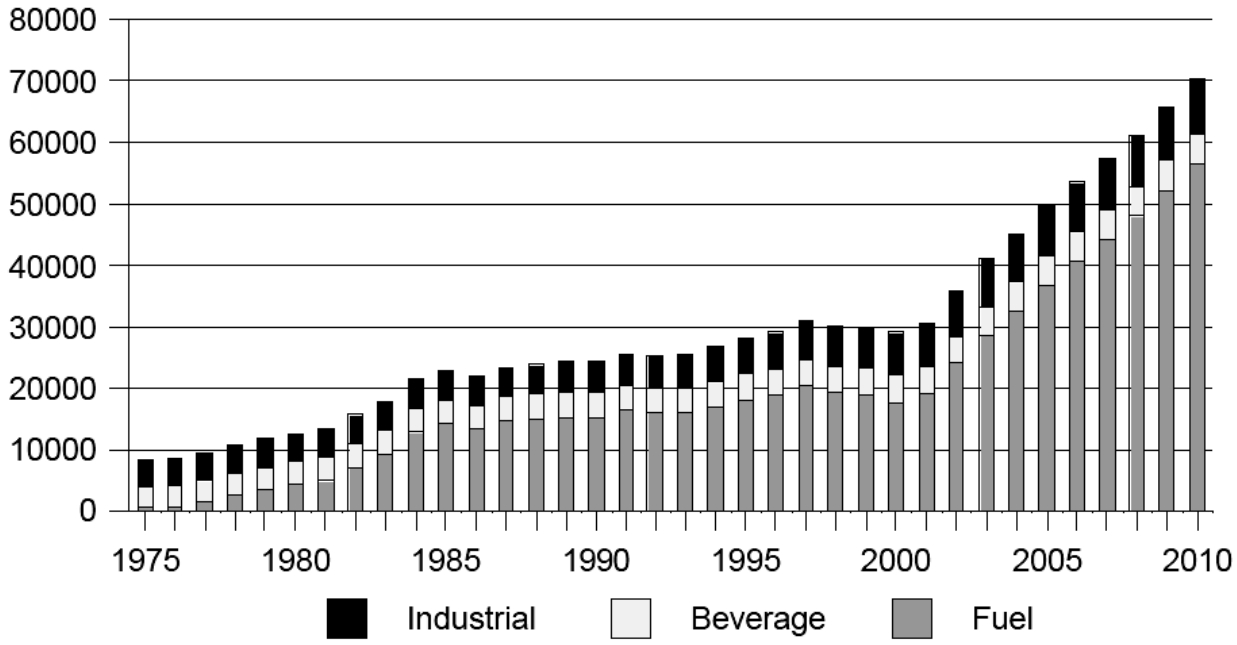


Figure 2.2: world ethanol application (millions of gallons)
Source: (Vincent, 2012)**.**

2.3 World Market of Ethanol

Today, bio-ethanol is the most dominant bio-fuel and its global production showed an upward trend over the last 25 years with a sharp increase from 2000. As of 2005, worldwide production capacity for bio-ethanol fuel was about 45 billion liters per year, with approximately 15% annual growth between 2000 and 2005. This value increased to 49 billion liters in 2006, when the Americans produced 75% of the total world ethanol output, followed by Asia/Pacific and Europe/Africa with respective values of 15 and 10% (Talebnia, 2006). The industrial alcohol market showed a rather modest rate of growth similar to the increase in Gross Domestic Product in many countries. The market for beverage alcohol in most developed countries is stagnating, due to increased health awareness.

In 2009, production of fuel ethanol reached an estimated 76 billion liters, an increase of 10 percent over 2008. The United States and Brazil accounted for 88 percent of global ethanol production in 2009. Most of the increased production occurred in the United States (Talebnia, 2006).After a significant downturn in the U.S. fuel ethanol market in 2008, U.S. production rose 16 percent to about 41 billion liters in 2009, accounting for approximately 54 percent of global ethanol production. According to one estimate, U.S. ethanol (which is mostly corn-

based) displaced more than 360 million barrels of imported oil for gasoline production. The highest sugar prices in years, combined with adverse weather conditions in a major producing region, resulted in a drop in Brazil's ethanol production from 27.1 billion liters in 2008 to 26.3 billion liters in 2009. All ethanol produced in Brazil is from sugar cane. All fueling stations in Brazil sell both pure ethanol and gasohol, a 25 percent ethanol/75 percent gasoline blend. Flex-fuel cars, which can use pure ethanol, gasoline, or any blend of the two, provide the flexibility to choose fuel based on price at the pump. They have been widely embraced by drivers and represent more than 95 percent of all new cars sold in Brazil. In recent years, significant global trade in fuel ethanol has emerged, with Brazil being the leading exporter. However, Brazilian ethanol export declined by almost 31 percent in 2009. International demands declined in great part because of the global economic crisis (Talebnia, 2006).

Table2.1:- World Fuel Ethanol Production by Country or Region (millions of Gallon)
Source: (RFA, EIO)

Country	2007	2008	2009	2010	2011	2012	2013	2014
USA	6,521	9,309	10,938	13,298	13,948	13,300	13,300	14,300
Brazil	5,019	6,472	6,578	6,922	5,573	5,577	6,267	6,190
Europe	570	734	1,040	1,209	1,168	1,179	1,371	1,445
China	486	502	542	542	555	555	696	635
Canada	211	238	291	357	462	449	523	510
Rest of World	315	389	914	985	698	752	1,272	1,490
WORLD	13,123	17,644	20,303	23,311	22,404	21,812	23,429	24,570

2.4 The State of Bioethanol in Ethiopia

Ethanol production in Ethiopia is linked with sugar factories. The total identified irrigable land for sugarcane plantation in the country is about 700, 000 hectares, estimated at a potential to produce one billion liters of ethanol (Yacob, 2013). At present, the main supply line in the domestic market is dominated by two sugar factories (Fincha and Metehara) with the combination of their annual production capacity at around 11.1 million liters.

In order to transform this potential into reality, the government developed a strategic plan in 2007 considering jatropha as a principal feedstock for biodiesel production and sugarcane as

a principal feedstock for bioethanol production. Among other things the strategy focused on establishing biofuel program, encouraging feedstock development, motivating customer demand, improving environmental sustainability, awareness conception and promotion of biofuels, and renewing energy policy to incorporate bioenergy in detail. As a continuation of this endeavor there have been repeated efforts to initiate using bioethanol for domestic use and particularly, blending 5% of ethanol with gasoline in the year 2008 followed by10% in the year 2011 and to increase the percentage in the years to come was the plan set out. With these of course the plan includes expansion of sugar factories and building new ones though delayed during implementation (Yacob, 2013).

Table 2.2: Ethiopia Ethanol production in litres
Source: (Ethiopia Sugar Corporation)

Year		Ethanol produced (litres)		
		Fincha sugar factory	Metehara sugar factory	Total
1991	1998/99	1,907,000		1,907,000
1992	1999/00	720,000		720,000
1993	2000/01	1,790,571		1,790,571
1994	2001/02	209,444		209,444
1995	2002/03	894,624		894,624
1996	2003/04	911,431		911,431
1997	2004/05	1,636,047		1,636,047
1998	2005/06	6,847,816		6,847,816
1999	2006/07	6,066,860		6,066,860
2000	2007/08	5,330,337		5,330,337
2001	2008/09	5,878,516		5,878,516
2002	2009/10	7,116,585		7,116,585
2003	2010/11	7,127,895	6,373,775	13,501,670
2004	2011/12	6,794,000	7,658,000	14,452,000
2005	2012/13	7,620,500.00	7,063,000.00	14,683,500.00
2006	2013/14	11,678,000.00	7,767,000.00	19,445,000.00
2007	2014/15	10,999,000.00	8,806,000.00	19,805,000.00

2.5 Feed stocks for Bio-ethanol Production

Application of agricultural wastes in bio-fuel industry is the focus of many researches aimed at achieving an effective and efficient waste management scheme.

Fossil fuel is a non renewable source of energy and the concern generated by its sustainability, economic and environmental impact led to the use of renewable sources for fuel. Energy from biomass is renewable and can contribute to sustainable development. In addition, biomass resources are often available locally and processing is feasible without high capital investment. Furthermore, dependence on biomass energy can help reduce green house gas emission thereby impacting on public health.

The various raw materials used in the manufacture of ethanol via fermentation are classified into three main types: sugars, starches and cellulose materials (Kudirat, 2012)

2.5.1 Sugars

Fermentation involves microorganisms that use the fermentable sugars for food and in the process produces ethanol and other byproducts. These microorganisms can typically use the 6-carbon sugars, one of the most common being glucose. Therefore, biomass materials containing high levels of glucose or precursors to glucose are the easiest to convert to ethanol. However, since sugar materials are in the human food chain, these materials are usually too expensive to use for ethanol production. One example of a sugar feedstock is sugarcane. Although fungi, bacteria, and yeast microorganisms can be used for fermentation, specific yeast (*Saccharomyces cerevisiae* also known as Bakers' yeast, since it is commonly used in the baking industry) is frequently used to ferment glucose to ethanol. Theoretically, 100 grams of glucose will produce 51.4 g of ethanol and 48.8 g of carbon dioxide. However, in practice, the microorganisms use some of the glucose for growth and the actual yield is less than 100%. Other biomass feedstocks rich in sugars include sugar beet, sweet sorghum, and various fruits. However, these materials are all in the human food chain and, except for some processing residues are generally too expensive to use for fuel ethanol production (Badger, 2002).

2.5.2 Starches

Another potential ethanol feedstock is starch. Starch molecules are made up of long chains of glucose molecules. Thus, starchy materials can also be fermented after breaking starch

molecules into simple glucose molecules. Examples of starchy materials commonly used around the world for ethanol production include cereal grains, potato, sweet potato, and cassava. Cereal grains commonly used in the US for ethanol production include maize and wheat. Starchy materials require a reaction of starch with water (hydrolysis) to break down the starch into fermentable sugars (saccharification). Typically, hydrolysis is performed by mixing the starch with water to form slurry which is then stirred and heated to rupture the cell walls. Specific enzymes that will break the chemical bonds are added at various times during the heating cycle (Badger, 2002).

2.5.3 Lignocellulosic Biomass

Agricultural residues are a great source of lignocellulosic biomass which is renewable, chiefly unexploited, and inexpensive. Such resources include: leaves, stems, and stalks from sources like corn cob, corn stover, sugarcane bagasse, rice hulls, woody crops, and forest residues. Also, other multiple sources of lignocellulosic waste from industrial and agricultural processes include citrus peel waste, sawdust, paper pulp, industrial waste, municipal solid waste, and paper mill sludge. In addition, dedicated energy crops for biofuels could include perennial grasses such as switchgrass and other forage feedstocks such as Miscanthus giganteus, M. sinensis, Bermuda grass, elephant grass, poplar etc. (Kudirat, 2012).

Cellulose materials represent the most abundant global source of biomass and have been largely unutilized. The global production of plant biomass, of which over 90% is lignocellulose, amounts to about 200×10^9 tons per year, where about 8 to 20×10^9tons of the primary biomass remains potentially accessible (Kudirat, 2012).

Cellulose is not used for food and the biofuels industries that use lignocellulosic materials do not compete for raw materials. Cellulosic biomass such as switch grass and agricultural wastes are cheaper to produce and requires fewer inputs in form of energy. Cultivation of such plants improves soil fertility and is accompanied by less soil erosion. Moreover, the process also represents a means of effective and efficient waste management as a large proportion of agricultural and municipal wastes are lignocellulosic. Another benefit of cellulosic ethanol is the reduction in greenhouse gas emission. Compared to gasoline, ethanol burns cleaner with greater efficiency thereby releasing up to 85% less carbon dioxide. In contrast, ethanol from corn which frequently involve the use of natural gas as energy source for processing may not reduce green house gas emission at all (Kudirat, 2012).

Lignocelluloses are complex substrates composed of a mixture of carbohydrate polymers i.e. cellulose and hemicelluloses tightly bound to lignin, a complex aromatic polymer, mainly by hydrogen bonds but also by some covalent bonds. Lignin interferes with cellulose hydrolysis because it acts as a physical barrier that prevents the contact of cellulase to cellulose. The first step in biofuels production from lignocelluloses therefore is delignification to liberate cellulose and hemicelluloses from their complex with lignin. It is a very crucial, rate limiting and difficult task (Kudirat, 2012).

Cellulose from wood, agricultural residues, etc must be converted to sugars, either by acid or alkali hydrolysis, or by action of cellulase enzymes and the sugar can be fermented to ethanol.

The chemical compositions (cellulose, hemicellulose and lignin content) of various feedstocks for bioethanol production in (see Table2.3 below) show that, on average, agricultural wastes with the highest cellulose content are corn cob, softwood stems, cotton seed hairs and saw dust. Forest residues comprise about 80% of the world's biomass (Demirbas, 2005) and in the United States alone 33.5-44.6 million metric tons of corn cob are available for harvest each year (Zych, 2008).

2.6 Composition of Lignocellulosic Materials

Lignocellulosic materials do not contain monosaccharides that are readily available for bioconversion but polysaccharides such as cellulose and hemicellulose, lignin, extractives, and ashes. The polysaccharides need to be hydrolysed by means of either enzymes or acids to fermentable sugars (Talebnia, 2008).

2.6.1 Cellulose

Cellulose is an unbranched homopolysaccharide composed of β-D glucose units linked by (1, 4) glycosidic bonds. However, the basic building block of cellulose is a dimer of two glucose units known as cellobiose (See Figure. 2.3). Cellulose is the most abundant material on Earth, and it is the main constituent of plants. It is also present in bacteria, fungi, algae and even in animals. In nature, cellulose chains have a degree of polymerization (DP) of approximately 10,000 and 15,000 glucopyranose units in wood and native cotton celluloses, respectively (Talebnia, 2008).

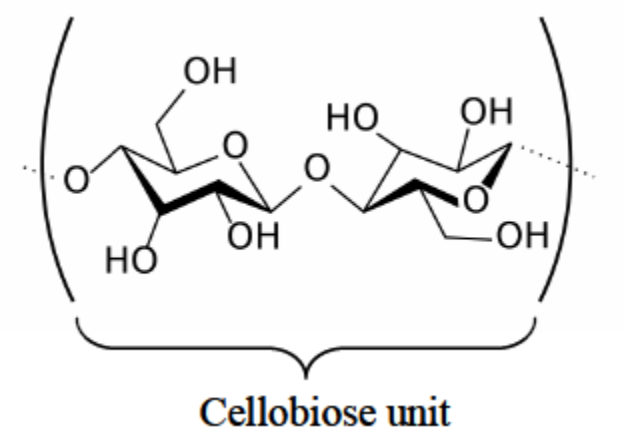


Figure 2.3: Chemical structure of cellulose
Source :(Talebnia, 2008)

2.6.2 Hemicellulose

Hemicellulose or polyose is a mixture of polymers comprising pentoses, hexoses hexuronic acids and deoxy-hexoses. Hemicelluloses differ from celluloses by composition of various sugar units and by much shorter and branched molecular chains.

In contrast to cellulose which is crystalline, strong, and resistant to hydrolysis, hemicellulose has a random, amorphous structure with little strength. Therefore, it is easily hydrolyzed by dilute acid or base, as well as hemicellulase enzymes (Talebnia, 2008).

2.6.3 Lignin

Lignin is a complex, hydrophobic, cross-linked, three-dimensional aromatic polymer of phenylpropane building blocks. The mechanical strength properties of plants are mainly due to incorporation of lignin into their cell walls, whereby huge plants such as trees can remain upright.

Lignin is one of the most complicated natural polymers with respect to its structure and heterogeneity, which make it extremely resistant to chemical and biological degradation (Talebnia, 2008).

Table 2.3: The contents of cellulose, hemicellulose, and lignin in common agricultural residues and wastes
Source: (Sun, Y. and Cheng, J. 2002)

Lignocellulosic material	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Hardwood stems	40 – 55	24 – 40	18 – 25
Softwood stems	45 – 50	5 – 35	25 – 35
Nut shells	25 – 30	25 – 30	30 – 40
Corn cobs	45	35	15
Grasses	25 –40	35 – 50	10 – 30
Paper	85 – 99	0	0 – 15
Wheat straw	30	50	15
Sorted refuse	60	20	20
Leaves	15 – 20	80 – 85	0
Cotton seed hair	80 – 95	5 – 20	0
News paper	40 – 55	25 – 40	18 – 30
waste paper from chemical pulps	60 – 70	10 – 20	5 – 10
Primary wastewater solids	8 – 15	Na	Na
Solid cattle manure	1.6 – 4.7	1.4 – 3.3	2.7 – 5.7
Coastal bermudagrass	25	35.7	6.4
Switchgrass	45	31.5	12
Swine waste	6.0	28	Na

Extractives: are woody compounds that are soluble in neutral organic solvents or water. The extractives usually represent a minor fraction (between 1-5%) of lignocellulosic materials. They contain a large number of both lipophilic and hydrophilic constituents. The extractives can be classified in four groups: (a) terpenoids and steroids, (b) fats and waxes, (c) phenolics constituents and, (d) inorganic components (Taherzadeh, 1997).

2.7 Corncobs as a feedstock of Ethanol

The maize plant comprise of the stalks, husks, shanks, silks, leaf blades, leaf sheaths, tassels and cobs. The corn cob carries the grain and together with associating husks, shanks and silks are harvested from the farm. The other parts are left on the farm to rot. Corn cobs form about 30% of maize agro-wastes (Kudirat, 2012).The agricultural residues from maize production

are potential sources of sugar for ethanol production. When maize is harvested in the field, the corn grain is separated from the cobs, stalks, and leaves. While the grain is transported for storing and processing, the stover is currently not widely collected. However, this biomass could be used for lignocellulosic ethanol production. Corn stover includes stalks, leaves, and corn cobs. As a renewable raw material, the corncobs from grain maize are a potential feedstock for the production of biogas, biodiesel and bioethanol to fulfill the increasing demand for biofuels (Pointner *et* al., 2014).

Before its use as a substrate for fermentation processes, the raw material has to be pretreated. Pretreatment is one of the many steps in the cellulose-to-ethanol process, but represents a currently critical step for hydrolysis. An effective pretreatment is performed at conditions that avoid degradation of pentose from hemicellulose, or glucose from cellulose, and limit formation of degradation products that inhibit the growth of fermentative microorganisms. The lignocellulosic structure is destroyed by treatment with high temperature and saturated steam in a reactor followed by a sudden pressure decrease (Pointner *et* al., 2014). Corncobs are a lignocellulosic material composed of cellulose, hemicellulose and lignin. These polymeric fibres consist of monomeric molecules. Cellulose is built of C_6 sugars; hemicellulose mainly of the C_5 sugars xylose and arabinose. Lignin consists of phenolic macromolecules. Corn cobs contain 32.3-45.6% cellulose, 39.8% hemicelluloses - mostly composed of pentosan and 6.7-13.9% lignin (Kudirat, 2012; Sun and Cheng, 2002) and can be converted to fermentable sugar for ethanol production.

According to study of (Samuel, 2011) was reported that the chemical compositions (cellulose, hemicellulose and lignin content) of various feedstocks for bioethanol production. Corncob contains 59.4 % cellulose, 6.5% hemicellulose, and 22.2% lignin.

The cobs produced from corn are underutilized, being mostly used as manure for agricultural production or burnt as fuel in households (Yah *et* al., 2010). Utilization of corncobs for second generation bioethanol production has been reported in several studies and represents a valid opportunity for the utilization of such feedstock.

2k8.8 Geographic Distribution

2.8.1 Overview of maize production in the world

In 2000, North America accounted for nearly 50% of the world maize production. The USA produced approximately 42%, China approximately 18% and Europe approximately 10%, whereas Australia produced less than 0.1 % (DH and AOGTR, 2008)

Table 2.5 shows the world production of maize. There are little data available on the area cultivated world for forage and silage. In the United States of America silage production accounts for approximately 10% of the total area planted to maize.

Table 2.4: World production of maize grain and green corn

		Year			
		1975	1985	1995	2006
Maize grain					
Area (ha)	World	121,444,141	130,503,715	136,461,796	144,376,477
	USA	27,366,480	30,436,000	26,389,000	28,590,000
	Australia	51,395	102,872	50,219	76,000
Yield (Kg/ha)	World	2,813	3,720	3,789	4,815
	USA	5,421	7,407	7,123	9,359
	Australia	2,593	2,872	4,826	5,000
Production (t)	World	341,661,957	485,527,301	517,139,871	695,228,280
	USA	148,361,957	225,527,008	187,967,992	267,598,000
	Australia	133,300	291,430	242,370	380,000
Green corn					
Area (ha)	World	871,011	855,213	980,925	1,053,038
	USA	278,460	253,500	286,960	260,140
	Australia	3,100	4,293	5,488	4,00
Yield (Kg/ha)	World	6,386	6,697	8,240	8,737
	USA	10,141	12,266	13,953	15,823
	Australia	8,497	11,469	13,494	10,000
Production (t)	World	5,562,206	5,962,206	8,083,182	9,200,824
	USA	2,824,000	3,109,600	4,004,100	4,116,260
	Australia	26,341	49,237	74,055	40,00

2.8.2 Overview of maize production in Ethiopia

Ethiopia is among the major maize producers in Sub Saharan African countries, where smallholder farmers dominate the major share of production. Maize, which is originated from South America, is first introduced in Ethiopia in the 16th to 17th Century (ECEA, 2009). It is classified as one of 'warm weather cereal crop" and widely cultivated at altitudes ranging from 1500 – 2200 meters above sea level of Western, Southwestern, and Southern parts of the country.

According to the data obtained from CIMMYT, Ethiopia is the third largest producer of maize in Eastern and Southern Africa, following South Africa and Tanzania. It accounts for about 10% of the area and 12% of the production of the region. Maize yield levels are also slightly above the regional average-about 1.7 metric tons/ha compared to 1.5 metric tons/ha for the whole region. In fact, yield of maize is the second highest following South Africa, which is about 2.3 metric tons/ ha (CIMMYT, 1999/2000).

In Ethiopia small-scale subsistence farmers, private commercial farmers and state farms grow maize. Small holders plant maize mainly as a subsistence crop while the large modern farms mainly produce for the market. According to CSA data, the average area planted for maize during 1997/98-2001/02 was about 1,497,300 hectares and during 2003/04 - 2007/08 was 1,549,613 hectare. The share of the smallholder sector was about 95% of total maize production.

Maize is widely grown in Ethiopia; only three regional states contribute to 94% of the total annual production. These regions are Oromia, Amhara and SNNP. According to a five years (2003/04 - 2007/08) CSA data, the share of Oromia region was on the average, 60% of the total Maize production in the country. This was followed by Amhara with 21.67% and SNNP with 12.55%. Thus the trend of the National maize production was totally dependent on the production field of the three regions. Accordingly, 16 zones from Oromia, 5 zones from Amhara and 7 zones from SNNP region are found to be producers of more than 100,000 quintals per year in all the years from 2003/04 - 2007/08. In Oromia region, 11 of the 16 zones on average produce more than 1,000,000 quintals annually. The major maize producing zones of Ethiopia and their relative share of the national maize production is shown table 2.2 below (ECEA, 2009).

Table 2.5: Major Maize Producing Administrative Zones of Ethiopia
Source: (CSA, 2003/04 – 2007/08)

Zone	Average production (in quintals)
West Gojam	3,209,274
Jimma	2,128,619
West Welega	1,795,239
East Welega	1,790,953
South Gonder	1,561,297
Arsi	1,460,934
Illubabor	1,171,244
Total	16,090,758

2.9 Overview of Ethanol production Process

Ethanol can be produced in two different ways. Either chemically, by hydration of ethylene, which is derived from crude oil or natural gas, or by fermentation of sugar containing feeds, starchy feed materials or lignocellulosic materials. About 5% - 10% of the ethanol produced in the world is a petroleum product. Petroleum ethanol product is made by the catalytic hydration of ethylene with sulfuric acid as the catalyst. It can also be obtained via ethylene or acetylene, from calcium carbide, coal, oil gas, and other sources. The two primary ways of producing fuel ethanol from cellulosic feedstock are: Biochemical conversion process and Thermo chemical conversion process (Cellulosic ethanol, 2010) (as cited in Wondale, 2012).

2.10 Biochemical Conversion

Biological conversion consists of exposing biomass to certain microorganisms. The secondary fuels produced are the result of metabolic activity of the microorganisms. Production of ethanol through fermentation through anaerobic digestion is the most common biological conversion processes. Ethanol fermentation from carbohydrates is probably one of the oldest processes known to man. Today, it is widely regarded as an important potential alternative source of liquid fuels for the transport sector (NUEP, 2013).

Typical lignocellulose-to-ethanol processes consist of at least four steps. These are pretreatment to enhance biomass digestibility, hydrolysis of cellulose to sugar monomers, fermentation of sugars to ethanol, and recovery of ethanol by distillation/evaporation from process stream (Ayele, 2011).

2.10.1 Pretreatment

Pretreatment is required to alter the biomass macroscopic and microscopic size and structure as well as its submicroscopic chemical composition and structure so that hydrolysis of carbohydrate fraction to monomeric sugars can be achieved more rapidly and with greater yields (Sun and Cheng, 2002;Ikechukwu, 2012).

The effect of pretreatment of lignocellulosic materials has been recognized for a long time. The purpose of the pretreatment is to remove lignin, reduce cellulose crystalline and increase the porosity of the materials. Pretreatment must meet the following requirements: Improve the formation of sugars or the ability to subsequently form sugars by acidic or enzymatic hydrolysis; avoid the degradation or loss of carbohydrate; avoid the formation of byproducts inhibitory to the subsequent hydrolysis and fermentation processes. Physical, chemical, physico-chemical, and biological processes have been used for pretreatment of lignocellulosic materials (Sun, Y. and Cheng, J. 2002).The purpose of the pretreatment was to reduce cellulose crystallinity and increase the porosity of the materials. Pretreatment must meet the following requirements: improve the formation of sugar, avoid the degradation or loss of carbohydrate, avoid the formation of by-product inhibitors and must be cost effective.

Dilute acid pretreatment

Acid pretreatment firstly developed in Germany in 1898. In this method concentrated or dilute mineral acids like sulfuric acid are used in order to break down hemicelluloses into monomeric sugars and simultaneously removing part of the lignin. This method needs a small amount of water since a small amount of energy is required to get an optimum temperature (Dehnavi, 2009). Dilute acid hydrolysis is the most employed technique for the hemicellulose breakdown. In this process, the use of diluted acids (1 to 4%) under moderate temperatures (120 to 160℃) has proven to be adequate for hemicellulose hydrolysis while promoting little sugar decomposition. (Mussatto, 2010: Vincent, 2010)**.**

Another study carried by (Bensah, *et* al., 2013) showed that under dilute acid (0.2–2.5% w/w) processes, high temperatures (120–210℃) and pressures are used to achieve reaction times in seconds or minutes and are thus suitable for continuous operations. The low acid consumption is a major advantage in terms of cost and process severity. Moreover, low acid concentrations (<1% w/v sulphuric/phosphoric) release essential nutrients (S and P) that enhance downstream fermentation. (Zhang *et* al., 2011) also studied that commonly used

dilute acid hydrolysis pretreatment for biofuel production apply from 0.05 to 2% H_2SO_4 (w/v) at between 120 and 220℃) for 2 to 90 minute. In general, at higher temperature and at longer reaction times of the pretreatment result in higher levels of hemicellulose sugar recovery. However, higher pretreating temperature and at longer pretreatment time cause degradation of xylose and glucose, which leads to production of inhibiter products such as furfural and 5-hydroxymethyl furfural (HMF). These toxic byproducts inhibit microbial growth and interface with subsequent ethanol fermentation. It also studied that corncobs used for xylose production are treated with 1.2 to 1.5% H_2SO_4 at 125℃) to minimize production of inhibit compounds. Dilute acid pretreatment is not effective in dissolving lignin, but it can disrupt lignin and enhance digestibility of cellulose and that around 100% hemicellulose removal is possible under this method (Ikechukwu, 2012).

Some advantages of this method are: (1) High yield of hemicelluloses sugar, (2) Remove of lignin and hemicelluloses in this method increases exposing of cellulose to enzyme, (3) Remove of heavy metals in the raw materials (Dehnavi, 2009).

2.10.2 Hydrolysis

Hydrolysis is the unit operation that depolymerizes the polysaccharide chains of cellulose and hemicellulose into fermentable oligosaccharides and/or monosaccharides (Alicia, 2013). After the pretreatment process, there are two types of processes to hydrolyze the feedstocks for fermentation into ethanol, most commonly used are acid (dilute and concentrated) and enzymatic hydrolysis. In addition, there are some other hydrolysis methods in which no chemicals or enzymes are applied. For instance, lignocellulose may be hydrolyzed by gamma-ray or electron-beam irradiation, or microwave irradiation. However, those processes are commercially unimportant.

Hydrolysis of cellulosic materials includes the processing steps that convert the carbohydrate polymers e.g. cellulose and hemicellulose into monomeric sugars. Cleavage of these polymers can be catalyzed enzymatically by cellulases or chemically by acids such as sulfuric acid (Mosier et al., 2005). The factors that have been identified to affect the hydrolysis of cellulosic biomass include porosity or accessible surface area, cellulose fiber crystallinity, and the content of lignin and hemicellulose (Talebnia, 2008).

Both enzymatic and chemical hydrolyses require a pretreatment to increase the susceptibility of cellulosic materials (Demirbas, 2005).

2.10.2.1 Acid hydrolysis

Dilute Acid Hydrolysis

The dilute acid process is conducted under high temperature and pressure, and has a reaction time in the range of seconds or minutes, which facilitates continuous processing. The combination of acid and high temperature and pressure dictate special reactor materials, which can make the reactor expensive. The first reaction converts the cellulosic materials to sugar and the second reaction converts the sugars to other chemicals. Unfortunately, the conditions that cause the first reaction to occur also are the right conditions for the second to occur (Demirbas, 2005).

The principle of acid hydrolysis is to apply temperature and pressure in order to soften lignocellulosic providing better penetration of the acid, and then degrade carbohydrate part of wood into monosaccharides. During treatment various products are formed: monosaccharides (xylose, arabinose, mannose etc.), some sugar-dehydration products (furfural, hydroxylmethylfurfural), while lignin and part of cellulose remain as solid residue. Research works on the dilute acid hydrolysis of different lignocellulosic materials have defined optimal process conditions: temperature 80-200℃, sulfuric acid concentration 0.25–8 wt%, and reaction time 10-2000 min. Sulfuric acid is a commonly used acid due to low cost, non-volatileness and affordable corrosion strength (Gladyshko, 2011). Another study carried by (Kumar, 2009) reported that acid concentration in the dilute-acid hydrolysis process is in the range of 2-5%. Acid hydrolysis typically uses dilute (4 wt %) sulfuric acid at high temperatures (120-200℃) for up to 2 hrs to break the cellulose chains into glucose. The challenges associated with this method include cost and production of compounds inhibitory to fermentation organisms. Some of the costs of using acid can be mitigated through acid recovery and by-product recovery. Gypsum is produced in large quantities during neutralization of the hydrolyzate with lime at the end of the process, which could be used to make building supplies like wallboard. Additionally, the remaining lignin can be burned for process heat. However, just like with dilute acid pretreatment, the production of inhibitory compounds like furfural, HMF, acetic acid, formic acid is possible, which impacts sugar recovery and ethanol yields (Alicia, 2013).

Despite all of the benefits of sulfuric acid hydrolysis, some limitations take place including high corrosion rates and expensive construction materials. Also, liquors have to be neutralized prior to fermentation of sugars, thus gypsum is formed. The large amounts of

gypsum negatively influence the downstream process, and also results in a low-value byproduct stream. Thus, the treatment entails considerable expenses, which limits wide commercial implementation in comparison with other possible methods of hydrolysis (Gladyshko, 2011).

According to the (Salve *et* al,2010) on dilute hydrolysis, higher sugar concentration liberated from acid hydrolysate compared to enzyme hydrolysate, It is found that, Acid hydrolysis of corn cob, gives the maximum yield of 24.50 per cent of bioethanol however , enzyme hydrolysis gives the maximum yield of 9.60 of bioethanol at the same condition. Another study on acid hydrolysis (Ali Zulfiqar, 2014) reported that maximum glucose recover obtained was 49.51%.

The biggest advantage of dilute acid processes is their fast rate of reaction, which facilitates continuous processing. Since 5-carbon sugars degrade more rapidly than 6-carbon sugars, one way to decrease sugar degradation is to have a two-stage process. The first stage is conducted under mild conditions to recover the 5-carbon sugars while the second stage is conducted under harsher conditions to recover the 6-carbon sugars (Demirbas, 2005).

2.10.3 Fermentation

Lignocellulose is often hydrolyzed by acid treatment. The hydrolysate obtained is then used for bioethanol fermentation by microorganisms such as yeast. Because such lignocellulose hydrolysate contains not only glucose, but also various monosaccharides, such as xylose, mannose, galactose, arabinose, and oligosaccharides, microorganisms should be required to efficiently ferment these sugars for the successful industrial production of bioethanol. The fermentation of ethanol is the biological process that converts fermentable sugars such as glucose and xylose to cellular energy with microorganisms which produce waste by-products ethanol and carbon dioxide anaerobically by the metabolic pathways of sugars. This is unlike yeast cells *S. cerevisiae* (baker's yeast), *Schizosaccharomycespombe be* that prefer fermentation even in the presence of oxygen and will produce ethanol given a suitable source of nutrition. *S. cerevisiae* is yeast that is most widely used in the production of ethanol from hexoses but cannot utilise pentoses. *P. stipitis* converts both hexoses and pentoses into ethanol at relatively high conversion (Samuel, 2011). In general, the conversion of lignocellulosic material to sugar and then ethanol is governed by (equation 2.1) below:

$$(C_6H_{10}O_5)\,n + nH_2O \longrightarrow nC_6H_{12}O_6 + \text{yeast} \longrightarrow 2nC_2H_5OH + 2nCO_2 \qquad (2.1)$$

From the above equation the first step is hydrolysis and the second step is fermentation. According to the reactions, the theoretical maximum yield is 0.51 kg bioethanol and 0.49 kg carbon dioxide per kg of xylose and glucose. The overall reaction of this fermentation of hexose sugar (glucose) by yeast has been expressed by Gay-Lussac which forms the basis of calculating fermentation efficiency as:

$$3C_5H_{10}O_5 \longrightarrow 5C_2H_5OH + 5CO_2 \quad (2.2)$$

$$C_6H_{12}O_6 \longrightarrow 2C_2H_5OH + 2CO_2 \quad (2.3)$$

Saccharomyces cerevisiae is the most favored organism for ethanol production from hexoses. *P. stipitis* and *Candidashehatae* are capable of fermenting both hexose (glucose) and pentose (xylose) sugars to ethanol (Joshi, 2011).

Xylose-fermenting microorganisms are found among bacteria, yeast and filamentous fungi. Today, xylose fermenting bacteria include both native and genetically engineered organisms, and many have characteristics useful for simultaneous saccharification and fermentation (Ayele, 2011).

One of the most effective bioethanol producing yeasts, *S. cerevisiae*, has several advantages owing to its high bioethanol production from hexoses, high tolerance to bioethanol and other inhibitory compounds in the acid hydrolysates of lignocellulosic biomass. However, because wild-type strains of this yeast cannotutilize pentoses, such as xylose and arabinose bioethanol production from a lignocellulose hydrolysate is inadequate. For xylose-using *S.cerevisiae*, high bioethanol yields from xylose also require metabolic engineering strategies to enhance the xylose (Ayele, 2011).

The optimal pH range for *Saccharomyces cerevisiae* varies between 4.0 and 6.0 depending upon the fermentation medium. The pH affects the efficiency of ethanol fermentation by influencing the activity of plasma proteins and intracellular enzymes. If enzymes are deactivated by pH < 4.0 the yeast will not be able to grow and produce ethanol efficiently (Uncu, 2009) observed an increase in ethanol production as well as fermentation efficiency with an increase in pH from 4.0 to 5.0 and found the optimum pH for *Saccharomyces cerevisiae* species around pH 4.5. Another study carried by (Yalçın and Özbaş, 2008 as cited in Uncu, 2009) showed that *Saccharomyces cerevisiae* worked well between a pH range of

4.0-4.5 and yielded slightly better results at pH 4.0 with fermentation of Kalecik Karası and Narince types of grapes.

One of the most successful microorganisms for bioethanol production is *Saccharomyces cerevisiae*. Although the wild-type strain has high bioethanol productivity and very tolerant to high ethanol concentrations and inhibitory compounds, it is unable to ferment pentoses (hemicelluloses). According to study (Sumphanwanich al, 2008) reported that 0.45 g ethanol/g glucose. *Pichia stipitis*, *Candida shek8hatae* and *Pachysolan tannophilus* are promising microbes that are capable of fermenting both hexoses and pentoses. However, *S. cerevisiae* is still the most commercialized and dominated strains for bioethanol production (Harun *et* al., 2009).

Microorganisms for bioethanol fermentation can best be described in terms of their performance parameters and other requirements such as compatibility with existing products, processes and equipment. The performance parameters of fermentation are temperature range, pH range, alcohol tolerance, growth rate, productivity, osmotic tolerance, specificity, yield, genetic stability, and inhibitor tolerance (Demirbas, 2004). All the recombinant strains are mesophilic organisms and function best between 25℃, and 33℃.

2.10.4 Separation

2.10.4.1 Distillation

Distillation is one of the steps of the purifications. Distillation is the method used to separate two liquid based on their different boiling points. However, to achieve high purification, several distillations are required. This is because all materials have intermolecular interactions with each other, and two materials will co-distill during distillation. (Onuki, 2005 as cited in Wondale, 2012).

Whatever method of preparation is used, the ethanol is initially obtained in a mixture with water. The ethanol is then extracted from this solution by fractional distillation. Although the boiling point of ethanol, 78.3℃, is significantly lower than the boiling point of water, 100℃,, these materials cannot be separated completely by distillation. Instead, an azeotrope mixture (i.e. a mixture of 95% ethanol and 5% water) is obtained, and the boiling point of the azeotrope is 78.15℃. In a distillation, the most volatile material (i.e. the material that has the lowest boiling point) is the first material to distill from the distillation flask, and this material is the azeotrope of 95% ethanol which has the lowest boiling point. If an efficient

fractionating column is used, 95% alcohol could be obtained first and then a small intermediate fraction of lower concentration, and then water. But no matter how efficient the fractionating column used, 95% alcohol cannot be further concentrated by distillation because the vapor has exactly the same composition as the liquid; towards distillation, then, 95% alcohol behaves exactly like a pure compound (Jackman, 1987as cited in Wondale, 2012).

2.10.4.2 Dehydration

After distillation, some amount of water remains in ethanol. Especially, this water is a big problem for fuel ethanol because the presence of this amount of water enhances the molecular polarity of ethanol when it is mixed with gasoline. Consequently, they separate into two phases, ethanol phase and gasoline phase. It is easy to imagine that this inhomogeneous fuel is not acceptable. Thus, dehydration can be another issue (Onuki, 2005).

For the ethanol to be usable as a fuel, water must be removed. Most of the water is removed by distillation, but the purity is limited to 95-96% due to the formation of a low boiling water-ethanol azeotrope. For blending with gasoline, purity of 99.5 to 99.9% is required, depending on temperature, to avoid separation. Currently, the most widely used purification method is a physical absorption process using molecular sieves. Another method is azeotropic distillation (Onuki, 2005as cited in Wondale, 2012).

3. METHODOLOGY

The experimental work was done in the laboratory of Addis Ababa university institute of technology, school of chemical and bio Engineering.

3.1. Materials

The materials used to run all experiments were listed below:

Chemicals: Sodium Hydroxide (NaOH, min. assay 98% BDH Chemicals Ltd pool England cellulose), Sulphuric acid (H_2SO_4, (98%, England)), Dextrose sugar, Yeast extract, Urea, $MgSO_4.7H_2O$, Yeast (Saccharomyces cerevisiae), (manufactured in france by S.I. Lesaffre with the strain'safinstant').

Equipments: Digital balance**s (**model-Sartorius with 0.01mg sensitivity, and model EP214C), Vacuum Filter (model-BN 3 STAATLICH, Berlin), Sieves (mesh size of 2.0 mm, Sortmks-3332, PFEUFFR, Germany), Shaking Incubator , Vertical Autoclave , pH- Meter , Ovens- Loading model 100 -800, Pycknometer, spectrophotometer (Perkin Elmer), heating mantle, condenser, Vessels, Fourier Transform Infrared spectroscopy (FTIR).

3.1.1 Characterization of corncob

Experiments were conducted to determine the moisture content, fixed carbon content, ash content and volatile matter content of air-dried biomass samples ground to particle size below 2.0 mm.

3.1.1.1 Proximate analysis

The proximate analysis gives moisture content (MC), volatile matter content, the fixed carbon content, the ash content (the inorganic residue remaining after combustion of the sample).

Moisture Content

Samples were weighed in clean preheated moisture crucible of known weight by using sensitive balance. The sample and crucible were kept in an oven 105℃ for an hour. The crucible was covered and transferred to desiccators, and weighed after reaching room temperature. The crucible was heated in the oven for another two hours and was re-weighed.

This was repeated until constant weight was obtained. The loss of weight was calculated as percent of weight and expressed as moisture content.

$$\text{Moisture content (\%)} = \frac{W1 - W2}{w1} \text{ x } 100 \quad (3.1)$$

Where:

W_1= Initial weight

W_2= weight after drying

Volatile Matter Content

A crucible was weighed empty, and then samples were put in it. The sample and the crucible were placed in a muffle furnace for 30min at 600^{o}C. The crucible was removed from furnace and placed in a desiccators to cool, then was reweighed. The process was repeated until constant weight was obtained.

$$\text{Volatile content (\%)} = \frac{W_{1-W_2}}{w_1} \text{ x100} \quad (3.2)$$

Where:

W_1= Original weight of the sample

W_2 = Weight of sample after cooling

Ash Content

A crucible was weighed empty, and then samples were put in it. The sample and the crucible were placed in a muffle furnace for 2 hours at 550℃. The crucible was removed from furnace and placed in a desiccators to cool, then was reweighed.

$$\text{Ash content (\%)} = \frac{W_{1-W_2}}{w_1} \text{x } 100 \quad (3.3)$$

Where:

W_1= Original weight of the sample

W_2 = Weight of sample after cooling

Fixed Carbon Content

This is the residue left after the moisture, volatile and ash is given up. It is deduced by subtracting from 100, the percentage of moisture, volatile matter and ash content. The fixed carbon content (FC) is given as

FC = 100 – (%moisture + %volatile matter + % ash) (3.4)

3.1.1.2 Chemical composition of corncob

Determination of extractives: Oven dried sample was placed into Trimble which is plugged with small amount of cotton and placed in a soxhlet extraction tube. An exhaustive ethanol extraction was complete in 24 hours using the Soxhlet method (Sluiter *et* al 2008).

Figure 3.1: soxhlet extraction unit set up

$$\text{Extractive (\%)} = \frac{W_{1-W_2}}{w_1} \text{ x } 100 \quad (3.5)$$

Where:

W_1 = oven dry sample

W_2 = extracted residue

Determination of cellulose content: The extractive free sample was treated with alcoholic nitric acid solution under reflux during four cycles of 1hr. After each cycle, the solution was removed for a fresh volume. The alcoholic nitric acid solution consisted of mixing one volume of 68% (w/w) solution of nitric acid with four volumes of 97% purity alcohol. At the end the cellulose was washed, dried and weighed (ibid).

Figure 3.2: Reflux set up

$$\text{Cellulose (\%)} = \frac{W_{1-W_2}}{w_1} \times 100 \quad (3.6)$$

Where:

W_1 = oven dry sample

W_2 = extracted residue

Determination of lignin: Extractive free sample was placed in flask and 72% sulfuric acid was added. The flask was kept in water bath at 30℃, during dispersion of the material for 1hr. after that adding deionised water and placed in autoclave for 121℃, to 1hr. Next the insoluble material (lignin) was filter by vacuum filtration. The lignin was washed until became acid free (with hot water) then dry and weight (Sluite *et* al, 2008).

$$\text{Lignin (\%)} = \frac{W_{1-W_2}}{w_1} \times 100 \quad (3.7)$$

Where:

W_1 = oven dry sample

W_2 = extracted residue

Determination of hemicellulose content

The hemicellulose content, W_H, was calculated by difference, assuming that extractives, cellulose, lignin, ash, and cellulose are the only components of the entire biomass (Ayeni, 2013):

$100 = W_C + W_H + W_E + W_L$

$$W_H = 100 - W_C + W_E + W_L \quad (3.8)$$

Where: W_C, W_H, W_E, W_L are cellulose content, hemicellulose content, extractive, and lignin content respectively.

3.2 Methods

3.2.1 Sample Preparation

The main aim of the research was ethanol production from corncob. First the raw material was collected form Zeway specific place Baco. Corn cobs were rinsed in water, drained and sundried for two days. Cobs were further treated by breaking to small pieces with the aid of wooden mortar and pestle in such a way that it is suitable to be dried and ground.

Then corncob was ground using milling machine to the size of 2mm and sieve analysis was performed. Flour whole size was greater than 2mm was again ground. Grinding of Corncob into fine powder gives increased surface area which enhances the contact between hemicellulose and cellulose.

a) b)

Figure 3.3 a) Corn cob sample; b) Ground sample

3.2.2 Acid pretreatment

According Zhang *et* al., 2011,dilute acid hydrolysis pretreatment for biofuel production apply from 0.05 to 2% H_2SO_4 (w/v) at between 120 and 220℃) for 2 to 90 minute (Thus, in this study dilute sulfuric 1.5% concentration was used and Corn cob powder was pretreated inside autoclave and heated at temperature of 120℃, for 30 minutes. After that, it was cooled and

filtered. The filtrated was preserved in another conical flask prepared for this purpose and kept it for fermentation. The residue was washed twice by distilled water to remove sulfuric acid from it and kept for hydrolysis purpose. Corn cobs powder was fed as batches and every batch contains 50 g of screened corn cobs powder with a ratio of 10:1(v/w) water to the sample.

3.2.3 Dilute Acid Hydrolysis

Dilute acid hydrolysis is an easy and productive process. Research works on the dilute acid hydrolysis of different lignocellulosic materials have defined optimal process conditions: temperature80-200℃, sulfuric acid concentration 0.25–8 wt%, and reaction time 10-2000 min (Gladyshko, 2011). Then, the acid hydrolysis procedure of the experiment started with adding of 3% to 5% (v/v) diluted sulfuric to the non soluble component from pretreatment steps and the corncobs were hydrolyzing in the reactor at three levels of temperature (120,130, and 140℃), time of (30, 60 and 90min). Next separate the solid particles from the liquid in the hydrolysate by vacuum filtration (to remove the non fermentable lignin portion). After separating the solid part, wash the solid part with distilled water two times. Finally, mix the soluble component with the previously filter solution from the pretreatment step for the next procedure.

pH Adjustment

Before addition of any micro-organism to the above prepared samples, pH of these samples has to be adjusted. Otherwise the micro-organism will die in hyper acidic or basic state. A pH of around 5.0 -5.5 was maintained (Wondale, 2012).

Pretreated and hydrolyzed solutions were mixed, shaken substrate primarily checked for pH using a digital pH meter. Since, the mixed sample was more acidic media, and then it would maintain the pH (5) by adding sodium hydroxide solution.

3.2.4 Fermentation

Microorganism used for fermentation

Baker's yeast, *Saccharomyces cerevisae* used for fermentation was cultured on yeast extract agar. In order to prepare the media should have the favorable condition for yeast growth or to

supply the required amount of nutrients. The following nutrients were mixed in their correct proportion.

Fermentation Medium: 200ml of production medium was prepared according to the requirements of Saccharomyces cerevisiae, containing 4 gm dextrose, 2gm malt yeast extract, 4gm peptone, 1 gm Urea, 1gm $MgSO_4.7\ H_2O$ and 200 ml make up distilled water. The pH was adjusted to 5, autoclaved at 121℃, and maintained for 15 minutes.

a) b)

Figure 3.4: a) Sterilization machine: b) Media after sterilization

After that, 1gm of yeast *Saccharomyces cerevisiae* (instant premium) was added to the above 200 ml media in a 250 ml conical flask. Next the conical flasks were properly covered with aluminum foil. Finally the conical flask was then placed in a shaking incubator for 24 hours, .a temperature of 30℃ and 200rpm (Sumphanwanich *et* al, 2008).

Sterilization

The reactor and all the equipments that were used for fermentation purposes were sterilized (autoclaved). The sterilization was carried out at a temperature of 121℃, for 15 minutes.

Fermentation

The prepared sample and media were mixed in the 500ml flasks with the ratio of 10 % (1% media with 10% sample). Then, it placed on shaking incubator at a temperature of 30 ^{o}C and at 200rpm for 72 hrs.

3.2.5 Distillation

The fermented product was distilled using batch distillation at a temperature of 85 ^{o}C for 2-3hrs. Temperature of sample to be distilled was measured by immersion of thermometer. The figure 3.5 below represents the overall set up of batch distillation.

Figure 3.5: distillation setup

3.4 Measurement of Reducing Sugars

Phenol Sulphuric Acid Method for Total Carbohydrate

The phenol sulphuric acid method to estimate total carbohydrates is described below.

In hot acidic medium glucose is dehydrated to hydroxymethyl furfural. This forms a green coloured product with phenol and has absorption maximum at 490 nm (Dubois *et* al (1956).

Standard Preparation:

A 0.009g/ml standard stock solution of glucose was prepared by dissolving 0.9g of glucose in 100.0ml distill water. Working standards were prepared by pipetting 25, 50, 75, and 100 µg aliquots of the standard stock solution into separate 100 ml volumetric flasks and diluting to volume with 50ml distill water. Five tubes were prepared for standard preparation; one tube for blank, the four tubes for glucose standard. 2ml of sample containing glucose standard was pipette. 2ml of 5% phenol was add to all tubes and mixed. Then 10ml of 96% concentrated

sulphuric acid was added, simultaneously the tubes were shaking to effect fast and complete mixing. To develop color the tubes are allowed to stand 10 min for shake, and the placed in water bath (25℃ - 30℃) to cool and display color. Blank solutions were prepared in the same way as above, except that the 2 mL of the standard solution was replaced by distilled water. The same procedure was used to prepare standard of xylose the only difference D- xylose used as a standard. The absorbance was measured at 490 nm from hexoses group glucose and 480nm from pentose groups' xylose (Khamlue *et* al, 2012).

The amount of total of total carbohydrate present in the sample solution was calculating using the standard graph.

$$y = mx + b \tag{3.9}$$

Where: y is absorbance
x is concentration
m is the slop and b is the intercept

$$\text{Con. of unknown sample} = \frac{(\text{absorbance of unknown sample}) - (y - \text{intercept})Df}{\text{Slope}} \tag{3.10}$$

Where:
D_f is dilution factor

$$\text{Sugar yield} = \text{gram of sugar/ raw material used x100\%} \tag{3.11}$$

$$\text{Ethanol yield (\%)} = (\text{gram ethanol produced/gram glucose used}) \times (100) \tag{3.12}$$

3.6 Experimental Design

Design expert® 7.0 software experimental method was used to determine the effect of three operating variables of the acid hydrolysis in ethanol production from corn cobs. These were time, temperature and acid concentration. The response variable was ethanol yield. Significance of the result was set from analysis of variance (ANOVA).

4. RESULTS AND DISCUSSION

In this section the study discussed proximate analysis and chemical composition of the sample, effect of acid hydrolysis on sugar and ethanol yield and finally chemical composition of the product were analyzing using FTIR.

4.1 Characterization of corncob

4.1.1Proximate analysis

Table 4.1: The results of proximate analyses of the corncob sample

Physical composition	Weight percentage (%wt. dry basis)
Moisture	6.8
Volatile	78.2
Ash	0.5
Fixed carbon	10

(Alabama, 2012) reported that Moisture content, Volatile content, Ash content and fixed carbon was 8.72, 80.72, 2.96, and 7.60 respectively. However, the results reported by (Alabama, 2012) are not in accordance with our result, probably due to the differences in species of varieties used. The moisture content is a measure of the amount of water in the corncob. Moisture content analysis used for the determination of proportionality of solid to liquid ratio in the pretreatment and hydrolysis method with increasing moisture content it affects the product quality. The analysis of total moisture is used to determine other properties such as volatile matter, ash content and fixed carbon. The sample of corncob with higher moisture content needs more heat for moisture vaporization. Ash is a measure of inorganic impurities in the corncob. In this study low ash content of corncob constituents, so decreasing sludge formation in the ethanol production. Finally, fixed carbon (FC) it is the carbon found in the material which is left after volatile materials are driven off this is used for the determination of carbon in the corncob.

4.1.2 Chemical composition analysis

Table 4.2: The results of chemical composition of corncob sample

Chemical composition	Weight percentage (w/w %)
Extractive	1.8
Cellulose	50
Hemicellulose	35.7
Lignin	12

Literature (Kudirat, 2012) data for corncob of chemical composition analysis range from 32.5 to 45.6% of cellulose, 39.8% hemicellulose, and 3% extractive. (Samuel, 2011) also reported, 59.4 % cellulose, 6.5% hemicellulose, and 22.2% lignin. The results from this study are in a comparable range with literature values as reported by the aforementioned researchers. Therefore, the determination of cellulose and hemicellulose can be applied to quantify the theoretical production of ethanol. However, Saccharomyces cerevisiae only converts glucose. In this study, corncob contained high contents of the total cellulose of approximately 50% cellulose. The low level of lignin present in the sample of corncob used in this study appeared more advantageous than the other types, for example, 18-25% in hard wood steam, 25-355% in Soft wood steam , 30-40% in Nuts shells, and 10-35% in grasses (See Table 2.3). The lower the lignin content the easer hydrolysis condition, and decrease formation of toxic chemicals such as, aromatic, polyaromatic, phenolic and aldehydic.

4.2 Effect of Acid hydrolysis on the ethanol yield

4.2.1 Measurement of Reducing Sugar

In this study, the total reduced sugar content through hydrolysis process was investigated. The powdered corncob through hydrolysis at different acid concentration, hydrolysis time, and temperature on the amount of sugar produced was investigated; glucose concentration and its absorbance, xylose concentration and its absorbance, and there results are shown below in Table 4.3, 4.4 respectively and the results of glucose calibration curve and calibration curve of xylose are shown in Figure 4.1 and 4.2 respectively.

Table 4.3: Glucose concentration and its absorbance

Glucose concentration, µg/ml	Absorbance
25	0.152017
50	0.41023
75	0.70350
100	0.97680

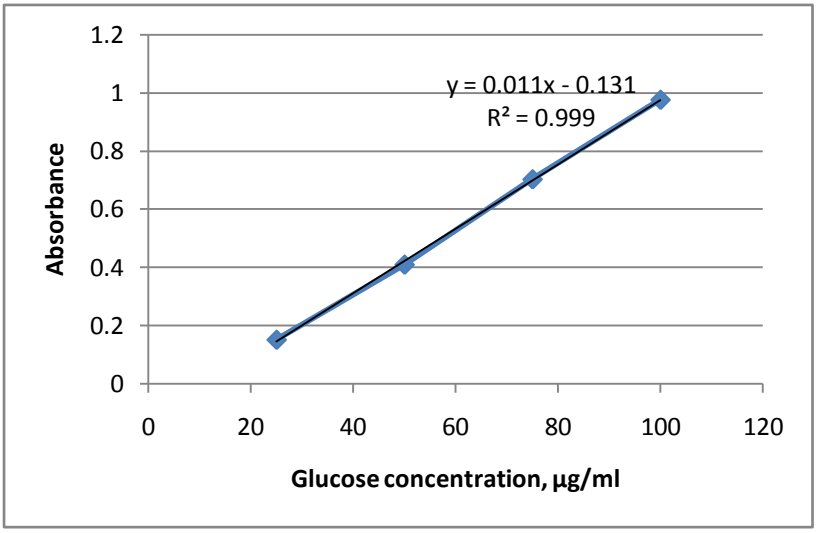


Figure 4.1: Calibration curve of glucose standard for determination of glucose content

The concentrations of unknown sugar samples were determined from a standard curve of glucose ($y = 0.011x – 0.131$; $R^2 = 0.999$)

Table 4.4: Xylose concentration and its absorbance

Xylose concentration, µg/ml	Absorbance
25	0.069966
50	0.710479
75	1.259572
100	1.784973

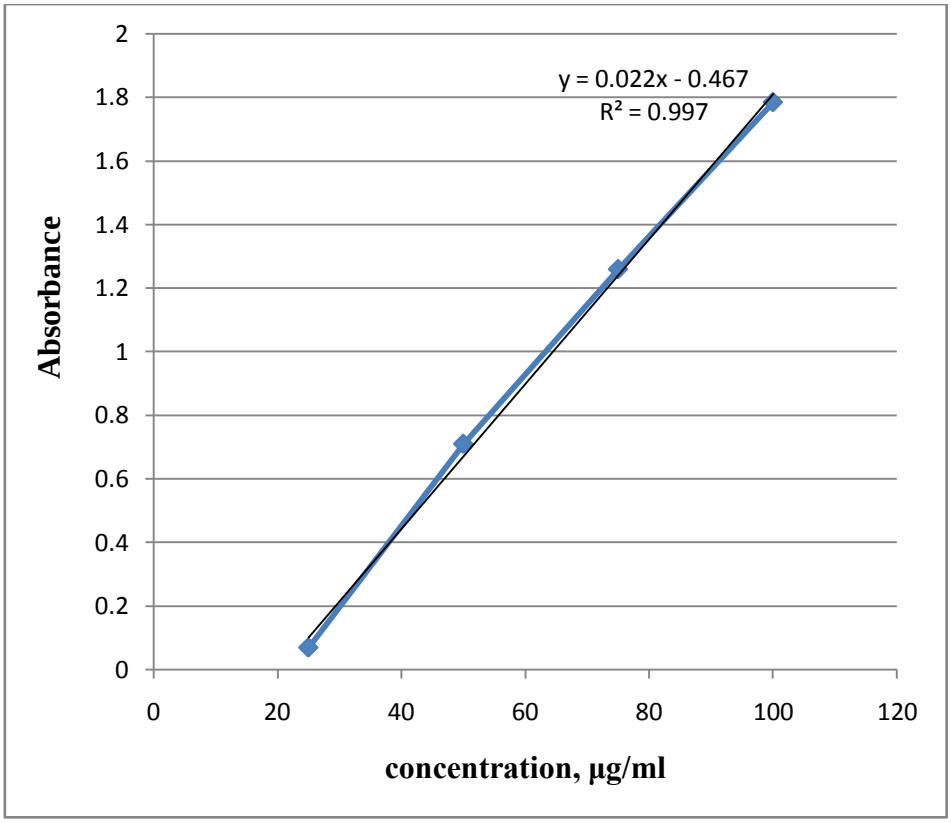


Figure 4.2: Calibration curve of xylose standard for determination of xylose sugar content

The concentrations of unknown sugar samples were determined from a standard curve of glucose ($y = 0.022x – 0.467$; $R^2 = 0.997$)

Table 4.5: Glucose (%), xylose (%) and ethanol produced at various temperature, acid concentration and reaction time.

Runs no	A: Acid Conc. (%)	B:temperature (^oC)	C: Time (min)	Glucose Yield (%)	Xylose Yield (%)	Ethanol Yield (%)
1	4	130	60	48.79	29.45	46.55
2	4	140	60	31.9	21.77	40.67
3	4	130	60	48.3	29.11	41.7
4	4	130	90	21.88	21.51	41
5	3	140	30	20.64	18.84	40.97
6	4	120	60	20.35	20.95	31.82
7	5	120	90	35	23.12	31.2
8	4	130	30	33.66	27.53	45.87
9	4	130	60	47.07	28.67	46.8
10	3	120	30	25.5	23.45	41.9
11	5	140	30	33.95	22.47	33.2
12	5	140	90	30.13	20.55	31.1
13	4	130	60	48.87	29.9	41.83
14	5	120	30	29.81	20.47	41.46
15	3	130	60	49.86	30.87	48.4
16	5	130	60	43.68	27.53	45.3
17	3	140	90	23.43	17	39.7
18	4	130	60	47.23	28.15	46.35
19	4	130	60	48.1	29.07	46.75
20	3	120	90	38.68	23.61	39.38

Regarding acid hydrolysis of corncobs, Table 4.5 above showed us the glucose concentration increased with time and temperature. Based on this, the maximum yield of glucose concentration was noted for 3% of acid concentration, at a temperature of $130°C$ and hydrolysis time of 60 min. For this condition the obtained glucose yield was 49.86%. But the glucose concentration was observed to decrease at high acid concentration, increasing time and temperature. This may be due to formation of other intermediates products. The observed result is coincide with Ali *et* al, 2014 and reported that the maximum yield of glucose was

obtained 49.51%. Table 4.5 above also indicated us the varying Ethanol yield at different acid concentration, time and temperature. To analyze the Experimental results, Design expert® 7.0.0 software was used.

From Table 4.5 above, the maximum ethanol yield 48.4% was obtained at an experiment number 15 at 130℃, of temperature, 3 % acid concentration, and at 60 minute time. While the minimum yield 31.1% was obtained at experiment number 12, at a temperature of 140℃, 5% acid concentration, and 90 minutes of hydrolysis time. The decrease and increase of the yield was depending on the level of factors. In contrast to this study, Sumphanwanich et al, (2008) obtained that 0.45 g ethanol/g glucose. This difference may be due to the different method of hydrolysis.

There resulting data, From Table 4.5, were analyzed using Design expert® 7.0.0 software to determine the effect of temperature, acid concentration, and time. The dependent variable used as a response parameter was the ethanol yield. All experiments were carried out in a randomized order to minimize the effect of unexpected variability in the observed response due to extraneous factors.

Table 4.6: Design summary

Study type	Response surface
Initial point	Central composite design
Center point	6
Design Model	quadratic polynomial
Run	20
Blocks	No

To determine whether or not the quadratic model is significant, it was crucial to perform analysis of variance (ANOVA), table 4.7 blows. The probability values (P-values) were used to perform as a device to check the significance of each coefficient, which also showed the interaction strength of each parameter. The smaller the p- values are, the bigger the significance of the corresponding coefficient.

Table 4.7: Analysis of variance (ANOVA)

Sources	Sum of Squares	Df	Mean Squares	F value	p-value prob>F
Model	452.52	4	113.13	13.51	< 0.0001*
A-Acid conc.	69.81	1	69.81	8.34	0.0113
B-Temperature	0.45	1	0.45	0.054	0.8199
C-Time	52.99	1	52.99	6.33	0.0237
B^2	307.64	1	307.64	36.75	< 0.0001
Residual	125.56	15	8.37		
Lack of Fit	95.28	10	9.53	1.57	0.3221**
Pure Error	30.28	5	6.06		
Cor Total	578.08	19			
*Significant ** not significant					

F- Value is a test for comparing model variance with residual (error) variance. If the variances are close to each other, the ratio will be close to one and it is less likely that any factors have a significant effect on the response. It is calculated by model mean square divided by residual mean square. Here the model F- Value of 13.51 implies the model is significant.There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise.Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, C, B^2 are significant model terms. The insignifican terms are depicted in appendx (E). Values greater than 0.1000 indicate the model terms are not significant. The "Lack of Fit F-value" of 1.57 implies the Lack of Fit is not significant relative to the pure error. There is a 32.21 % chance that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit is good -- we want the model to fit. Coefficent of varation, the standard deviation expressed as a percentage of the mean; predicted Redual Error sum of squares, which is the a measure of how the model fits each piont in the design; the R- squared, measure of the amount of the amount of varian around the mean explained by the model; Adj R- squared, a measure of the amount of variation in new data explained by the model, and Adquate precision, this is a signal to disterbance ratio due to random error, presented table 4.8, below, are used to decide whether the model can be used or not.

Table 4.8: Model adequacy measures

Std. Dev.	2.89	R-Squared	0.7828
Mean	41.00	Adj R-Squared	0.7249
C.V.	7.06	Pred R-Squared	0.6008
PRESS	230.75	Adeq Precision	10.671

The “pre R- squared” of 0.6008 is as close to the “Adj R- square” of the 0.7249 in less than 0.2 difference as one might expect. The difference between Adj R-Squared and Pred R-Squared is 0.1241 (i.e. they are reasonably close to each other). This indicated a close fit of the model to the actual response data. “Adeq precition” measures the signal to disturbance ratio due to random error. A ratio grater than 4 is desirable. Here the ratio of 10.671 indicates an adequate signal. Therefore, the model can be used to navigate the design space.

Diagonastic pot

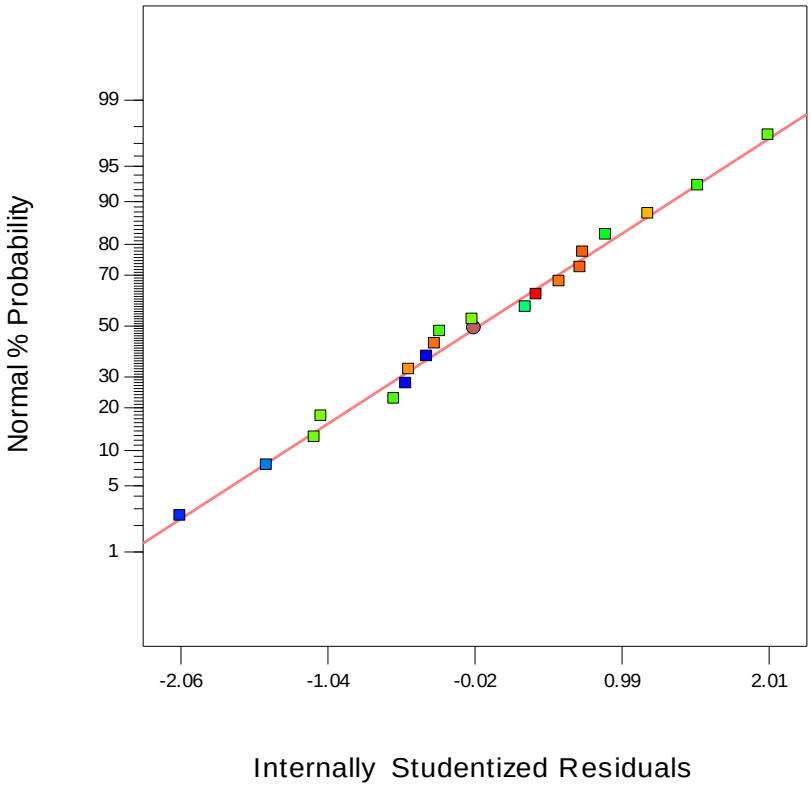


Figure 4.3: Normal plots of residuals

The normal probability plot, (Fig. 4.3), indicates the residuals following a normal distribution, in which case the points follow a straight line. This shows that the quadratic polynomial model satisfies the assumption of ANOVA.

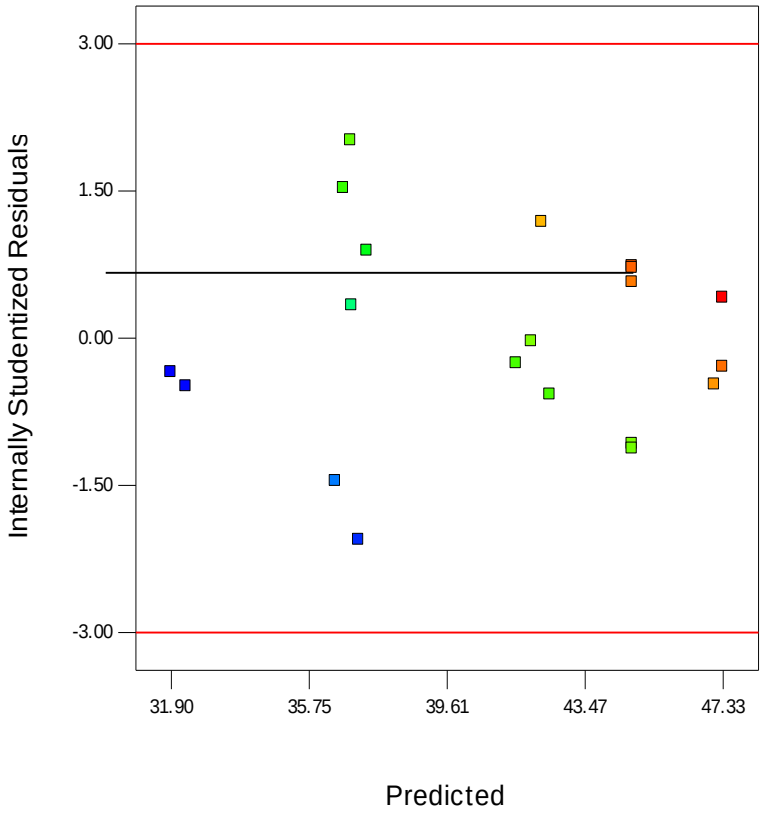


Figure 4.4: Plot of residuals versus model predicted values

If the model is correct and the assumptions are satisfied, the residuals should be structure less; in particular, they should be unrelated to any other variable including the predicted response. A simple check is to plot the residuals versus the fitted (predicted) values. A plot of the residuals versus the rising predicted response values tests the assumption of constant variance. The plot shows random scatter which justifying no need for an alteration to minimize personal error.

4.3 Determination of the optimum operating conditions

The effects of the operating conditions on the ethanol yield were investigated and the optimal values were determined in this study.

4.3.1 Effect of temperature on ethanol yield

The resulting plot of temperature versus the ethanol yield, when Acid concentration and hydrolysis time were actual factors, was depicted in Figure 4.5 below. From the plot as temperature increases from 120℃ to 130℃, ethanol yield increased to 48.4 % by weight. Beyond 130℃, decrease the yield was observed which is due to further conversion of other by product. Therefore, the optimum temperature was found to be 130℃ and the yield at this temperature was 48.4%.

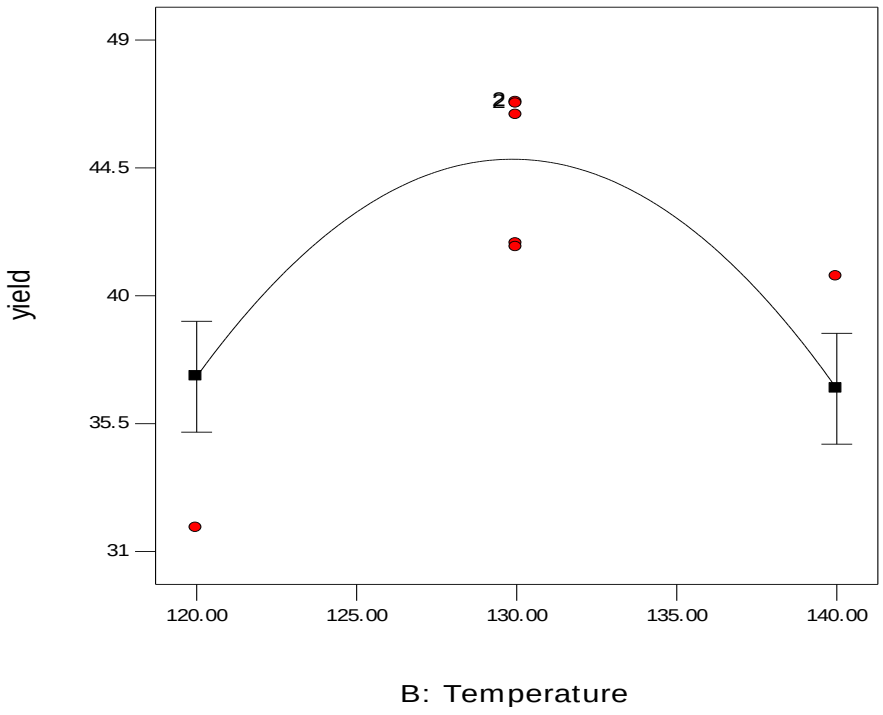


Figure 4.5: Effect of temperature on the ethanol yield

4.3.2 Effect of time on ethanol yield

The resulting plot of time versus the ethanol yield, when Acid concentration and hydrolysis temperature were actual factors, was depicted in Figure 4.6 below. As shown from the plot increasing acid concentration from 30 to 90 minute, ethanol yield decreased the reason was due to formation of degradation product.

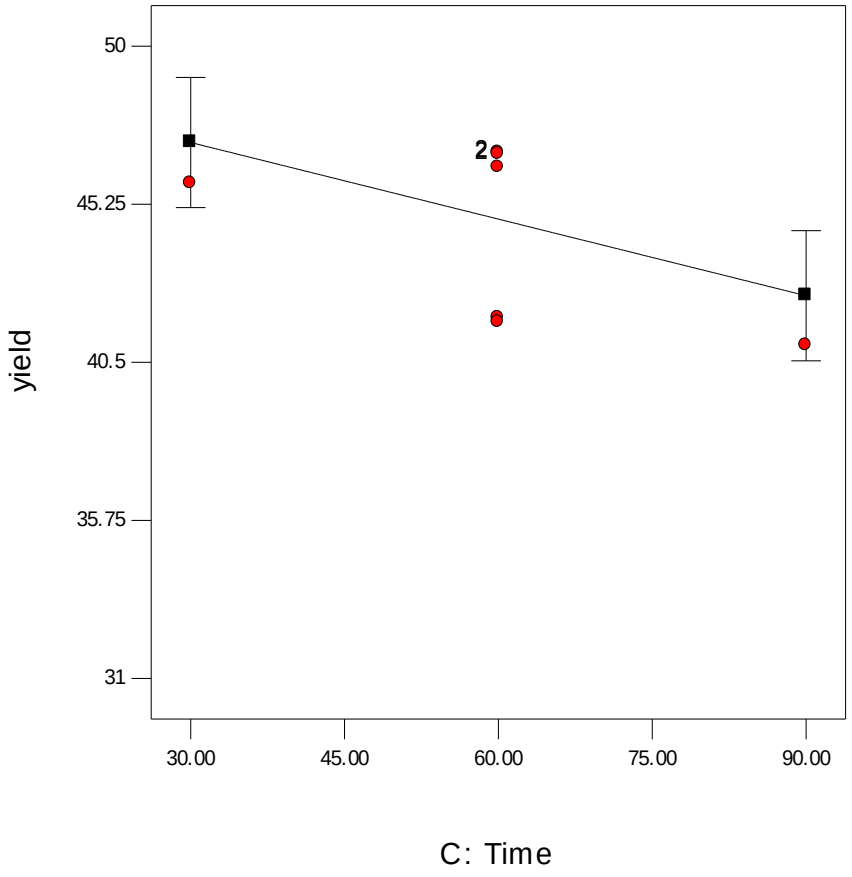


Figure 4.6: Effect of time on the ethanol yield

4.3.3 Effect of acid concentration on the ethanol yield

The resulting plot of acid concentration versus the ethanol yield, when temperature and hydrolysis time were actual factors, was depicted in Figure 4.7 below. As shown from the plot increasing acid concentration from 3% to 5%, ethanol yield decreased the reason was due to degradation of pentoses, hexoses, and the lignin present. These products can include furfural, acetic acid, 5-hydroxymethyl-2-furaldehyde (HMF), and formic acid. Therefore, the

optimum acid concentration was found to be 3% and the yield at this acid concentration was 48.4%.

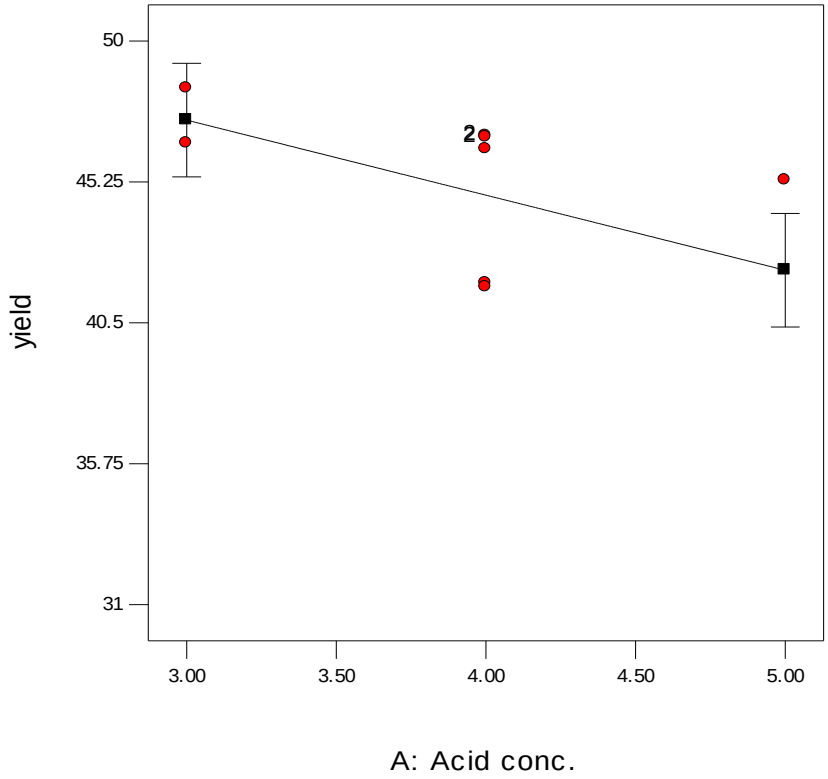


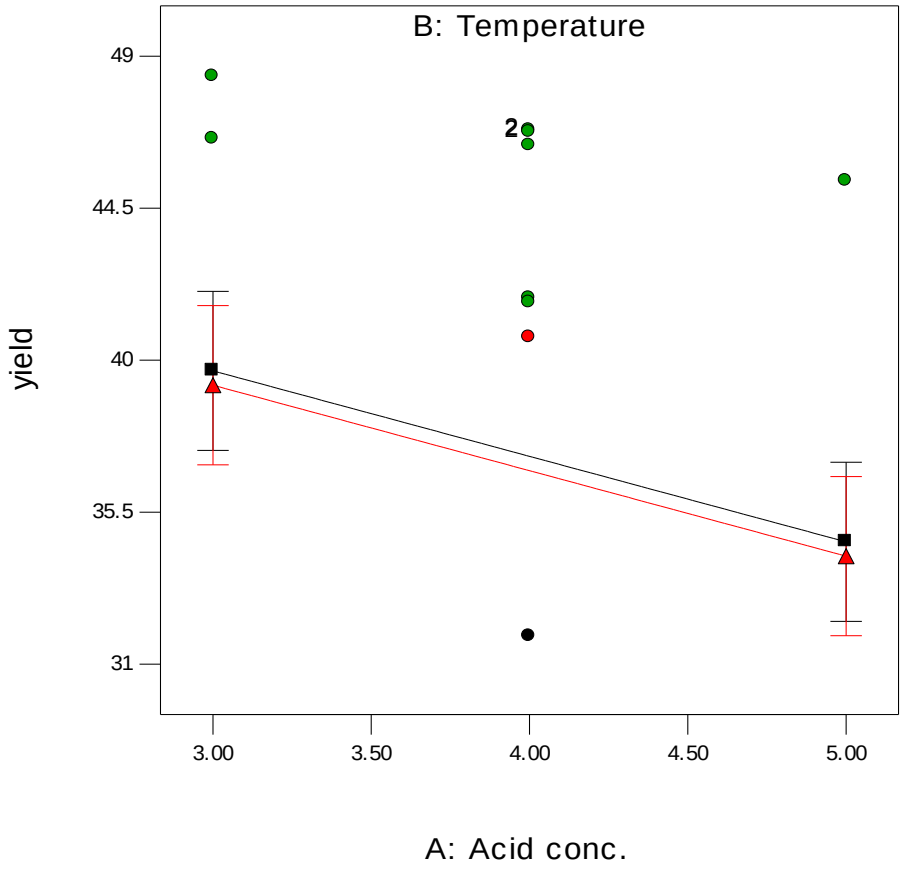
Figure 4.7: Effect of Acid concentration on the ethanol yield

4.4 Effects of Experimental variables on acid hydrolysis

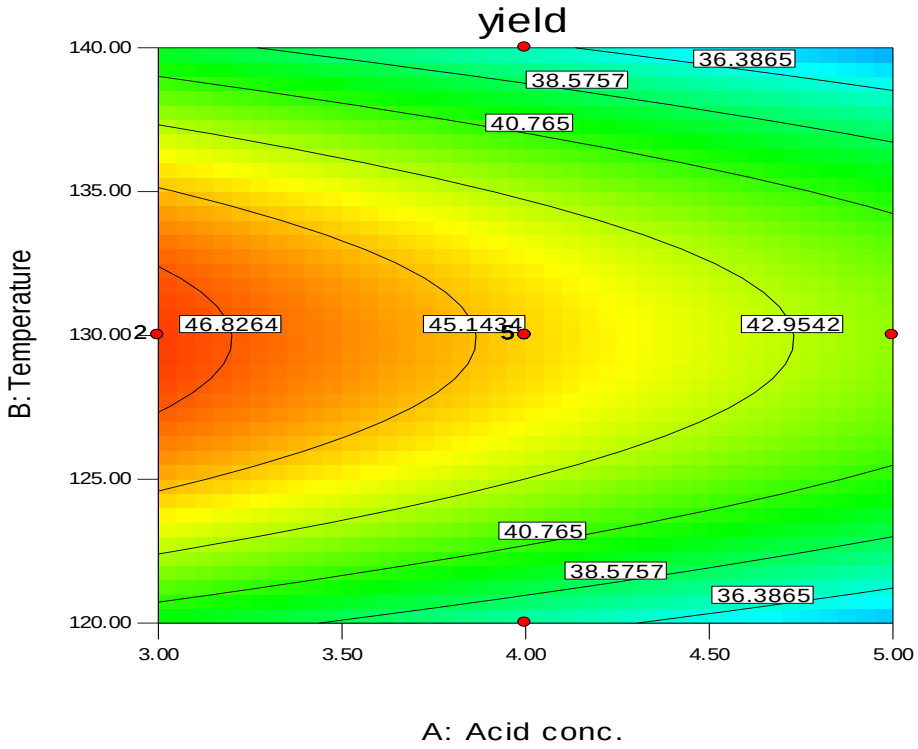
Ethanol production can be affected by many parameters starting from sample preparation to distillation, the hydrolysis steps has a complex connection with independent variables. The best way of showing the effects of this parameter for the yield of ethanol are to generate response surface plots of the equation. The three dimensional i.e. interactions, contours and response surfaces effect were plotted in figures (4.8), and (4.9) below as a function of the interactions of any two of the variables by holding the other value of the variable at center.

Interaction effects are effects that independent variable impose on one another. All controllable factors are obvious variables which affect the output of the response variable. In this research, there are three controllable factors in the acid hydrolysis step, namely: hydrolysis temperature, hydrolysis time and acid concentration. The main effects of acid concentration and hydrolysis time depended on the level of hydrolysis temperature.

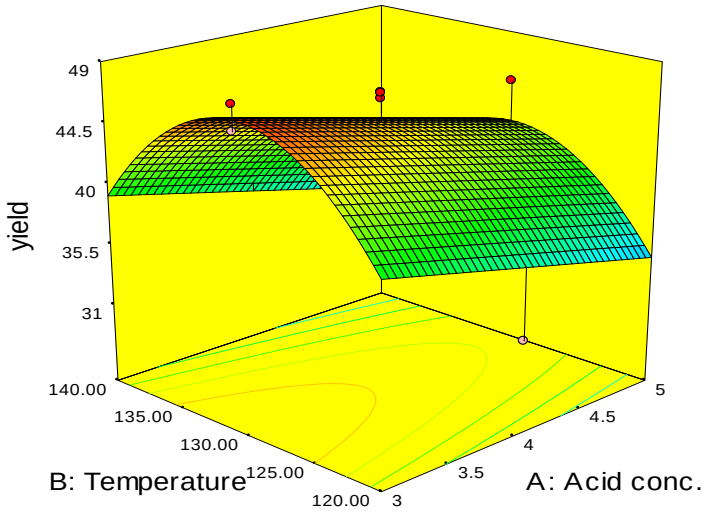
An interaction is the failure of the one factor to produce the same effect on the response at different levels of another factor. From this it is possible to conclude that, there is no interaction effect. For the interaction figures, black and red line indicates low and high level of parameters respectively. Figure 4.8(a) below shows that there is no interaction among each factor. This shows us an increment in acid concentration decrease the ethanol yield.



(a) The effects of temperature and acid concentration on the yield of ethanol, when the temperature was at the center point.



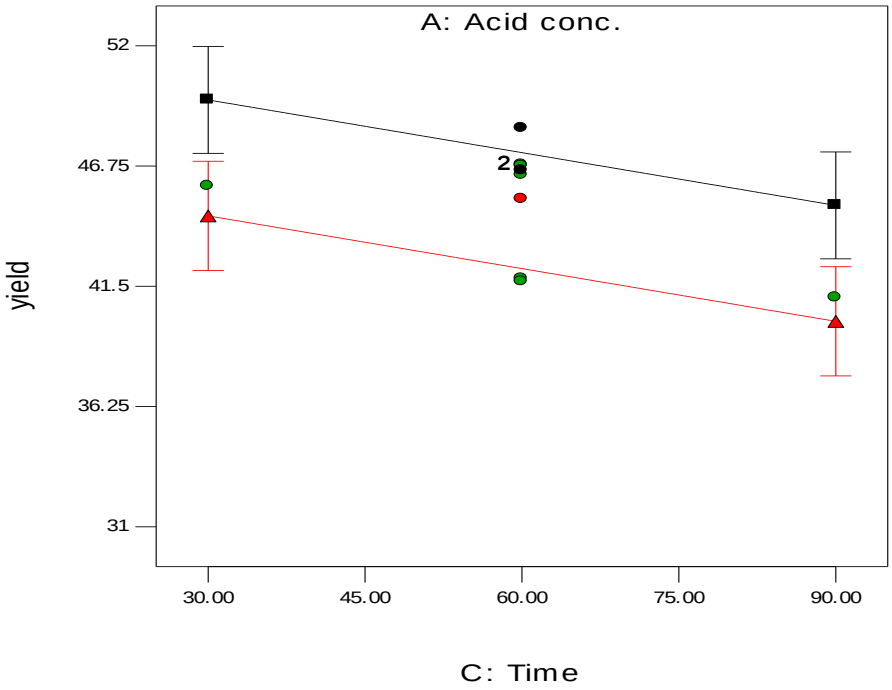
(b) Contour plot of the effects of acid concentration and temperature on the yield of ethanol.



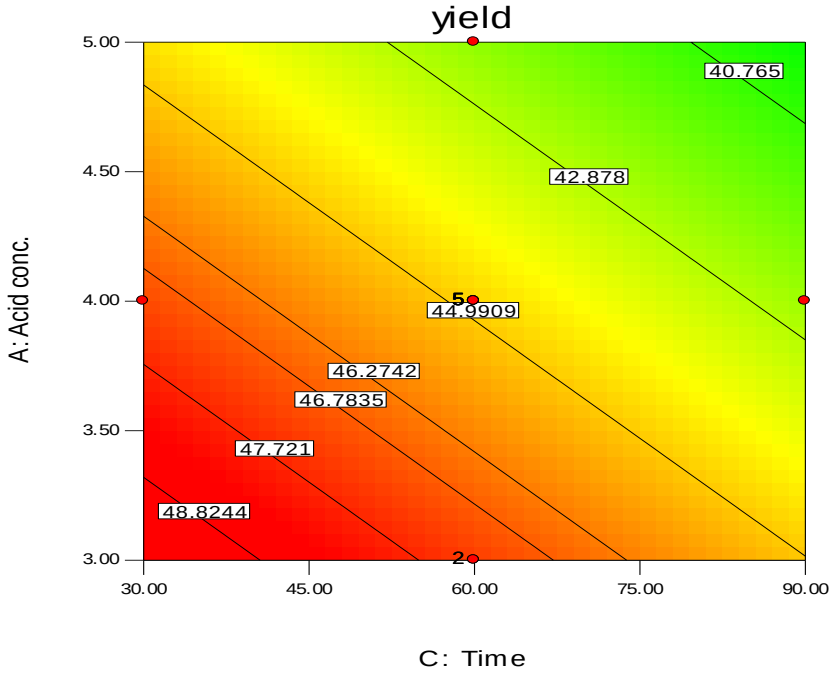
(c) Surface plot of the effects of temperature and acid concentration on the yield of ethanol.

Figure 4.8: Effect of acid concentration and temperature on the yield of ethanol when time was at the center point (a, b, and c)

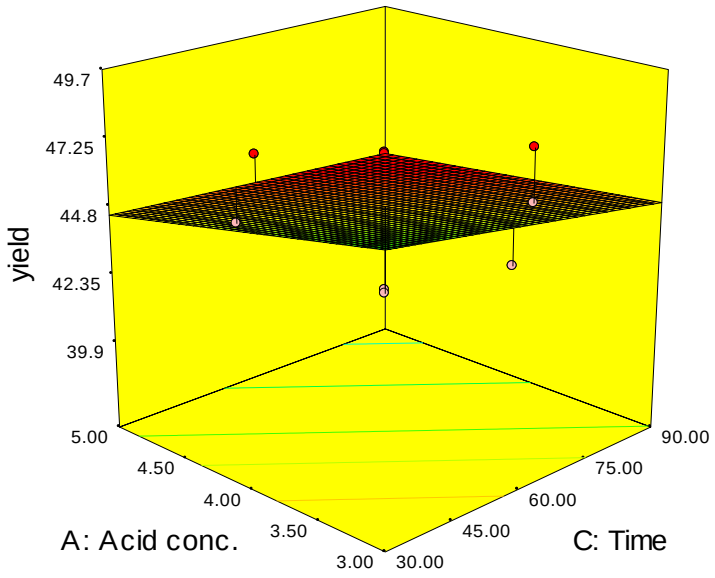
In contour and 3D surfaces graph figures 4.8 (b) and (c) above also shown that the effect of acid concentration and temperature on the ethanol yield. At the lower and higher levels of temperature, the production level of ethanol yield decrease. However, at lower acid concentration increases the yield. This is because it has effect of the hydrolysis treatment. At lower temperature the cellulose might not hydrolysis to simple glucose and at higher acid concentration and temperature cellulose forms other degradation products. Hence both acid concentration and temperature have strong relationship for the yield of ethanol production. Similarly, low levels of acid concentration and moderate temperature; it had a positive effect on the sugar yield. However, at the higher levels of both temperature and acid concentration, the yield of sugars declined as a result ethanol yield also decreases. Certainly this is due to presence of the strong interaction between these two variables. As temperature increased from 120℃ to 130℃ and 3% acid concentration, the yield of ethanol also increases to its optimal 47.2%. But when the temperature increased beyond 130℃ there was decrease in the yield of ethanol. This is because, sugar degradation products such as pentose sugar monomers may dehydrate to the inhibitor furfural, hexose sugars (e.g. glucose) may degrade to the toxic hydroxymethyl-furfural (HMF) which leads to decreased glucose yield. These inhibitors have toxic effects on the fermenting organisms, thus reducing the ethanol yield and productivity.



(a) The interaction effects of acid concentration and time on the yield of ethanol, when the temperature was at the center point.



(b) Contour plot of the effects of acid concentration and time on the yield of ethanol.



(c) Surface plot of the effects of time and acid concentration on the yield of ethanol.

Figure 4.9: Effect of acid concentration and time on the yield of ethanol when temperature was at the center point (a, b, and c).

The effects of acid concentration and time on the yield of ethanol, temperature was selected at the center point, are shown in figure 4.9 above. The maximum yield of ethanol was observed at low acid concentration and minimum hydrolysis time. At increasing acid concentration, and time the yield of ethanol became decreases since the possible formation of other molecules instead of glucose formation or the conversion sugars such as glucose and xylose in to other fermentation inhibiters.

4.5 Optimizations

Optimization of ethanol yield was carried out by a multiple response method called desirability (D) function to optimize different combinations of process parameters. The goal of optimization was to maximize economic benefit or increasing ethanol yield by minimizing process cost.

To investigate the optimum values of ethanol production from corncob using dilute acid hydrolysis are summarized as follows:

Table 4.9: Optimization criteria for optimum yield of ethanol

Name	Goal	Lower Limit	Upper Limit
Acid concentration (%)	Minimize	3	5
Temperature ^{o}C	Minimize	120	140
Time (min)	Minimize	30	90
Ethanol yield (%)	Maximize	31.1	48.4

Table 4.10: Optimum possible solutions

Solution No	Acid conc.	Temperature	Time	Yield (%)	Desirability	
1	3.00	123.75	30.00	46.6961	0.925	
2	3.00	123.86	30.00	46.8004	0.925	
3	3.00	124.14	30.00	47.0618	0.925	
4	3.00	123.50	30.06	46.4497	0.925	
5	3.00	124.30	30.00	47.1984	0.925	Selected
6	3.00	124.40	30.00	47.285	0.924	
7	3.01	123.92	30.00	46.8232	0.923	
8	3.00	123.40	30.63	46.3001	0.922	
9	3.00	124.05	30.84	46.911	0.921	
10	3.02	122.51	30.00	45.3238	0.918	
11	3.00	123.86	31.56	46.6762	0.917	
12	3.00	124.71	32.09	47.3897	0.913	
13	3.00	121.39	30.00	43.9865	0.912	
14	3.00	124.18	33.39	46.8307	0.908	

The desirability lies between 0 and 1 and it represents the closeness of a response to its ideal value. If a response falls within the unacceptable intervals, the desirability is 0, and if a response falls within the ideal intervals or the response reaches its ideal value, the desirability is 1. Based on the above analysis best local maximum for ethanol yield 47.2% was found at acid concentration 3%, temperature 124.3℃ and time 30 minutes and the value of desirability obtained was 92.5%.

4.6 Validation of the model

According to the central composite design result using Design-Expert® 7 software, an experiment with acid concentration, temperature and hydrolysis time were conducted in order to study the outcome or effect of the design. The experiment was carried out at the optimized conditions. Based on the second-order models, numerical optimization was carried out to maximize the yield of ethanol, using the response optimizer in Design expert®7. The optimal values test factors were 3 % Acid concentration, 124.3°C temperature and 30 minutes time (obtained from Table 4.10). Ethanol yield of 46.55% (average) obtained and was in good agreement with the predicted one. Therefore the model is considered to be accurate and reliable for predicting the yield of ethanol.

4.7 Fourier Transform Infrared spectroscopy (FTIR) for Bioethanol Characterization

Alcohols have characteristic IR absorptions associated with the O-H, C-O and the C-H stretching vibrations. When run as a liquid film the region 3500-3200 cm-1 with a very intense and broad band indicated the O-H stretch of alcohols, while the region 1260-1050 cm-1 confirms the C–O stretch. The bands at around 2880 and 2930 cm-1 were assigned as the symmetric stretching modes of the $–CH_2$ and$–CH_3$ groups, respectively (Bodîrlău, 2007). This assures that the product obtained from corncob was ethanol due to the confirmation of these regions as shown below.

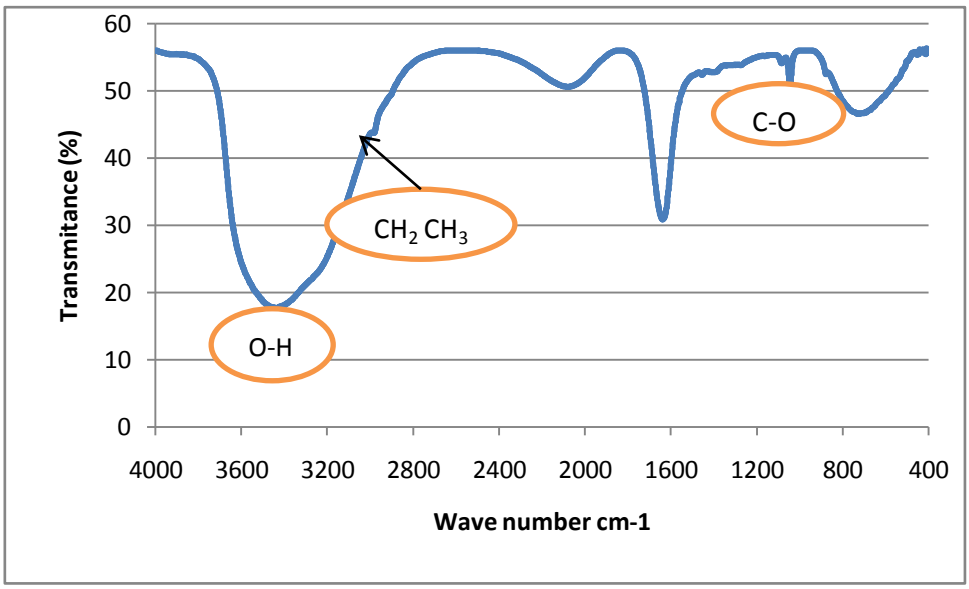


Figure 4.10: FTIR result of the ethanol yield at acid concentration of 3%, temperature of 130℃, and for a hydrolysis of 60 minutes

5. CONCLUSION AND RECOMMENDATION

5.1Conclusion

The main difficulty in ethanol production from conventional sources is that raw material availability is limited; on the other hand 75% food-material inflation (worldwide) is attributed to using conventional feedstock for ethanol production. As biofuels are very essential for the environment and the economy when they are produced from lignocellulosic biomass, selection of the cheap and appropriate raw material is big task.

This study examines the possibility of corncob for ethanol production. The conversion of corncob to ethanol was carried out with dilute acid pretreatment, dilute acid hydrolysis, fermentation and distillation process steps.

The experimental design was conducted by central composite design (CCD) to study the effects of three variables, acid concentration (3, 4, and 5%), temperature (120, 130, and 140℃), and time (30, 60, and 90minutes). The optimum operating condition was found to be at a temperature of 130℃, acid concentration 3%, and a hydrolysis time of 60minutes. At these optimum operating conditions the maximum yield of ethanol was found to be 48.4 %

Quadratic model was employed to correlate the operating variables with the response. From the analysis of variance, acid concentration and time were found to have the most significant effect on productivity of bioethanol by using F-test ($p<0.05$). The maximum, observed, value of ethanol productivity recorded was 48.4 % g /g and this is in a good agreement with the predicted value of 47.2 % g /. Based on this study, it is evident that the chosen method of optimization was efficient, and reliable. From this, it can conclude that the selected model was adequate to fit the data of response variable. Chemical characterization of the bio-ethanol produced was performed using FTIR. From result, it was observed that the ethanol produced from corncob contains O-H, C-O, -CH_2, and CH_3 functional groups; which confirm the presence of ethanol in the product.

5.2 Recommendation

Based on the current investigation the following recommendations are forwarded:

- Alternative extraction methods of ethanol such as enzymatic extraction need to be done in order to investigate the variation that could be arise on the quality and quantity of the ethanol yield as a result of using different extraction methods.
- It is also, recommend that in this study acid hydrolysis variables are optimised; future studies should include optimisation of pretreatment process, optimisation of fermentation process and optimisation of distillation process variables to obtain maximum yield of ethanol from corncob.
- Further investigation should be done to analyze the potential of bio-ethanol production from corncob using combination of yeasts, because xylose can't be converted by Saccharomyces cerevisae.
- An economic feasibility analysis of the overall conversion process is necessary for the purpose of commercialization.

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APPENDICES

Appendix A: Properties of Ethanol

Physical properties	Description

Molecular formula	CH_3CH_2OH
Molar mass	40.06844(232) g/mol
Appearance	Colorless clear liquid
Density	0.789 g/cm3
Melting point	- 114.3 ^oC
Boiling point	78.4^oC
Solubility in water	Fully miscible
Ack2idity (pKa)	15.9
Viscosity	1.200 mpa.s(cp) at 20^oC
Dipole moment	5.64 fc.fm (1.69 D) (gas)
EU classification	Flammable (F)
Flash point	286.15K(13^oC)

Appendix B: Density Measurements using pycnometer

The specific gravity of the ethanol was determined by using Specific gravity bottle (pycnometer). The 25ml pycnometer was cleaned and dried first and then weighed (W_0), then after the bottle was filled with ethanol, stopper inserted and reweighed to give (W_1). The ethanol was substituted with water after washing and drying the bottle and weighed to give (W_2). The expression for specific gravity (Sp.gr) is:

$$Sp.gravity = \frac{(W1 - Wo)}{(W2 - Wo)} \qquad (B1)$$

Where: -W0- weight (g) of empty bottle

W1- weight (g) of bottle + sample (ethanol)
W2 - weight (g) of bottle + water

Appendix C: Density versus Percent Alcohol of Aqueous Ethanol Solutions at 20^{o}C

$D^{20}4$	% alcohol by volume	$D^{20}4$	% alcohol by volume
0.9973	0.50	0.9504	32.00
0.9963	1.00	0.9468	34.00

0.9954	1.50	0.9431	36.00
0.9945	2.00	0.9392	38.00
0.9936	2.50	0.9352	40.00
0.9927	3.00	0.9311	42.00
0.9918	3.50	0.9269	44.00
0.9910	4.00	0.9227	46.00
0.9902	4.50	0.9183	48.00
0.9893	5.00	0.9139	50.00
0.9885	5.50	0.9095	52.00
0.9878	6.00	0.9049	54.00
0.9870	6.50	0.9004	56.00
0.9862	7.00	0.8958	58.00
0.9855	7.50	0.8911	60.00
0.9847	8.00	0.8865	62.00
0.9840	8.50	0.8818	64.00
0.9833	9.00	0.8771	66.00
0.9826	9.50	0.8724	68.00
0.9819	10.00	0.8676	70.00
0.9805	11.00	0.8629	72.00
0.9792	12.00	0.8581	74.00
0.977k8	13.00	0.8533	76.00
0.9765	14.00	0.8485	78.00
0.9752	15.00	0.8436	80.00
0.9739	16.00	0.8387	82.00
0.9726	17.00	0.8335	84.00
0.9713	18.00	0.8284	86.00
0.9700	19.00	0.8232	88.00
0.9687	20.00	0.8180	90.00
0.9660	22.00	0.8125	92.00
0.9632	24.00	0.8070	94.00
0.9602	26.00	0.8013	96.00
0.9571	28.00	0.7954	98.00
0.9539	30.00	0.7893	100.00

Appendix D: Fit summary

1Table D.1:Sequential Model Sum of Squares [Type I]

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob> F	
Mean vs Total	33615.90	1	33615.90			
Linear vs Mean	144.88	3	48.29	1.78	0.1908	
2FI vs Linear	16.39	3	5.46	0.17	0.9145	
Quadratic vs 2FI	316.09	3	105.36	10.46	0.0020	Suggested
Cubic vs Quadratic	70.36	4	17.59	3.48	0.0848	Aliased
Residual	30.36	6	5.06			
Total	34193.98	20	1709.70			

Select the highest order polynomial where the additional terms are significant and the model is not aliased.

Appendix E: ANOVA for Response Surface Reduced Quadratic Model

Table E.1: Backward Elimination Regression with Alpha to Exit = 0.100

Removed	Coefficient Estimate	t for H0 Coeff=0	Prob > |t|	R-Squared
B- temprature	-0.21	-0.21	0.8369	0.8250
C^2	-0.91	-0.51	0.6193	0.8208
AB	-0.72	-0.69	0.5021	0.8137
AC	-0.82	-0.81	0.4342	0.8044
BC	1.29	0.87	0.4013	0.7939
A^2	0.93	0.93	0.3673	0.7820

Hierarchical Terms Added after Backward Elimination Regression B-Temperature

Appendix F: Laboratory work pictures

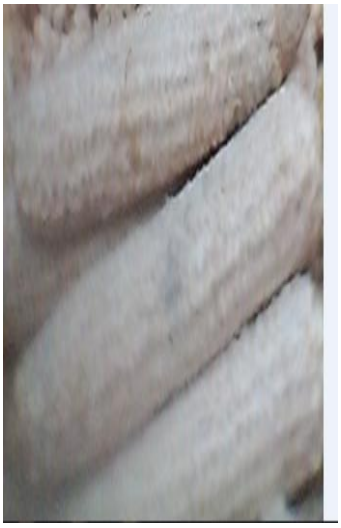


Fig.1: corncob sample

Fig.2: ground corncob

Fig.3: Grinding Machine

Fig.4: sieve analysis

Fig.5: Sample ready for pretreatment

Fig.6: autoclave reactor

Fig.7: Hydrolysate product

Fig.8: Vacuum filtration

Fig .9: Sterilization machine

Fig.10: Media after sterilization

Fig.11: Filtered sample

Fig .12: pH adjacent

Fig.13: Shaker incubator

Fig.14: Distillation setup

Fig.15: produced ethanol

Fig.16: Hydrolysate Sample

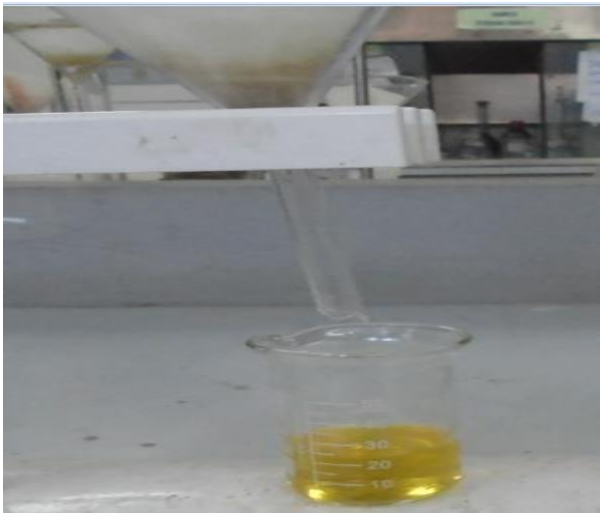
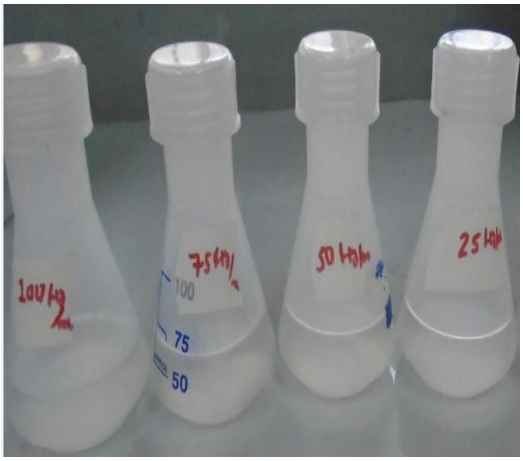


Fig.17: Filtration



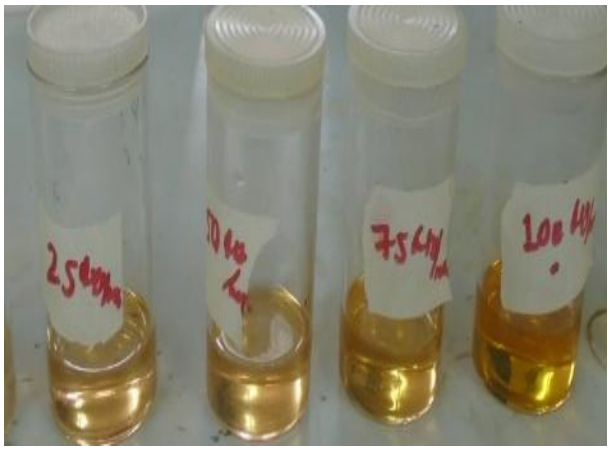


Fig.18: Stock standard of glucose and Xylose Fig.19: Glucose standard after the addition of phenol Sulphuric acid.

Fig. 20: spectrophotometer