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PROCESS ENGINEERING STREAM

Optimization of fermentation condition for bio ethanol production from corn cobs

By:

Tilahun Teshome

A Thesis submitted to Jimma Institute of Technology School of Chemical Engineering in Partial Fulfillment of the Requirements for the Degree of Master of Science in process Engineering

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External Examiner	Signature	Date
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Internal Examiner	Signature	Date
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DECLARATION

I, Tilahun Teshome, hereby declare that the work on which this thesis is based on and entitled: **Optimization of fermentation condition for bio ethanol production from corn cobs** is my original work not submitted for another degree in this or any other university, and all resources of material used for this thesis had been duly acknowledged. The work was under the guidance of Dr. Kumsa Delessa, Assistant professor in Addis Ababa Institute of Technology (AAIT), Addis Ababa University and Mr. Abreham Bekele, Lecturer in School of Chemical Engineering in (JIT), Jimma University.

Mr. Tilahun Teshome	____________	____________
Name	Signature	Date

This thesis has been submitted for examination with my approval as University Advisor and Co-advisor.

Dr. Kumsa Delessa	____________	____________
Advisor	Signature	Date
Mr. Abreham Bekele	____________	____________
Co-advisor	Signature	Date

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ABSTRACT

Lignocellulosic biomass can be utilized to produce ethanol, a promising alternative energy Source for petroleum based fuels. The bioconversion of lignocellulosic to bio fuel from cheap non edible materials such as corn cob for renewal energy is very important. Corn cobs are abundant, inexpensive, reusable, contains sufficient amount of cellulosic material, which is the best source of fermentable sugars. In this study, corn cobs were mechanically treated followed by drying, acidic hydrolysis and alcoholic fermentation. In this paper, optimization of fermentation process by Response Surface Methodology (RSM) was performed using Box behnken design. The process here in included physical and chemical pre-treatment of biomass, which was then followed by acid hydrolysis as a potential step. The concentration of reducing sugar in the hydrolyzate thus obtained was then analyzed by Benedict solution. After fermenting the hydrolysate with Saccharomyces cerevisiae for several days, distillation was done. Analysis of hydrolysate was done by FTIR. Pre-treatment is used for lignocellulosic biomass for improving the hydrolysis of the corn cob as it contains a high amount of cellulose and removal of lignin and hemicellulose. Cellulose is converted into the reducing sugars and then to ethanol. Distillation and fermentation process were performed to acquire maximum yield of ethanol. The corn cob was pre-treated with Sulphuric acid and sodium hydroxide solutions.

Different parameters of fermentation conditions were optimized. The effect of temperature, substrate concentration and PH on ethanol yield was studied. The maximum yield of ethanol was achieved at temperature of 32.718℃, substrate concentration of 125g/l, and PH of 4 with maximum ethanol yield of 42.598% at this condition.

Keywords: Bioethanol; fermentation; Saccharomyces cerevisiae; optimization; Response surface Methodology

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List of acronyms

AFEX	Ammonium fiber explosion
CAP	Concentrated acid hydrolysis
CC	Corn cob
DAP	Dilute acid hydrolysis
EtOH	Ethanol
FTIR	Fourier Transform Infrared spectroscopy
LC	lignocellulosic
LCB	lignocellulosic biomass
LHW	liquid hot water
RSM	Response surface methodology
pH	power of hydrogen
SNNP	south nation nationality and people
USA	United state of America

1. INTRODUCTION

1.1 Background

One of the most challenges of twenty-first century is to meet the growing demand of energy for heating, transportation and industrial processes; to provide raw materials for chemical industries in sustainable ways. Hence, bio fuels have emerged as an ideal alternative to meet these requirements in a sustainable approach. However, bio fuels are distinctive among available alternative energy sources in their general compatibility with existing liquid transport fuel. The worldwide production and usage of bio fuels have increased considerably in recent years, from 18.2 billion liters in 2000 to 60.6 billion liters in 2007, with about 85 % of this being bio ethanol (Kumar and Reetu , 2015).

Bio-ethanol can be produced by using different technologies. One of the most important technology, the fermentation, produce the bio-ethanol by means of biological transformation of natural starch and sugars resources such as energy-rich crops, (first-generation bio fuels) and lignocellulosic biomass (second-generation bio fuels (Bharathiraja et al., 2014) .

Large scale production of fuel ethanol is mostly based on sucrose from sugarcane in Brazil or starch, mainly from corn, in the USA. Current ethanol production based on sugar substance, corn and starch may not wanted due to their food and be feed value (Sarkar et al. , 2012). Bio ethanol can be produced from a variety of raw materials. These grouped into three main categories. Materials containing large amounts of sucrose that can be fermented, such as sugar cane; Starchy materials such as corn containing polysaccharides that can be hydrolyzed to obtain sugars suitable for fermentation; and lignocellulosic biomass, that contains a complex of several polysaccharides that can similarly be broken down into fermentable sugars (range from paper to wood).

Lignocellulosic biomass wastes constitute a significant renewable substrate for Bioethanol production that do not compete with animal feed and food production. These cellulosic materials also contribute to environmental sustainability furthermore, lignocellulosic biomass can be supplied on a large-scale basis from different low-cost raw materials such as municipal and industrial wastes, wood and agricultural residues (Limayem and Ricke, 2012).

Currently, the second generation bio-products such as biodiesel, bio ethanol, bio hydrogen and methane from lignocellulosic biomass are highly produced from wastes rather than from energy crops (jatropha, switch grass, hybrid poplar and willow) because the latter competes for land and water with food crops that are already in high demand. The use of food crops such as corn and sugarcane to produce bio fuels is mostly being discouraged due to the current worldwide rise in food prices. In order to reduce food-feed-fuel conflicts, it is necessary to integrate all types of bio waste into a biomass economy (Mtui, 2009). Corn cob, a waste product of corn contains large amount of sugars that can be further utilized to produce various compounds.

The bioconversion of lignocellulosic to bio fuel from cheap non edible materials such as corn cob for renewal energy is very important (Yah et al., 2010). Corn cobs contains sufficient amount of cellulosic material, which is the best source of fermentable sugars (Biosci et al., 2014).

1.2 Statement of the problems

Air pollution caused by the combustion of fossil fuels can affect environment seriously which leads to the problem of global warming. Due to this reason finding alternative energy that are environmentally and commercially feasible is becoming a critical issue of the world. To avoid such problems, alternative and non edible agricultural biomass must be investigated.

In different parts of the world the amount of agricultural wastes are abundantly available. The use of food crop (like corn, maize) for bio fuel production may cause inflation of cost of these crops leading to food insecurity. One of these wastes is corn cob. However, this corn cob is used as a fuel for fire, animal feed and thrown simply to the environments in some parts of Ethiopian rural area. Converting this corn cob to bio-ethanol using different technology is a better to environmental management and economically efficient.

A few researchers have done bio ethanol production from corn cob without optimizing fermentation conditions and characterizing the product properties (Yah et al., 2010). The focusing area of previous work was optimization on acid hydrolysis process to get maximum ethanol yield rather than optimizing different conditions in fermentation process. It was also recommended that future studies should include optimization of fermentation and distillation process variables to obtain maximum yield of ethanol from corncob (Mebrhit 2016). Hence, this study includes; optimization of fermentation conditions and product characterization to obtain optimal point of parameters and get maximum amount of yield during ethanol production.

Fermentation process is affected by different conditions such as (temperature, pH, substrate concentration, mixing rate and fermentation time), but for the purpose of this work fewer parameters: temperature, substrate concentration and pH were selected for investigation to study the effects of those parameters on yield of ethanol production from corn cob.

1.3 Objectives

1.3.1 General objective

- To optimize the fermentation conditions so as to produce maximum yield of ethanol from corn cob.

1.3.2 Specific objectives

- To determine proximate composition of corn cob.
- To investigate effects of fermentation process parameters such as (temperature, substrate concentration and pH).
- To determine optimum fermentation operating parameters (substrate concentration, temperature and pH) during ethanol production from corn cob.
- To characterize final product physical properties (density, pH, viscosity, flash point, functional group).

1.4 Significance

All energy sources have an impact on the environment. Concerns about the greenhouse effect and global warming, air pollution, and energy security have led to increasing interest and more development in renewable energy sources such as bio-fuel, solar, wind, geothermal, and hydrogen.

This study is important as corn cob is a widely available plant and is an alternative feedstock for ethanol production and addresses problems related with energy security, promote rural development through job creation, promote environmental conservation and decrease greenhouse gas emission.

1.5 Scope of the research

This paper focuses on the optimization of fermentation condition for bio ethanol production from corn cob and product characterization.. The methodologies that were used in this paper were proximate analysis of corn cob, composition determination of corn cob, physical pretreatment, dilute-acid hydrolysis, fermentation and distillation. The statistical data was generated from

laboratory experiments and analyzed using design expert 11 (ANOVA) to analyze the effect of fermentation process parameters on the yield of bio ethanol and to draw a generalizing conclusion for each parameter on the optimum yield of product. The yield of total reduced sugar in the hydrolysate was analyzed by using a benedicts solution through determination of concentration via absorbance. The water-ethanol mixture separation process was conducted using a distillation unit and the final product; ethanol was characterized using, refractive index to determine the density by measuring the specific gravity, vibro-viscometer to determine its viscosity and FTIR was determine the functional groups found.

2. LITERATURE REVIEW

2.1 Introduction

Energy is one of the most important factors to global prosperity. In view of continuously increasing the price of petroleum and dependence upon fossil fuel resources, considerable attention has been focused on alternative energy resources, the search for renewable energy sources has becomes a matter of extensive concern. Substituting petroleum with bio fuel can minimize air pollution, improve rural economies by creating job opportunities and raising farm incomes, diversify energy portfolios, reduce dependence on foreign oil and improve trade balances in oil-importing nations. To reduce the net contribution of GHGs to the atmosphere, bio ethanol has been recognized as a potential alternative to petroleum derived transportation fuels and cooking fuels (Kefale, Redi, and Asfaw , 2012).

Bioethanol is a renewable and sustainable liquid fuel that is expected to have a promising future in tackling today's global energy crisis and the worsening environment quality. In 2011, the world's Bioethanol production was stated to be above100 billion liters and was expected to increase up to 3–7% In the year 2012–2015, which shows that Bioethanol is already being seen as one preferable alternative energy source to substitute the fossil fuel (Aditiya et al. 2016).

Bio fuels can be grouped into three major categories, namely first-generation, second-generation and third-generation types. The main difference among them is the type of feedstock used in the production process, their current and future availability. First generation bio fuels 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 bio fuels used in the transport sector and account for over 90% of global bio fuel usage. Second-generation fuels are generally those made from non edible lignocellulosic (LC) biomass, either residues of forest management or food crop production (e.g. corn stalks or rice husks) or whole plant biomass (e.g. grasses or trees grown specifically for bio fuel purposes) (Vohra et al., 2013).

Bio-ethanol can be produced by using different technologies. One of the most important technology, the fermentation, produce the bio-ethanol by means of biological transformation of

natural starch and sugars resources such as energy-rich crops, and lignocellulosic biomass (Bharathiraja et al. , 2014) .

2.2 Ethanol and its characteristics

Ethanol, also known as "ethyl alcohol" or "grade alcohol", is a flammable, colorless chemical compound, one of the alcohols that are most often found in alcoholic beverages. In common parlance, it is often referred to simply as alcohol. Its molecular formula is C_2H_6O, variously represented as EtOH, C_2H_5OH or as its empirical formula C_2H_6O (Rutz 2008).

Bioethanol are produced by microbial fermentation (as opposed to petro chemically-derived alcohol) that is used as a transportation bio fuel. It is produced through distillation of the ethanolic wash emanating from fermentation of biomass derived sugars and can be utilized as a liquid fuel in internal combustion engines, either neat or in petrol blends.

Table 2.1 properties of ethanol

Property	Value
Density and phase	0.789 g/cm3, liquid
Solubility in water	Fully miscible
Melting point	−114.3 °C (158.8 K)
Boiling point	78.4 °C (351.6 K)
Acidity (pKa)	15.9 (H+ from OH group)
Viscosity	1.200 cP at 20 °C
Dipole moment	1.69 D (gas)
Flash point (ºC)	12-13

Source: bio fuel technology hand book

2.3 Bio ethanol and its application as fuel

The use of ethanol as an automotive fuel has a long history. The first prototypes of internal combustion engines built in the nineteenth century by Samuel Morey in 1826 and Nicholas Otto in 1876 were able to use ethanol as fuel (Solange I Mussatto et al. 2010). The first car produced by Henry Ford in 1896 could use pure ethanol as fuel and in 1908 the Ford Model-T, the first car manufactured in series, was a flexible vehicle able to use ethanol as a fuel, in the same way as gasoline or any mixture of both Alcohols have been used as fuels since the inception of the automobile.

The term alcohol often has been used to denote either ethanol or methanol as a fuel. With the oil crises of the 1970s, ethanol became established as an alternative fuel. Many countries started programs to study and develop fuels in an economic way from available raw materials.

The interest then waned as the price of oil dropped, until 1979 when we had another oil crisis. Since the 1980s, ethanol has been considered as one possible alternative fuel in many countries. Countries including Brazil and the USA have long promoted domestic bio ethanol production.

In addition to the energy rationale, ethanol/gasoline blends in the USA were promoted as an environmentally driven practice, initially as an octane enhancer to replace lead.

Ethanol also has value as oxygenate in clean-burning gasoline to reduce vehicle exhaust emissions (Kumar and Reetu, 2015). As bio ethanol can be produced from biomass of crop plants, it offers opportunities to improve the income levels of smallholder farmers. At a community level, farmers can cultivate energy crops that fetch an income while also meeting their food needs. Ethanol derived from biomass is the only liquid transportation fuel that does not contribute to the green house gas effect. Ethanol represents closed carbon dioxide cycle because after burning of ethanol, the released carbon dioxide is recycled back into plant material as plants use it to synthesize cellulose during photosynthesis. Ethanol contains 35 % oxygen that helps complete combustion of fuel and thus reduces particulate emission that poses health hazard to living beings. The toxicity of the exhaust emissions from ethanol is lower than that of petroleum(Kumar and Reetu, 2015). Thus, the use of even 10 % ethanol blends reduces GHG emissions by 12–19 % compared with conventional fossil fuels. Burning E 85 (85 % ethanol) reduces the nitrogen oxide, particulate and sulfate emissions by 10, 20 and 80 %, respectively, compared to conventional gasoline.

2.4 Worldwide market of ethanol

Ethanol production worldwide has strongly increased since the oil crises in 1970. Its market grew from less than a billion liters in 1975. The estimated world ethanol production in 1998 was 33.3 billion liters (Taylor , 2006). Approximately 9% of the ethanol is produced synthetically, and consequently, fermentation is responsible for 91% of global ethanol production. Brazil is the dominant producer of alcohol with a production of 16.1 billion liters in 1998. to more than 39 billion liters in 2006, and is expected to reach 100 billion liters in 2015 (Solange I Mussatto et al. , 2010). Actually, the American continent is the biggest worldwide producer of ethanol, with United States and Brazil representing an important role in this sector.

Table 2.2: World fuel ethanol production by country or region ((Million Gallons))

Data Source: Available at www.ethanolrfa.org/pages/annual-industry-outlook

Country	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
World	13,123	17,644	20,303	23,311	22,404	21,812	23,429	24,570	25,682	26,504
USA	6521	9309	10,938	13,298	13,948	13,300	13,300	14,300	14,806	15,250
Brazil	5019	6472	6578	6922	5573	5577	6267	6191	7093	7295
Europe	507	734	1040	1209	1168	1179	1371	1445	1387	1377
China	486	502	542	542	555	555	696	635	813	845
Canada	211	238	291	357	462	449	523	510	436	436
Others	315	389	914	985	698	752	1272	1490	1147	1301

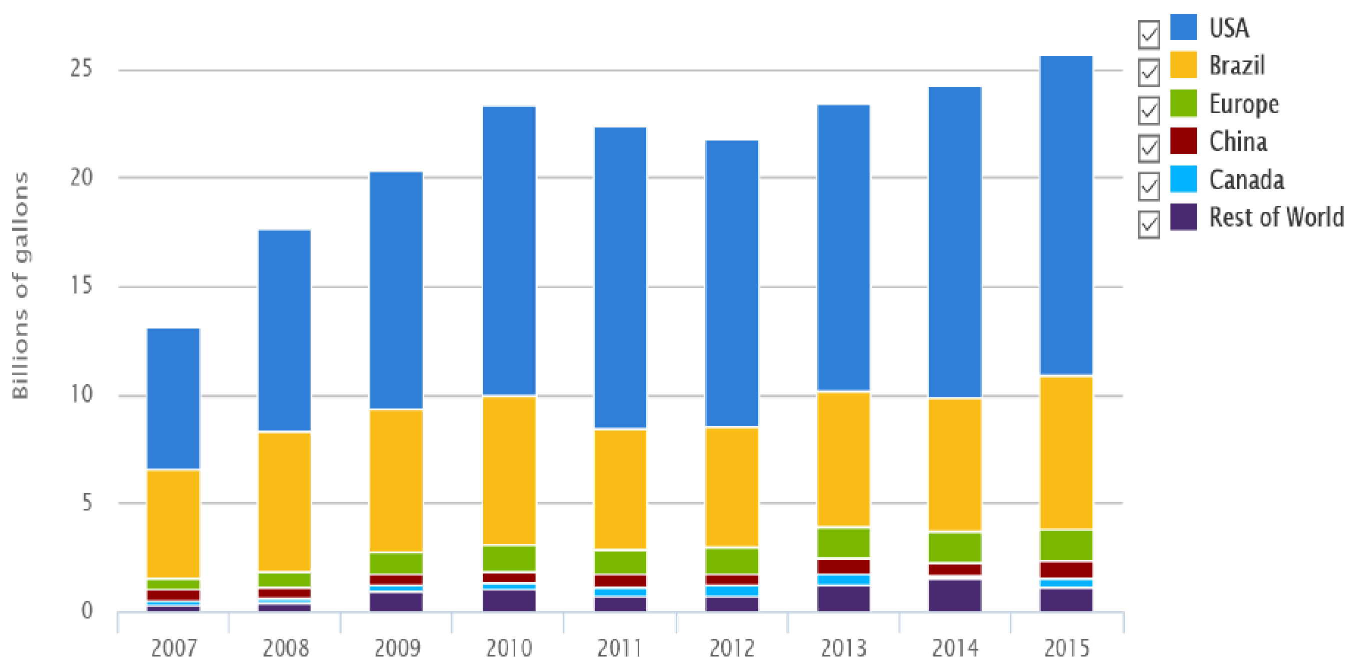

Figure 2.1: global bio ethanol production (source: www.afdc.energy.gov/data)

2.5 Status of bio ethanol in Ethiopia

Ethanol production in Ethiopia has been started since 1998/99 in Fincha sugar factory with the capacity of 1,907 m3 per year. The capacity of ethanol production is the sum of individual sugar factories ethanol production capacity. Ethanol is produced as a byproduct in the sugar factories. The demand for new sugar plants, desired number of sugar mill, is based on the countries

response to address the progressive sugar consumption every year. The initial per capita sugar consumption of the country is taken as a reference and this consumption level increases following the population change. The population estimation and projection until the year 2050 is used based on the World Bank (Nigatu , 2017). Ethiopia has several sugar factories (Fincha, Metehara, Wonji Shoa, Tendaho and Welkait, and currently under construction Omo-kuraz-1, Kesem, two of the Tana Beles factories and Arjo Dedisa) which are run and administered by Sugar Development Agency. Among molasses derived products ethanol takes the largest part, but its utilization must attract the attention of the government policy makers in order to utilize as a bio ethanol.

Table 2.3 number of sugar factory and their respective ethanol production capacity

No	Sugar factory	Sugar production capacity(ton/year)	Ethanol capacity(toe/year)
1	Tendaho -2factories	619,000	63,000
2	Omokuraz -4factories	1,390,000	130,810
3	Wolkayit	484,000	41,654
4	Wonji shoa	220,700	12,800
5	Metehara	136,692	12,500
6	Finchaa	270,000	20,000
7	Arjo - dediessa	-	-
8	kessem	260,000	30,000
9	Belles-two factories	484,000	41,654
Total	14	3,864,392	352,418

Source, Compiled from Sugar Corporation (Nigatu , 2017).

2.6 Feedstock's for Ethanol production

Bioethanol can be produced from different kinds of raw materials, mainly from three kinds of agricultural raw materials: sugar containing feedstock (e g. sugarcane, sugar beets, fruits, etc.), starch materials (corn, wheat, rice, barley, etc.) and lignocellulosic materials (wood, straw, grasses). Globally, bio ethanol production from rice straw, wheat straw, corn cob and sugarcane bagasse is now gaining importance (Mahapatra and Manian,2016). Bio ethanol can be made synthetically from petroleum or by microbial conversion of biomass materials through fermentation. In 1995, about 93% of the ethanol in the world was produced by the fermentation

method and about 7% by the synthetic method (Badger , 2002). The fermentation method generally uses three steps: (1) the formation of a solution of fermentable sugars, (2) the fermentation of these sugars to ethanol, and (3) the separation and purification of the ethanol, usually by distillation

2.6.1 Sugar feedstock's

Fermentation involves micro-organisms 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. Brazil developed a successful fuel ethanol program from sugarcane for a number of reasons: (1) Brazil traditionally relied heavily on imported oil for transportation fuels, which caused a severe economic drain on the country; (2) Brazil can attain very high yields of sugarcane; and (3) Brazil has also experienced periods of poor sugar markets. As a result, the Brazilian government established programs supportive of the industry with the result that Brazil has been able to successfully produce and use sugarcane for fuel ethanol production. 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 feedstock's rich in sugars (materials known as saccharides) 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).

2.6.2 Starches feedstock

Starch is a homo-polymer, made up of D-glucose monomers (Mahapatra and Manian,2016).In order to produce bio ethanol from starch sources, it is necessary to break the chains of this carbohydrate to obtain glucose units that can be converted to ethanol by yeasts. Starch can be converted to fermentable sugar by the method of hydrolysis. Hydrolysis is the reaction of starch using water, which is normally used to break down starch into fermentable sugar. Specific

enzymes that will break the chemical bonds are added at various times during the heating cycle (Badger 2002).

2.6.3 Lignocellulosic biomass

Lignocellulosic biomass is one of the most suitable alternative energy sources which can be harnessed to meet up the challenges of energy security. Biomass has been a significant contributor in achieving sustainable development goals (Ecology et al., 2017). The total potential for bio ethanol production from crop residues is about 16 times higher than the current world bio ethanol production from all other sources combined (Mahapatra and Manian ,2016).

The lignocellulosic biomass, which represent the largest renewable reservoir of potentially fermentable carbohydrates on earth (Mtui ,2009) , is mostly wasted in the form of pre-harvest and post-harvest agricultural losses and wastes of food processing industries. The main components of LCB are cellulose, hemicelluloses, lignin and inorganic materials (Singh and Satapathy ,2018). Cellulose is the main component in lignocellulosic material followed by hemicelluloses then lignin. Cellulose and hemicelluloses are polysaccharides which are tightly bound to lignin by covalent cross-linkages or non-covalent forces whereas, lignin is an aromatic polymer made up of phenylpropanoid precursors. Apart from the three basic chemical compounds (cellulose, hemicellulose, and lignin), lignocellulosic biomass content water, proteins, minerals and other compounds.

The organic component of biomass plays a major role in processing and producing bio fuels.

Cellulose and hemicellulose are sugar rich fractions of interest for use in fermentation processes, since microorganisms may use the sugars for growth and production of value added compounds such as ethanol, food additives, organic acids, enzymes, and others (S I Mussatto and Teixeira ,2010). Cellulose is a major structural component of cell walls. It provides mechanical strength and contributes major fuel. The solar energy is absorbed through the process of photosynthesis and stored the energy as cellulose or hemicellulose. It has been estimated that around 7.5×10^{10} tonnes of cellulose are consumed and regenerated every year(Singh and Satapathy , 2018). This is the main reasons why cellulose is considered as the most abundant organic compound on the Earth. The composition and proportion of these compounds vary from species to species depending upon plant cell wall structure.

2.6.4 Composition of lignocellulosic biomass

Lignocellulosic material can generally be divided into three main components: cellulose (30-50%), hemicellulose (15-35%) and lignin (10-20%) (Limayem and Ricke ,2012)

2.6.4.1 Cellulose

Cellulose ($C_6H_{10}O_5$) n is made up of a linear chain of D-glucose linkages by β-(1, 4)- glycosidic bonds. This linear chain together makes the cellulose fiber. Due to the intra and intermolecular hydrogen bonds Linked between the cellulose fibers made it insoluble in water and other organic solvents (Singh and Satapathy, 2018). The single molecule structure of cellulose is given in Fig. 1.

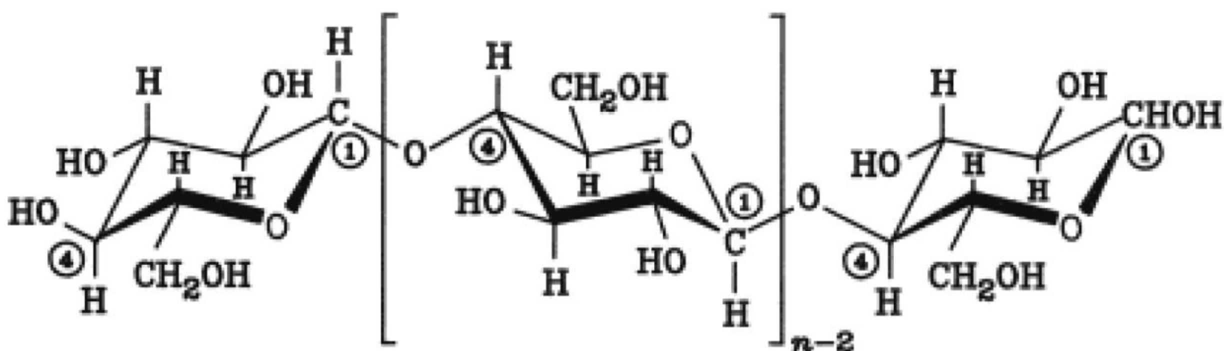

Figure 2.2: Structure of single cellulose molecule Source (Singh and Satapathy, 2018)

2.6.4.2 Hemicellulose

Hemicelluloses ($C_5H_8O_4$) m is heterogeneously branched biopolymers with different pentoses (β D-xylose, α-L-arabinose), hexoses (β-D-mannose, β-D-glucose, α –D-galactose) and some uronic acids (α-D-glucuronic, α-D-4-0-methyle-galacturonic)(Singh and Satapathy 2018).Hemicellulose is a short, highly branched polymer of pentoses (e.g. D-xylose and L-arabinose) and hexoses (e.g. D-manose, D-galactose, and D-glucose) with 50–200 units(Kumar and Reetu, 2015). As compare to cellulose fibers, they are easy to hydrolyse because of their branched structure, amorphous. In the fermentation process, hemicelluloses are more relatively sensible to temperature, retention time and hence must be controlled to avoid the formation of furfurals and hydroxymethyl furfurals, which inhibits fermentation(Singh and Satapathy,2018).

2.6.4.3 Lignin

Lignin $(C_{31}H_{34}O_{11})$ n is the stuff that makes the biomass woody in nature (Singh and Satapathy , 2018). Lignin is present in all lignocellulosic biomass. Therefore, any ethanol production process will have lignin as a residue. It is a large complex polymer of phenylpropane and methoxy groups, a non carbohydrate polyphenolic substance that encrusts the cell walls and cements the cells together. It is degradable by only few organisms, into higher value products such as organic acids, phenols and vanillin (Ã and Hooijdonk , 2005). It is a giant polymer molecule with both aliphatic and aromatic portions synthesized from phenylpropanoid precursors (Fig. 2). In general, lignin is made up of three basic building blocks such as P-counmaryl, coniferyl and sinapyl alcohol(Singh and Satapathy , 2018).

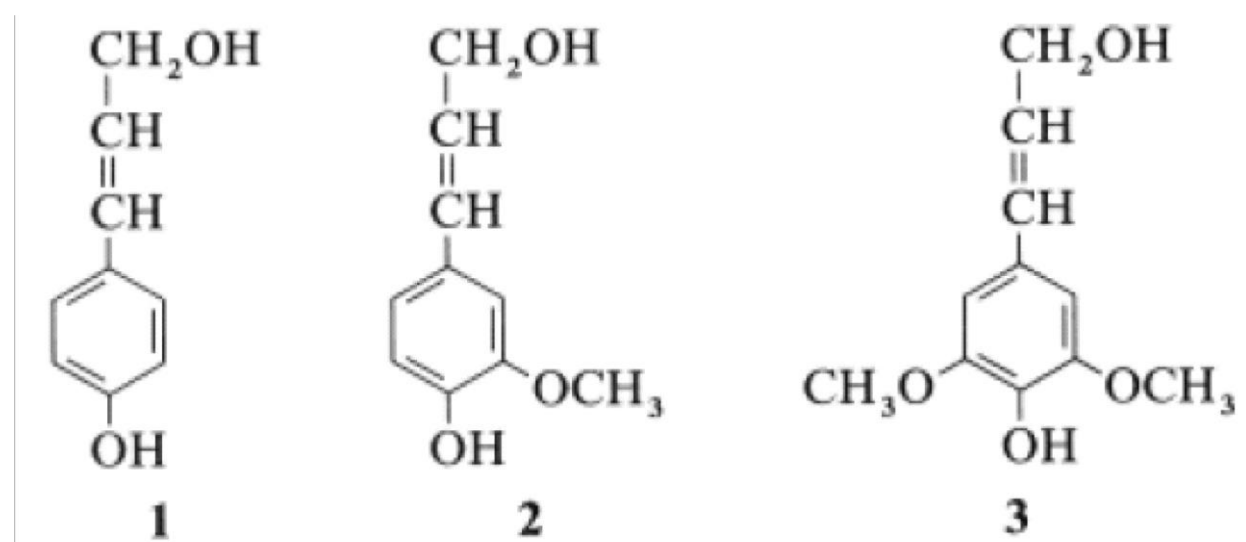

Figure 2.3: P-coumaryl- , coniferyl- and sinapyl alcohol: dominant building blocks of the three dimensional polymer lignin (adopted from (Singh and Satapathy,2018))

Table 2.4: main components of lignocellulosic biomass (adopted from (S I Mussatto and Teixeira , 2010))

Lignocellulose waste	Cellulose (wt %)	Hemicellulose (wt %)	Lignin (wt %)
Barley straw	33.8	21.9	13.8
Corn cobs	33.7	31.9	6.1
Corn stalks	35.0	16.8	7.0
Cotton talks	58.5	14.4	21.5
Ota straw	39.4	27.1	17.5
Rice straw	36.2	19.0	9.9
Rye straw	37.6	30.5	19.0
Soya stalks	34.5	24.8	19.8
Sugarcane bagasse	40.0	27.0	10.0
Sunflower stalks	42.1	29.7	13.4
Wheat straw	32.9	24.0	8.9

2.7 Corn cob as ethanol feedstock's

Corn (maize) is a major food crop in many parts of the world. In Ethiopia, corn is processed to a variety of diets and the maize plant comprises of the stalks, husks, shanks, silks, leaf blades, leaf sheaths, tassels and cobs. Corncobs form about 30% of maize agro-wastes(Zakpaa and Johnson , 2009). Currently the corncobs are burnt as fuel in households of peasant rural farmers. the corn cob carries the grain and together with associating husks, shanks and silks are harvested from the farm (Potentials et al. 2012). Production of bio-ethanol from maize agro waste has been attempted with enzymes from different sources for hydrolysis of lignocellulose and with different organisms for fermentation. 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 hemicelluloses, or glucose from cellulose, and limit formation of degradation products that inhibit the growth of fermentative microorganisms. The lignocelluloses structure is destroyed by treatment with high temperature and saturated steam in a reactor followed by a sudden pressure decrease (Potentials

et al, 2012). Corncobs are a lignocellulosic material composed of cellulose, hemicellulose and lignin. These polymeric fibres consist of monomeric molecules.

Table 2.5: proximate analysis of corn cob (Anukam et al. , 2017)

Constituents	Weight percentage (%wt.dry basis
Moisture content	5.1
Volatility content	65.1
Ash content	8.5
Fixed carbon content	21.3

2.8 Pathways of bio ethanol production from cellulosic feed stocks

Lignocellulosic biomass can be converted into bio ethanol in two different approaches, (i.e. biochemical or thermo chemical conversion) (Limayem and Ricke , 2012). Both approaches involve degradation of the recalcitrant cell wall structure of lignocellulose into fragments of lignin, hemicellulose and cellulose. Each polysaccharide is hydrolyzed into sugars that are converted into bio ethanol subsequent followed by a purification process. The thermo chemical process includes gasification of raw material at a high temperature of 800 ºC followed by a catalytic reaction. Application of high levels of heat converts raw material into synthesis gas (syngas) such as hydrogen, carbon monoxide and CO_2. In the presence of catalysts, the resulting syngas can be utilized by the microorganism Clostridium ljungdahlii to form ethanol and water can be further separated by distillation (Limayem and Ricke, 2012).where as the thermo chemical approach, biochemical conversion involves physical (i.e. size reduction) or/and thermo-chemical with possible biological pretreatment. Biochemical pretreatment is mainly used to overcome recalcitrant material and increase surface area to optimize cellulose accessibility to cellulases. Overall, biochemical approaches include four unit-operations namely, pretreatment, hydrolysis, fermentation and distillation (Limayem and Ricke , 2012). Currently the biochemical approach is the most commonly used process.

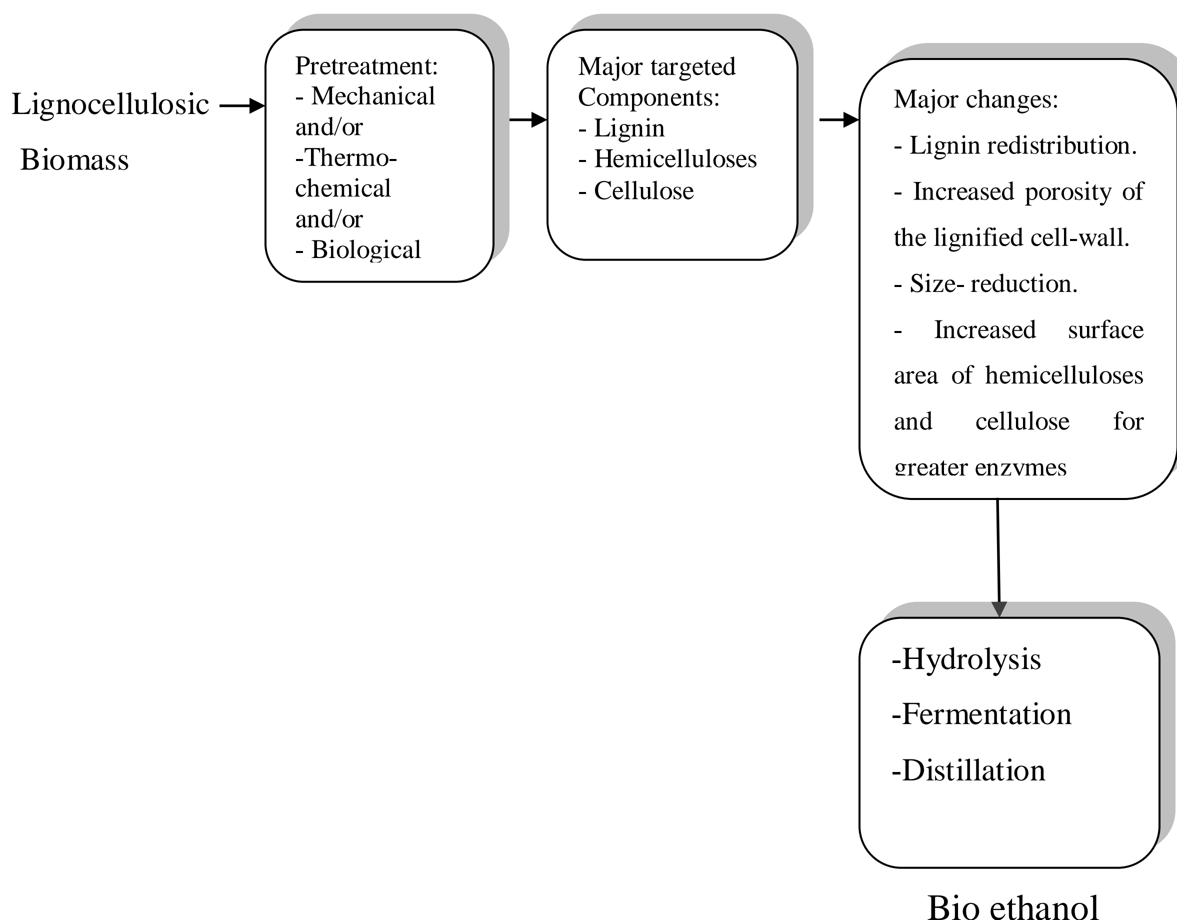

Figure 2.4: schematic diagram of lignocellulosic biomass

2.8.1 Pretreatment

The fundamental step in bioconversion of lignocellulosic biomass to bio ethanol is size reduction and pre treatment. The objective of pre-treatment technology is to alter or remove structural and compositional impairments in order to improve the rate of enzyme hydrolysis and increase yields of fermentable sugars from cellulose and hemicelluloses (Mahapatra and Manian , 2016). Pre-treatment has been viewed as one of the most expensive processing steps within the conversion of biomass to fermentable sugars. During pre-treatment, the matrix of cellulose and lignin bound by hemicelluloses chains needs to be broken in order to reduce the degree of crystallinity of cellulose and increase the fraction of amorphous cellulose. Amorphous cellulose is the most suitable form for enzymatic attack. Additionally, the main part of hemicellulose should be hydrolyzed and lignin should be released or degraded (Mahapatra and Manian, 2016). The purpose of pre-treatment is to remove lignin and the hemicellulose, reduce cellulose crystallinity

and increase the porosity of the materials. Pre-treatment must also meet the following requirements: (1) improve the formation of sugars or the ability to subsequently form sugars by enzymatic hydrolysis, (2) avoid the degradation or loss of carbohydrate, (3) avoid the formation of by-products inhibitory to the subsequent hydrolysis and fermentation processes, (4) to reduce energy demands, and (5) be cost-effective (Mahapatra and Manian , 2016). There are different methods for pretreatment technology of lignocellulosic biomass prior to enzymatic hydrolysis. These methods could be classified into Physical pretreatment, Physico-chemical pretreatment, Chemical pretreatment, and Biological pretreatment (Singh and Satapathy , 2018).

2.8.1.1 Physical pretreatment

Physical pretreatment is the process of applying mechanical force such as ball milling, two roll milling, hammer milling, colloid milling, vibrato energy milling, chipping, grinding irradiation by gamma rays, electron beam or microwaves etc. to the lignocellulosic biomass to reduce its size. This process provides more surface areas, decrease degree of polymerization of cellulose molecules and most importantly decrystallisation(Singh and Satapathy , 2018). The size of the materials is usually 10–30 mm after chipping and 0.2–2 mm after milling or grinding. Vibratory ball milling has been found to be more effective in breaking down the cellulose crystallinity of spruce and aspen chips and improving the digestibility of the biomass than ordinary ball milling (Sun and Cheng , 2002).

Pyrolysis:

Pyrolysis is one of the physical pretreatment processes where less input of energy is required. In this process the materials are treated at a temperature greater than 300 °C, whereby cellulose rapidly decomposes to produce gaseous products such as H_2 and CO and residual char. The decomposition is much slower and less volatile products are formed at lower temperatures (Sarkar et al. , 2012). Mild acid hydrolysis (1 N H_2SO_4, 97 °C, 2.5 h) of the residues from Pyrolysis pretreatment has resulted in 80–85% conversion of cellulose to reducing sugars with more than 50% glucose(Mahapatra and Manian , 2016).The process can be enhanced with the presence of oxygen.

2.8.1.2 Physico-chemical pretreatment

Combined chemical and physical treatment systems are of importance in dissolving hemicellulose and alteration of lignin structure, providing an improved accessibility of the cellulose for hydrolytic enzymes (Mtui,2009) . The most successful physicochemical pretreatments include thermo chemical treatments such as steam explosion or (steam disruption), liquid hot water (LHW), ammonia fiber explosion (AFEX) and CO2 explosion (Mtui ,2009). In these processes, chipped biomass is treated with high pressure saturated steam, liquid ammonia or CO_2 and then the pressure are swiftly reduced, making the materials to undergo an explosive decompression.

Steam explosion (auto hydrolysis):

Steam explosion is typically initiated at a temperature of 160 – 260°C (corresponding pressure of 0.69 – 4.83 MPa) for several seconds to a few minutes before the material is exposed to atmospheric pressure. The processes cause hemicellulose degradation and lignin transformation due to high temperature, thus increasing the potential of cellulose hydrolysis (Mtui ,2009). The hydrolysis of hemicellulose into glucose and xylose monomers is carried out by the acetic acid formed from the hemicellulose acetyl groups during this pretreatment (Ecology et al. 2017). A number of factors such as resistance time, the size of biomass, moisture content and temperature affect the pretreatment. The presence of H_2SO_4, CO_2 or SO_2 as a catalyst can enhance the actual efficiency of this process. Without these catalysts, the acidic catalyst has been found most effective for minimized the production of inhibitor compounds, recovery of hemicellulose sugar and better enzymatic hydrolysis. This pretreatment has been found effective for agricultural residue and hardwoods pretreatment.

The advantages of steam explosion pre-treatment include low energy requirements compared to mechanical comminution and no recycling or environmental costs. Conventional mechanical methods require 70% more energy than steam explosion to achieve the same size reduction (Mahapatra and Manian, 2016). Limitations of steam explosion include destruction of a portion of the xylan fraction, incomplete disruption of the lignin-carbohydrate matrix, and generation of compounds that may be inhibitory to microorganisms used in downstream processes. Because of the formation of inhibitory products that inhibit microbial growth, enzymatic hydrolysis and fermentation, pre treated biomass needs to be washed by water to remove the inhibitory materials along with water soluble hemicellulose (Mahapatra and Manian, 2016).

Liquid hot water:-

Liquid hot water, also known as hot compressed water and as its name indicates, water is used at high pressure up to 5 MPa and high temperature 170–230 °C instead of steam(Ecology et al. 2017). Bagasse, corn stalk and straws of wheat, rice and barley pretreated by liquid hot water have been reported to effect 80 - 100% hemicellulose hydrolysis, resulting to 45 - 65% xylose (Mtui 2009). The advantages of liquid hot water include low-temperature requirement, no inhibitory compounds formation at high temperature, and low-priced solvent of liquid hot water process (Ecology et al. ,2017).

Ammonia fiber explosion (AFEX):

It is one of the alkaline Physico chemical pretreatment processes. In this treatment, biomass is exposed to hot liquid ammonia at 90- 100 ºC for 30 min under high-pressure and then the sudden release of pressure disrupts the structure of LCB leading to increasing digestibility and simultaneously delignifying it (Singh and Satapathy 2018). This process can modify or effectively reduce lignin content in biomass without disturbing hemicellulose and cellulose fractions. The optimum condition for pretreatment of LCB by AFEX process varies according to nature of the material. For example, in switch grass optimum conditions of pretreatment were 100 ºC, ammonia loading of 1:1 kg of ammonia per kg of dry matter and 5 min retention time. It has been applied to various lignocellulosic raw materials like rice straw, municipal solid wastes, newspaper, sugar beet pulp, sugar cane bagasse, corn stover, switchgrass, miscanthus, apsen chips, etc. AFEX works only moderately and is not attractive for biomass that contains high amounts of lignin. Since grasses contain relatively lower amounts of lignin (15-20%) than hardwood and softwood (20-35%), grasses can be more easily digested by AFEX treatment (Mahapatra and Manian ,2016).

2.8.1.3 Chemical pretreatment of lignocellulosic biomass

Acid hydrolysis:-

One of the oldest and most used conversion technologies for lignocellulose to fermentable sugars was acid hydrolysis. There were two basic types of acid hydrolysis: dilute and concentrated acid hydrolysis. Both the process of concentrated and dilute sulfuric acid was carried out at high temperatures (373 and 495 K). Such conditions could degrade sugars, reducing the carbon source and ultimately reduced the efficiency of bio ethanol (Sławik,2014). There are different acids

which can be used in the pretreatment of LCB. For example, sulphuric acid and phosphoric acid are broadly used for treating LCB because of its efficient in hydrolyzing celluloses. Similarly, hydrochloric acid and nitric acid are used and have better cellulose to sugar conversion rate than sulphuric acid (Singh and Satapathy,2018). However, both acids are more expensive than sulphuric acid.

Dilute acid pretreatment (DAP):- Industrially made acids can be diluted and used in the pretreatment of biomass for ethanol production. These acids include H_2SO_4, HNO_3, and HCL. Different concentration of acids could be used however, 0.2-2.5% w/w is used for treating the LCB in high temperature at 120-210 ^oC and pressures (Singh and Satapathy, 2018). DAP is effective in terms of low acid consumption and process severity. Another advantage is that low acid concentration releases essential nutrients such as sulphur and phosphorus that enhance downstream fermentation of sugars to ethanol. Sometimes two stages processes could be used where both dilute and concentrated acid treatment have to perform. In such case, most of the hemicelluloses solubilized in dilute acid and celluloses hydrolyzed in concentrated acid (Singh and Satapathy, 2018). Pretreatment with dilute sulfuric acid was the hydrolysis of hemicellulose and making cellulose more available for enzymatic hydrolysis. Pentose was degraded more rapidly than hexose. To decrease sugar degradation was two-stage process. The first stage was conducted under mild process conditions to recover pentose. The second stage was conducted under harsher conditions to recover the hexose (Sławik, 2014). Dilute acid hydrolysis was probably the most commonly applied method among the chemical pretreatment methods

Concentrated acid pretreatment (CAP): - Concentrated acids such as H_2SO_4 and HCl have been used to treat lignocellulosic materials. Among the acids, sulphuric acid is most widely used while other acids such as HCL, HNO_3 (Sławik 2014) and H_3PO_4 were also used in pre treating the biomass. Concentrated acids such as sulphuric (65-86% w/v), hydrochloric (41%) and phosphoric (85% w/w) were generally used in pre treating the dried LCB (5-10% moisture) at low temperature (30-60 ^oC). Although they are powerful agents for cellulose hydrolysis, concentrated acids are toxic, corrosive and hazardous and require reactors that are resistant to corrosion(Sun and Cheng ,2002). In addition, the concentrated acid must be recovered after hydrolysis to make the process economically feasible.

2.8.1.4 Biological pretreatment:

For the degradation of lignocellulose biomass, natural microorganisms possessing enzymes (bacteria, brown-white and soft rot fungi) are employed that are capable cell wall deconstruction. Biological pretreatment method does not produce any unwanted products as compared to chemical and physical pretreatment. Additionally, high pressure, acids, alkali, high temperature or any reactive species are not compulsory for this pretreatment (Ecology et al., 2017). White- and soft-rot fungi contain lignin-degrading enzymes like lignin peroxidases, manganese-dependent peroxidases, polyphenol oxidases, and laccases which are effective for the lignin degradation. Degradation by microorganisms, mode of action and features depends on targeted biomass component. For example, white and soft-rot fungi are considered most useful for degradation of lignin by using their lignin-degrading enzymes, and brown-rot fungi mainly attack cellulose.

2.9 Fermentation

After pretreatment and hydrolysis have released simple sugars, fermentation is used to turn as much of that sugar as possible into liquid fuel (Schnepf 2010). Fermentation involves microorganisms that use the fermentable sugars as food and produces ethyl alcohol and other by-products. These microorganisms typically utilize 6-carbon sugars like glucose. One of the most effective bio ethanol producing microbes is yeast, *Saccharomyces cerevisiae.* Use of yeast has its advantage owing to its high bio ethanol production from hexoses and its high tolerance to bio ethanol and other inhibitory compounds (Mahapatra and Manian , 2016). Under anaerobic condition S.cerevisiae produces ethanol from hexoses as the overall shows below in equation 2.1 and 2.2, but *S. cerevisiae* cannot utilize the main C-5 sugar - xylose - of the hydrolyzate. Native organisms such as Pichia and Candida species can be used in place of S. cerevisiae and they can utilize xylose but their ethanol production rate is at least fivefold lower than that observed with S. cerevisiae (Sarkar et al.,2012). Different microorganisms have shown different yields of ethanol depending on their monomer utilization.

$$C_6H_{12}O_6 \xrightarrow{yeast} 2C_2H_5OH + 2CO_2 \quad \text{...(2.1)}$$

Theoretically, 100 grams of glucose will produce 51.4 g of bio 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% (Taylor 2006).

2.10 Distillation

Bio ethanol obtained from a fermentation conversion requires further separation and purification of ethanol from water through a distillation process. Fractional distillation is a process implemented to separate ethanol from water based on their different volatilities. This process consists simply of boiling the ethanol-water mixture. Because the boiling point of water (100℃) is higher than the ethanol-boiling point (78.3℃), ethanol will be converted to steam before water. Thus, water can be separated via a condensation procedure and ethanol distillate recaptured at a concentration of 95% (Limayem and Ricke , 2012). Typically, most large scale industries and bio refineries use a continuous distillation column system with multiple effects. Liquid mixtures are heated and allowed to flow continuously all along the column. At the top of the column, volatiles are separated as a distillate and residue is recovered at the bottom of the column.

2.11 Factors Affecting Fermentation

Microorganisms for bio ethanol 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 (Balat , 2011).

2.11.1 Effect of temperature

Temperature has an important factor on the growth rate of the microorganisms and the rate of ethanol production. Wine and beer fermentations are generally conducted below 20°C, whereas higher temperatures (30-38°C) are being examined for industrial alcohol production by yeast cultures (fiseha Amare , 2016). Too high temperature kills yeast, and low temperature slows down yeast activity and growth. Thus, specific range of temperature is required (Onuki 2016).

All the recombinant strains are mesophilic organisms and have best function between 30 to 38 °C. Operating at greater temperatures is desirable for the following reasons: High fermentation temperature increases growth rate and productivity exponentially, Plant capital cost is less due to higher productivity per unit volume of ferment or vessel and cooling equipment investment is lowered.

Operating costs are less since less energy is required to maintain desired fermentation temperature and recover the ethanol. Contamination risk is less as fewer organisms exist at high temperatures.

2.11.2 Effect of pH

A very important factor for cellular growth is external pH. Most alcoholic yeast fermentations are conducted below pH 4.5, although this may not be the optimal pH for growth or ethanol production. Yeast cultures can grow over a wide range from 3 to 8 with an optimum for growth generally in the slight acidic range. Shifts in pH can also affect the final ratio of organic waste products produced by yeast cultures. Thus, the optimal pH for a fermentation process must support a balance among ethanol production, cellular growth, and physicochemical effect on waste product pathways. Low pH values in yeast fermentation help to inhibit growth of contaminating bacterial cultures. Bacterial cultures generally have a pH optimum around 7-7.5, with less tolerance than yeast to acid conditions.

2.11.3 Ethanol concentration

Concentration of ethanol in the fermentation broth can directly affect the growth rate of the culture and its ability to convert sugar to ethanol. Inhibitory and toxicity level of ethanol vary from culture to culture. Higher temperature lowers the tolerance of the organism. At temperatures above 35 °C, current strains lose viability at ethanol concentrations of 10 % (w/v) (Hettenhaus, 1998).

3. METHODOLOGY

3.1 Materials

The materials used to run all the experiments were listed below

Equipments

Plastic bags to collect and transport samples to the laboratory, knife for cutting the corn cob in to pieces, oven to dry the sample, crushers to crush the dried sample, Sieves to sieve the crushed sample to the particle size of 2mm. vacuum, balances to weigh samples. digital pH meter to measure the pH of the hydrolyzate before fermentation, thermostats to control temperature of the sample under experiment (fermentation and distillation) isothermally at the set point, rack:- to hold samples, vessels to hold samples and additives for hydrolysis, fermentation and distillation experiments, graduated cylinders of different volumes for volume measurement, autoclave (Sanoclave) for sterilization and hydrolysis, pycnometer for density measurement, shaker to shake sample and its additives after hydrolysis and before fermentation and fermentation and distillation set ups to ferment and distill respectively.

Chemicals

Sulfuric Acid (H_2SO_4, (98%, England)), used as a pretreatment and hydrolysis of corn cob sodium Hydroxide (NaOH, min. assay 98% BDH Chemicals Ltd pool England cellulose),) used to adjust the pH of soluble cellulose and hemicelluloses before fermentation, Benedict's solution used to determine reduced sugars, yeast extracts (Agar) ,urea, dextrose sugar, Mg SO_4.7 H_2O, and yeast (*Saccharomyces cerevisiae*) used as media preparation (manufactured in france by S.I. Lesaffre with the strain"safinstant")..

3.1.1 Sample collection

Corn cob sample was collected from SNNP in the town of Wolkite which is the south western region of Ethiopia. Sample preparation process include: manual size reduction (knife cutting), and grinding after the samples was collected. 4kg of corn cob was used for experiment. The corn cob was size reduced to about 125μm size. Sample drying was carried out in oven (100 ℃ for 1hr) to obtain easily crushable material. After drying, the sample was milled. The maximum particle sizes of corn cob sample were 125μm. The sample of larger particle size than 125μm was ground over and over again until all particle size became 125 μm. The sample was kept at low temperature until the next stage of experiment. Grinding of corn cob into powder form

increases the surface area of the sample which enhances the contact between hemicellulose and cellulose with dilute acid to reduce cellulose crystallinity.

Figure 3.1: Corn cob sample

3.2 Characterization of corn cob

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

3.2.1 Determination of moisture content

2 g of the ground corn cob sample was put in crucible, after the crucible has been heated and weighed. The moisture content was determined by oven drying at 105°C for an hour until constant weight was obtained (Bhavsar et al. 2018). The sample was taken from the oven and cooled in a desiccators, and then weighed using digital balance. The percent moisture content was determined using the following formula.

$$\text{Moisture content } (\%) = \frac{w_1 - w_2}{w_1} \tag{3.1}$$

Where; W_1=weight of the sample before drying

W_2=weight of the sample after drying

3.2.2 Determination of volatile content

A crucible was weighed empty, and then 1.5 g sample was put in it. The sample and the crucible were placed in a muffle furnace for 7 min at 950 ^{o}C (Bhavsar et al. 2018). The crucible was

removed from the furnace and placed in a desiccators to cool, then was reweighed. The percent volatile matter content was determined using the formula given below:

$$\text{Volatile content }(\%) = \frac{W_1 - W_2}{W_1} * 100 \quad (3.2)$$

Where W_1=original weight of the sample

W_2=weight of the sample after cooling

3.2.3 Determination of ash content

A crucible was weighed empty, and then 3 g of corn cob sample was put in it and placed in a temperature controlled furnace at 550°C for about 2 hours for proper ashing. The crucible was removed from the furnace and placed in a desiccators to cool, then was reweighed. The percent ash content was determined using the formula:

$$\text{Ash content }(\%) = \frac{W_2}{W_1} * 100 \quad (3.3)$$

Where

W_1=original weight of the sample

W_2=weight of the sample after cooling

3.2.4 Determination 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:

$$FC = 100 - (\%\text{ moisture} + \%\text{ volatile matter} + \%\text{ ash}) \quad (3.4)$$

3.3 Determination of chemical composition of corn cob

3.3.1 Extractives content

2.5 g of dried raw corn cob was loaded into the cellulose thimble. With the Soxhlet extractor set up, 150 mL of acetone was used as solvent for extraction. Residence times for the boiling and rising stages was carefully adjusted to 70 °C and 25 min respectively on the heating mantle for a 4 h run period. After extraction, the sample was air dried at room temperature for few minutes. Constant weight of the extracted material was achieved in an oven at 105°C. The %(w/w) of the extractives content was evaluated as the difference in weight between the raw extractive-corn cob and extractive-free corn cob.

Extractive content (%) = $\frac{M1-M2}{M1}$*100…………………………………….. (3.5)

Where, m1=weight of sample (g)

M2=weight of sample after oven dried

3.3.2 Hemicellulose

1 g of extracted dried corn cob was transferred into a 250 mL Erlenmeyer flask. 150 mL of 500, mol/m^3 NaOH was added. The mixture was boiled for 3.5 h with distilled water. It was filtered after cooling through vacuum filtration and washed until neutral pH. The residue was dried to a constant weight at 105 °C in an oven. The difference between the sample weight before and after this treatment is the hemicellulose content (%w/w) of dry biomass.

Hemicellulose content (%) = $\frac{M1-M2}{M1}$*100…………………………………… (3.6)

Where, m1=mass of oven dried before extraction, g

M2 =mass of oven dried after extraction, g

3.3.3 Lignin

0.3 g of dried extracted raw corn cob was weighed in glass test tubes and 3 mL of 72% H_2SO_4 was added. The sample was kept at room temperature for 2 h with carefully shaking at 30 min intervals to allow for complete hydrolysis. After the initial hydrolysis, 84 mL of distilled water was added. The second step of hydrolysis was made to occur in an autoclave for 1 h at 121 °C. The slurry was then cooled at room temperature. Hydrolyzate was filtered through vacuum using a filtering crucible.

Lignin content (%) = $\frac{M2}{M1}$*100………………………………………….. (3.7)

Where, M1=mass of oven dried sample before hydrolysis, g

M2= mass of oven dried sample after hydrolysis, g

3.3.4 Cellulose

The cellulose content (%w/w) was calculated by difference, assuming that extractives, hemicelluloses, lignin, and cellulose are the only components of the entire biomass.

Cellulose content (%) = 100-extractive-hemicellulose-lignin…………………………… (3.8)

3.4 Methods

3.4.1 Acid treatment of corn cob powder

According to the (Singh and Satapathy, 2018), dilute acid hydrolysis pretreatment for bio fuel production apply from 0.2 to 2.5 % H_2SO_4 (w/v) at between 120 and 220ºC for 2 to 90 minutes.
In this study dilute sulfuric 1.1% concentration and 80 gram of corn cob powder with a ratio of 1:10(w/v) sample to solution was used and pretreated inside autoclave at a temperature of 130ºC for 60 minutes. After that it was cooled and filtered using filter vacuums.
The residue was washed four times by distilled water to remove sulfuric acid from it till the pH becomes 5-5.5 which is on the recommended interval during pretreatment.

Procedure

80 g of grinded corn cob sample was added in to 1000 ml conical flasks and 1.1 % dilute sulfuric acid concentration was added to the sample. Then the conical flasks capped with the help of aluminum foil. The samples were heated to 130ºC temperature for 45 minutes in a vertical auto clave. Then the samples in the autoclave were removed and cooled after the given time and t temperature. The soluble portion was separated from the non soluble portion by filtration. The filtrate is preserved in another conical flask prepared for this purpose and kept it for fermentation.

3.4.2 Measurement of Reducing Sugars

Benedict's solution for determining of glucose concentration

In this study, the total reduced sugar content through hydrolysis process was investigated by Benedict's solution method. The concentration of total reducing sugar (TRS) content of hydrolyzate which was obtained from hydrolysis was determined using digital spectrophotometer) by measuring absorbance vs. sugar concentration at 540nm wave length. Quantitative Benedict solution and standard glucose solution were used for assays to plot the calibration curve. Benedict's solution is designed to detect the presence of reducing sugars. In hot alkaline solutions, reducing sugars reduce the blue copper (II) ions to brick red copper (I) oxide precipitate. As the reaction proceeds, the color of the reaction mixture changes progressively from blue to green, yellow, orange and red. When the conditions are carefully controlled, the color developed and the amount of precipitate formed depends upon the amount of reducing sugars present. Hence, in most conditions, a sufficiently good estimation of the concentration of glucose-equivalent reducing sugars present in a sample can be obtained.

Standard preparation

A 0.01g/ml standard stock solution of glucose was prepared by dissolving 1gram of glucose in 100.0ml distill water. Working standards were prepared by pipetting 5, 4, 3, 2, 1, 0.5 and 0%. prepare 6 test tubes with 5ml of distill water for each test tubes and dissolve these glucose solution in 5ml distill water of test tubes for each one remains free since the glucose samples are 5 in numbers. Then shake the sample until the glucose is completely dissolve in the distil water. And prepare 6 other test tubs with 2ml of Benedict's solution for each. Then 1 ml of each of the standard solutions were pipetted out and taken into test tube which contains the Benedict's solution. The mixture was kept in water bath at a temperature of 90℃ for 5 minutes after rapid cooling, filtered and then the absorbance was recorded at 540 nm using UV-visible spectrophotometer.

The concentration of sugar in each sample was read from the calibration curve of the standard glucose solution.

$$Y=mx+b \tag{3.5}$$

Where:

Y=absorbance

m = slope, x = concentration and b = intercept

$$CTRSUS = \frac{\text{(absorbance of unknown sample)} - \text{(Y}-\text{intercept)}}{\text{slope}} \tag{3.6}$$

Where:

CTRSUS=concentration of total reducing sugar of unknown sample

The yield of total reduced sugar was calculated from equation

$$Y = C * \frac{V}{M} * 100 \tag{3.7}$$

Where: Y= yield of total reduced sugar

V = liquid volume

M = amount of biomass

Figure 3.2: Sample preparation to determine reducing sugar

Figure 3.3 visible spectrophotometer

3.5 Fermentation

The fermentation process was carried out in a shaker incubator, at different temperature and, stirring rate of 175 rpm, for a 72h. All assays were performed with 10% (v/v) of inoculums. The prepared hydrolyzates were adjusted to pH of different conditions which is optimum for *Saccharomyces cerevisiae* using 2M sodium hydroxide solution.

Media Preparation

The culture medium was prepared in 250 mL test tube by composed of (g/l), Yeast extract (10); Dextrose (20); Urea (5); Mg SO4.7 H2 O (5); Peptone (20).

Procedures in Media Preparation

The media was sterilized at 121 ^{o}C for 15min. After the media was cooled, 0.50 g of *Saccharomyces cerevisiae* were added into 100ml prepared media at 250 mL conical flask. The conical flasks were properly covered with aluminum foil and placed to a shaker incubator for 24 h, at 30 ^{o}C and 200rpm

Figure 3.4: Shaking incubator (media preparation)

3.5.1 Sterilization

The reactor and all the equipment that were used for fermentation purposes were sterilized (autoclaved). The sterilization was carried out at a temperature of 121 ^{o}C for 15 minutes.

The Procedure for Fermentation

The hydrolyzate sample was conditioned at (25, 30, and 35) temperatures. Those temperatures were favorable for fermentation by Saccharomyces *cerevisiae.* pH of the sample was adjusted using 2M NaOH to make solution pH from (3-5.0) to establish a favorable condition for *S. cerevisiae*. The hydrolyzate sample with 10% inoculums was placed into shaker incubator at 25, 30 and 35°C and 175 rpm for 3 days. After 72 h fermentation, the samples were taken out and introduce into distillation to separate the hydrous ethanol.

$C_6H_{12}O_6$ (l) → 2 C_2H_5OH (l) + 2CO_2 (g)……………………………………………..….. (3.8)

A→ 2B +2C……………………………………………………………………...……. (3.9)

Where: A B and C glucose, ethanol and carbon dioxide respectively.

3.5.2 Ethanol Separation

Distillation

After fermentation, we have to make the purity of ethanol higher. Distillation is one of the steps of the purifications. Distillation is the method to separate two liquid utilizing 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. This means that proportion between two materials, in this case ethanol and water can be changed, and still, there are two materials in layers, the liquid and the vapor layers.

3.6 Determination of the Properties of Ethanol

3.6.1 Density and specific gravity test

Empty pycnometer was weighed. The pycnometer was filled with sample (ethanol), the excess was wiped off, the weight was recorded, and the density calculated using the formula:

Density (g/ml) = (Mass)/(volume) or Density $= (M2 - Mo)/(M1 - Mo)$

Where,

M2= mass of empty bottle in (g), M1 = mass of empty bottle + water in (g)

Secondly, distilled water was filled into the pycnometer, weighed and recorded. The specific gravity was calculated using the formula:

Specific gravity (spg) = (density of ethanol)/(density of water)

3.6.2 Viscosity Test

50 ml of ethanol was turned into A-arm of U-tube capillary viscometer through the orifices to the marked point. A sucker was used to lift the sample to the B-arm of the capillary to the marked point. A stop watch was used to regulate the time it took the ethanol to return (fow) to the mark under the B-arm, and the time noted. Viscosity calibration curve was then used to convert viscosity in seconds to centistokes.

3.6.3 Flash point test

The cup in the apparatus was dried. 50ml of sample (ethanol produced) was placed in a brass cup to touch the prescribed mark on the inside of the cup. The cover was then fitted into position on the cup. The Bunsen burner was used to provide heat to the lower side of the apparatus. The heating was adjusted to provide a temp rise of about 7°F per minute, and the sample was continuously stirred. As the sample approach the temperature of the flash, the injector burner was lighted on and then injected into the sample at about 12 seconds interval until a distinct flash was observed within the container and the injector burner put off. At this point the close flash point was noted with the aid of a thermometer. The flash point was then recorded

3.6.4 PH Test.

PH meter was first inserted in a buffer solution to standardize the apparatus then placed into the sample (ethanol) and the readings were obtained.

3.6.5 Yield of Ethanol

Bio ethanol yield from each fermented sample was determined as follows;

$$\text{Yield} = \frac{\text{sample weight}}{\text{mass of sample distillate}} * 100 \quad (3.8)$$

3.6.6 FT-lR determination of Bio ethanol

The functional groups of corn cob bio-ethanol were determined by using prinks Elmer spectrum 65 FT-IR with the help of IR correlation charts in Addis Ababa University, 4kilo campus. The IR spectrum was reported by % transmittance. The wave number region for the analysis was 4000-400cm-1(in the mid-infrared range).

3.7 Data analysis

The experiments were designed to determine the effect of fermentation conditions on the yield ethanol production from corn cob. A fully randomized experimental design was conducted to determine the optimal point. Randomization ensures that the conditions in one run neither depend on the conditions of the previous runs nor predict the conditions in the subsequent runs. Randomization is essential for drawing conclusions from the experiment, in correct, unambiguous and defensible manner. Temperature, substrate conc. and pH were taken as experimental factor. ANOVA was performed using Design expert® (V.11.0.0) trial version.

Response surface methodology (RSM) was extensively used in for experimental data analysis as this model predicts experimental modifications like changes in operational conditions, various processing steps, which ultimately help in designing an experimental setup with minimum requirements and maximum yields. The experiment was done through a combination of values for actual design factors on each level from Design Expert.

A Response Surface Method was employed to provide a scope for improvement and optimization of the designed response which is influenced by various variables. The response variable was fitted to the following second-order polynomial model which is generally able to describe relationship between the responses and the independent variables.

$Y = \beta 0 + \beta 1A + \beta 2B + \beta 3C + \beta 11\ A2 + \beta 22\ B2 + \beta 33\ C2 + \beta 12\ AB + \beta 13\ AC + \beta 23BC$………….(3.9)

Where; Y is predicted response, A, B and C are temperature, substrate conc. and PH.
$\beta 0$ is intercept, $\beta 1$, $\beta 2$, $\beta 3$, are linear coefficient, $\beta 11$, $\beta 22$, $\beta 33$, are squared coefficients, $\beta 12$,

$\beta 13$, $\beta 23$, are interaction coefficients:

Data analysis was carried out by DESIGN EXPERT software@11 (Box-behken) to evaluate the effects of the process variables; temperature, PH and substrate concentration. The response variable was ethanol yield after fermentation. This design of the experiment helps us to optimize of process parameters using Response Surface Methodology (RSM). Significance of the result was set from analysis of variance (ANOVA).

Temperature

To determine the optimum temperature for maximum yield of bio ethanol production by selected isolates after hydrolysis process, each flask containing 100 ml sample of hydrolyzate were inoculated with 10% (v/v) yeast isolates and incubated at a different temperature in between 25 and 40 under stationary conditions. A 10% yeast concentration was selected with a stirring rate of 175 rpm.

To determine the optimum pH for maximum yield of bio ethanol production by selected isolates after hydrolysis process, each flask containing 100 ml sample of hydrolysate were inoculated with 10% (v/v) yeast isolates and incubated over a selected point of temperature with a pH in between 3 and 5 under stationary conditions. A required fermentation process for 10% yeast concentration was achieved at a stirring rate of 175 rpm.

PH

To determine the optimum pH for maximum yield of bio ethanol production by selected isolates after hydrolysis process, each flask containing 100 ml sample of hydrolyzate were inoculated with 10% (v/v) yeast isolates and incubated over a selected point of temperature with a pH in between 3 and 5 under stationary conditions. A required fermentation process for 10% yeast concentration was achieved at a stirring rate of 175 rpm.

Substrate concentration

To determine the optimum substrate concentration for maximum yield of bio ethanol production by selected isolates after hydrolysis process, each flask containing 100 ml sample of hydrolyzate were inoculated with 10% (v/v) yeast isolates, incubated over a selected point of temperature and pH with different substrate concentration between 50 hours and 200 g/l at a stationary conditions. A required fermentation process for 10% yeast concentration was achieved at a stirring rate of 175 rpm.

Table 3.1 Design Summary of factorial designs

Design Summary of Design expert® 11 software	
Study type	Response surface
Initial design	Box-Behnken
Design model	Quadratic polynomial
Run	17
Block	No block

Table 3.2 minimum and maximum value of factor

Factor name	Unit	low	High
Temperature	℃	25	40
Substrate conc.	g/ml	50	200
PH		3	5

4. RESULTS AND DISCUSSION

4.1 Characterization of Corn Cob

4.1.1 Proximate analysis

Moisture content determination of corn cob

Moisture Content was determined according equation 3.1 by continuously putting the measured sample in oven dry until it achieved constant weight.

Weight of sample = 2g, the amount after drying= 0.529 g

Moisture content (%) = $\frac{2-0.529}{2}*100 = 7.4\%$

Moisture content of corn cob which studied by (Anukam et al. 2017) was 5.1% which was less than this studies .this may due to personal error and equipment error.

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 sample of corn cob with higher moisture content needs more heat for moisture vaporization.

Determination of Volatility contents of corn cob

The amounts of volatility content were determined according to equation 3.2

Original weight of sample=1.5 g

Weight of sample after cooling = 0.402 g

Volatility content = $\frac{1.5-0.402}{1.5}*100 = 73.2\%$

Determination of Ash content of corn cob

The amounts of ash contents were determined according to equation 3.3

Original weight of sample=3 g

Weight of sample after cooling = 0.0729 g

Ash content = $\frac{0.0729}{3}*100 = 2.4\%$

Ash content of corn cob which studied by (Anukam et al. 2017) was 8.5 % which was higher than this studies. This may due to personal error and equipment error. Ash is a measure of

inorganic impurities in the corn cob. In this study low ash content of corn cob constituents, so decreasing sludge formation in the ethanol production.

Determination of fixed carbon content of corn cob

The amounts of fixed carbon content were determined according to equation 3.4

Fixed carbon content = (100- moisture content-volatility content-ash content)

Fixed carbon content= 100 -7.4 -73.2 -2.4 =17%

Fixed carbon content of corn cob which studied by (Anukam et al. 2017) was 21.3 % which was higher than this studies. This may due to personal error and equipment error. Finally, fixed carbon 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 corn cob.

Chemical composition of corn cob

Determination of extractives

The amounts of extractives were determined according to equation 3.5

Amount of the sample 2.5g,

Weight of sample after oven dried 1.9625g

Extractive content $= \frac{2.5 - 1.9625}{2.5} * 100 = 21.5\%$

Determination of hemicellulose

The amounts of hemicellulose were determined according to equation 3.6

Amount of the sample 1 g,

Weight of sample after oven dried 0.76 g

Hemi cellulose content $= \frac{1 - 0.76}{1} * 100 = 24\%$

Determination of lignin

The amounts of extractives were determined according to equation 3.7

Lignin content = $\frac{0.055}{0.3} * 100 = 18.33\%$

Determination of cellulose

The amounts of cellulose were determined according to equation 3.8

$$100 - 21.5 - 24 - 18.3 = 36.2\%$$

Small difference was observed on the contents of cellulose, hemicelluloses and lignin compare to the research done by (S I Mussatto and Teixeira 2010) which was 33.7%,31.9%,6.1%

respectively. This difference is expected because the comparison was done between corn cobs that are grown in different field, different geographic location and different weather environment were plants are cultivated.

4.2 Determination of the content of polysaccharides by Benedict solution

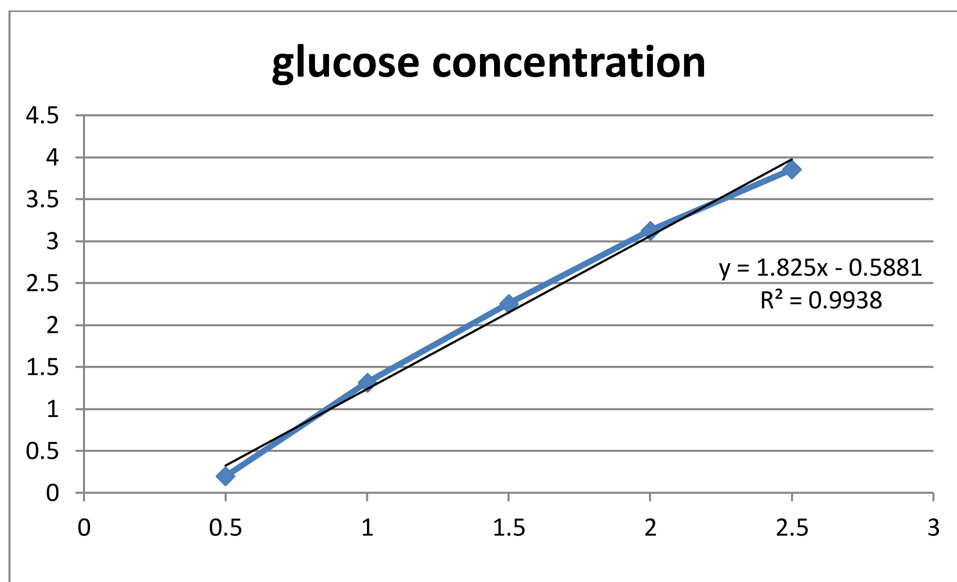

Figure 4.1: glucose conc. vs. absorbance

The concentration of unknown sample from the standard curve and the absorbance values was determined by using equation (3.6). Now by substituting numerical values, the concentration of unknown sample was determined.

$C=\frac{0.889+0.588}{1.825}$=0.809315 g/ml

4.3 Statistical Analysis of the Experimental Results

4.3.1 Analysis of variance (ANOVA)

Table 4.1: The "Model Summary Statistics" table lists other statistics used to compare models

Source	Std. Dev.	R^2	Adjusted R^2	Predicted R^2	Press	
Linear	5.99	0.0367	-0.1856	-0.7528	847.57	
2FI	6.74	0.0594	-0.5050	-2.7362	1806.67	
Quadratic	1.01	0.9852	0.9662	0.7920	100.58	**suggested**
Cubic	0.4919	0.9980	0.9920		*	Aliased

The statistical summary for each model is given in above table. A quadratic model was Suggested compared to a cubic model because it has a higher value of **Adjusted R^2** and **Predicted R^2** and also it is not aliased.

Table 4.2: Result using Design expert® 11 software

Std	Run	Factor 1 A -temperature ℃	Factor 2 B- Substrate conc. g/l	Factor 3 C –PH	Response 1 Yield %
6	1	40	125	3	32.65
3	2	25	200	4	29.5
16	3	32.5	125	4	42.1
15	4	32.5	125	4	42.8
8	5	40	125	5	32
10	6	32.5	200	3	42
2	7	40	50	4	33.8
13	8	32.5	125	4	43
14	9	32.5	125	4	42
4	10	40	200	4	32.7
5	11	25	125	3	34.5
7	12	25	125	5	29
12	13	32.5	200	5	39.7
1	14	25	50	4	32
17	15	32.5	125	4	43
11	16	32.5	50	5	44
9	17	32.5	50	3	42

To determine whether or not the quadratic model is significant, it was crucial to perform analysis of variance (ANOVA), table 4.3 the probability (P-values) values were used 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. From Table 4.3 it was observed that the Values of “Prob > F” less than 0.0500 indicate model terms are significant. In this case A, B, C, AC, BC, A^2 are significant model terms.

The coefficient for the linear effect of temperature, substrate concentration and PH was highly significant. It was also observed that there is an interaction effect between temperature and substrate concentration

Table 4.3: Analysis of variance (ANOVA) for Response Surface Quadratic Model

source	Sum of squares	df	Mean square	F-value	P -value	
Model	475.44	7	67.92	75.32	<0.0001	Significant
A-temperature	4.73	1	4.73	5.24	0.0478	
B–substrate conc.	7.80	1	7.80	8.65	0.0165	
C -PH	5.20	1	5.20	5.77	0.0398	
AB	0.4900	1	0.4900	0.5434	0.4798	
AC	5.88	1	5.88	6.52	0.0310	
BC	4.62	1	4.62	5.13	0.0498	
A^2	446.72	1	446.72	495.37	<0.0001	
Residual	8.12	9	0.9018			
Lack fit	7.15	5	1.43	5.91	0.0550	Not significant
Pure error	0.9680	4	0.2420			
Cor total	483.56	16				

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 75.32 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. Values greater than 0.1000 indicate the model terms are not significant. The "Lack of Fit F-value" of 5.91 implies the Lack of Fit is not significant relative to the pure error. There is a7.09 % chance that a "Lack of Fit F-value" this large could occur due to noise.

Table 4.4 : Regression coefficients and the corresponding 95% CI High and Low

Factor	Coefficient Estimate	df	Standard error	95%CI Low	95%CI High	VIF
Intercept	42.29	1	0.3165	41.57	43.00	
A -temperature	0.7687	1	0.3357	0.0092	1.53	1.0000
B–substrate conc.	-0.9875	1	0.3357	-1.75	-0.2280	1.0000
C –PH	-0.8062	1	0.3357	-1.57	-0.0467	1.0000
AB	0.3500	1	0.4748	-0.7241	1.42	1.0000
AC	1.21	1	0.4748	0.1384	2.29	1.0000
BC	-1.07	1	0.4748	-2.15	-0.0009	1.0000
A^2	-10.27	1	0.4614	-11.31	-9.23	1.0000

The regression coefficients and the corresponding 95% CI (Confidence Interval) High and Low were presented in table 4.4 above. If zero was in the range High and Low 95% Confidence interval, the factors has no effect. From the 95% CI High and Low values of each model term, it could be concluded that the regression coefficients of temperature, PH and the interaction terms of temperature and substrate concentration have highly significant effect in ethanol production

By the designed experimental data from table 4.4, the quadratic polynomial model for ethanol production from corn cob by fermentation was retreated and shown as below:

Final Equation in Terms of Coded Factors

Ethanol yield $=+42.29+0.7687*A-0.9875*B-0.8062*C+0.3500*AB+1.21*AC-1.07*BC-10.27*A^2$……………………………………………………………………………………4.1

The equation in terms of actual factors can be used to make predictions about the response for given levels of each factor. Here, the levels should be specified in the original units for each factor. This equation should not be used to determine the relative impact of each factor because the coefficients are scaled to accommodate the units of each factor and the intercept is not at the center of the design space

Table 4.5: Model adequacy measures

Std .Dev	0.9496	**R^2**	0.9832
Mean	37.46	**Adjusted R^2**	0.9702
Cv %	2.54	**Predicted R^2**	0.9003
		Adeq precision	21.9728

The Predicted R^2 of 0.9003 is in reasonable agreement with the Adjusted R^2 of 0.9702; i.e. the difference is less than 0.2. Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 21.973 indicates an adequate signal. This model can be used to navigate the design space.

Since the R^2 value is closer to 1.0 it indicates that the regression line perfectly fits the data. Similar to that in this investigation, R^2 obtained was 0.9832, which was close to 1. Results imply that the predicted values were found to be in good agreement with experimental values (R^2= 0.9832and Adj-R2= 0.9702), indicating the achievement of the RSM. The model's goodness of fit was checked by regression coefficient (R^2). In this case, the value of the coefficient (R^2 =0.9832) from Table 4.5 indicated that only 1.68% of the total variance was not explained by the developed regression model. The obtained R^2 values suggest good adjustments to the experimental results. The adjusted determination coefficient (Adj-R^2= 0.9702) was also satisfactory for confirming the significance of the model. Pred R-Squared indicating that the model will probably explain a high percentage (about 90.03%) of the variability in new data. "Adeq precision" measures the signal to noise ratio. a ratio greater than 4 is desirable. In this study 22.403 indicates an adequate signal.

Diagnostic plot

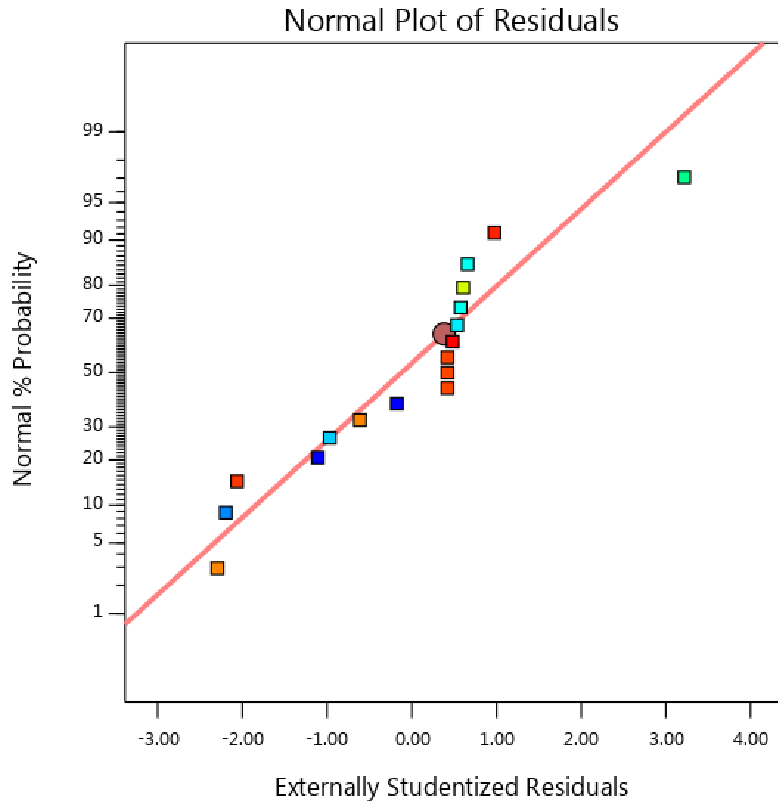

Figure 4.2 Normal plots of residuals

From the plot as shown above, the normal probability plot indicates the residuals following by the normal % probability distribution, in the case of this experimental data the points in the plots shows fitted to the straight line in the figure, this shows that the quadratic polynomial model satisfies the assumptions analysis of variance (ANOVA) i.e. the error distribution is approximately normal.

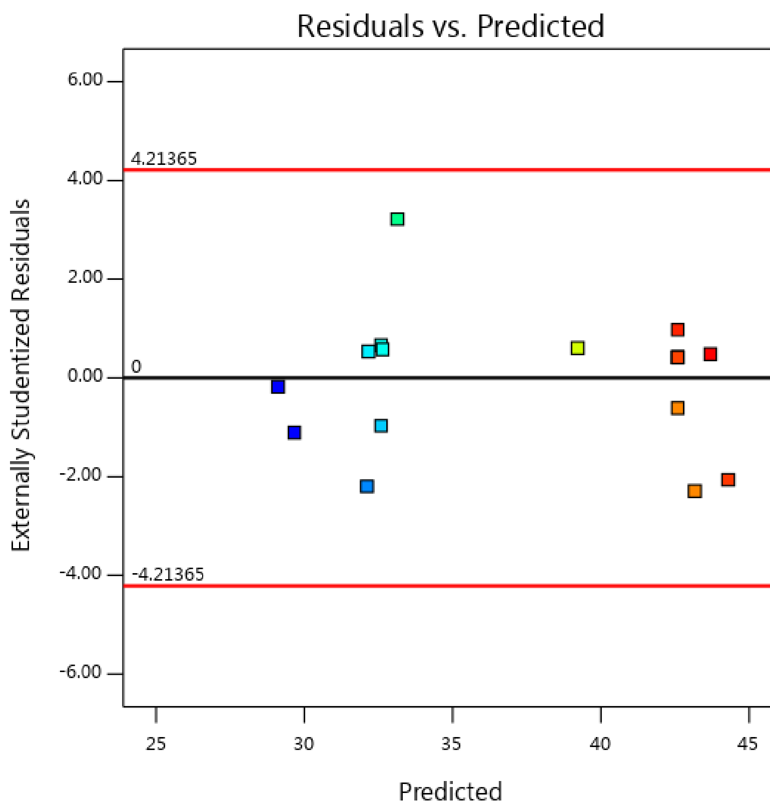

Figure 4.3: Residual versus predicted values

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.

4.3.2 Effects of experimental variables on fermentation

Ethanol production can be affected by many parameters. 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 shown below as a function of the interactions of any two of the variables by holding the other value of the variable at middle. For the interaction figures, black and red line indicates low and high level of parameters respectively.

Design-Expert® Software
Factor Coding: Actual

yield (%)
Design Points
95% CI Bands

Std # 9 Run # 17
X1 = B: substrate conc = 50
X2 = C: PH = 3

Actual Factor
A: temperature = 32.5

C- 3
C+ 5

Y = yield (%) = 42
CI = (41.4176, 44.9102)

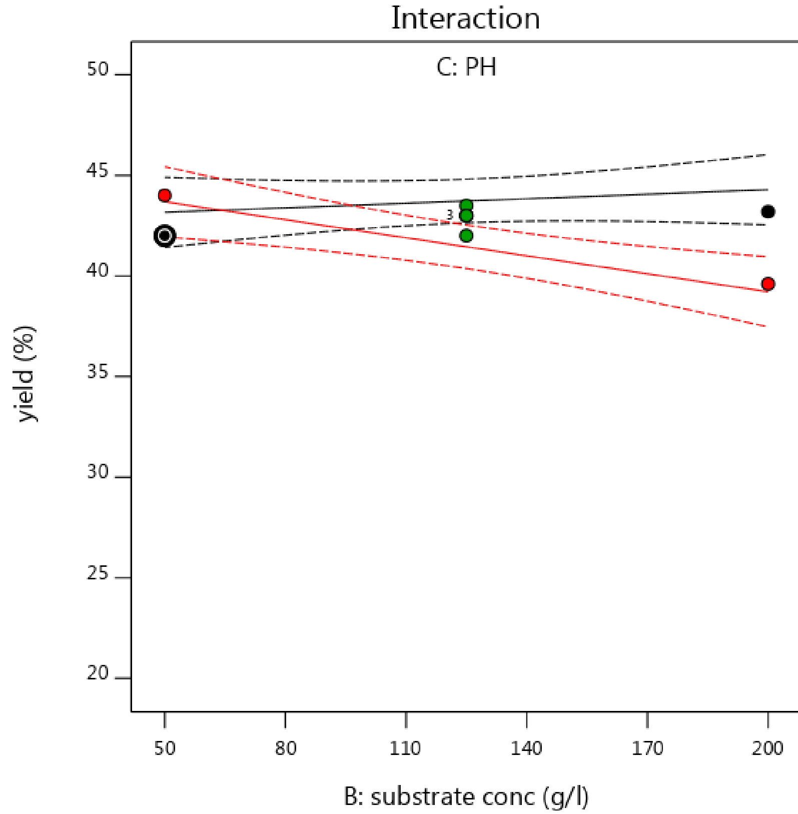

Figure 4.4: The effects of substrate conc. and acid PH on the yield of ethanol, when the temperature was at the center point

Design-Expert® Software
Factor Coding: Actual

yield (%)
Design Points
29 44

X1 = B: substrate conc
X2 = C: PH

Actual Factor
A: temperature = 32.5

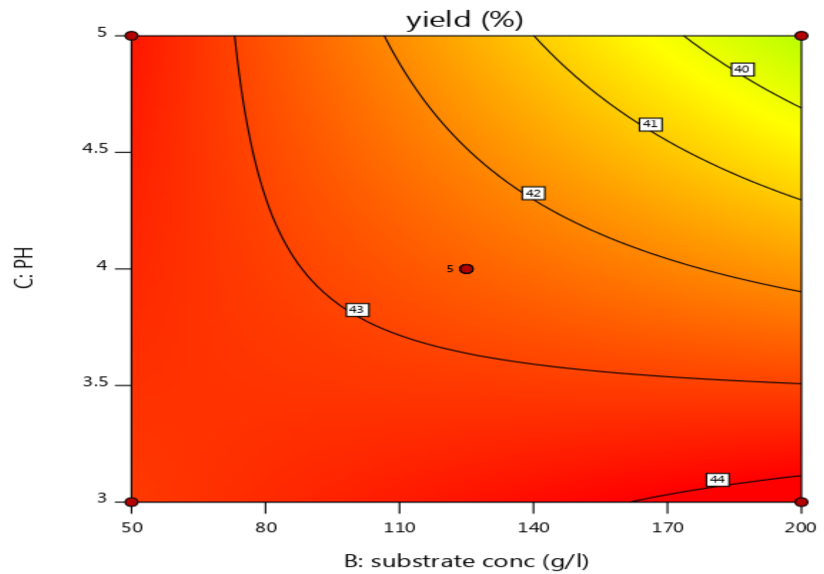

Figure 4.5: Contour plots of the effects of substrate conc. and PH on ethanol yield

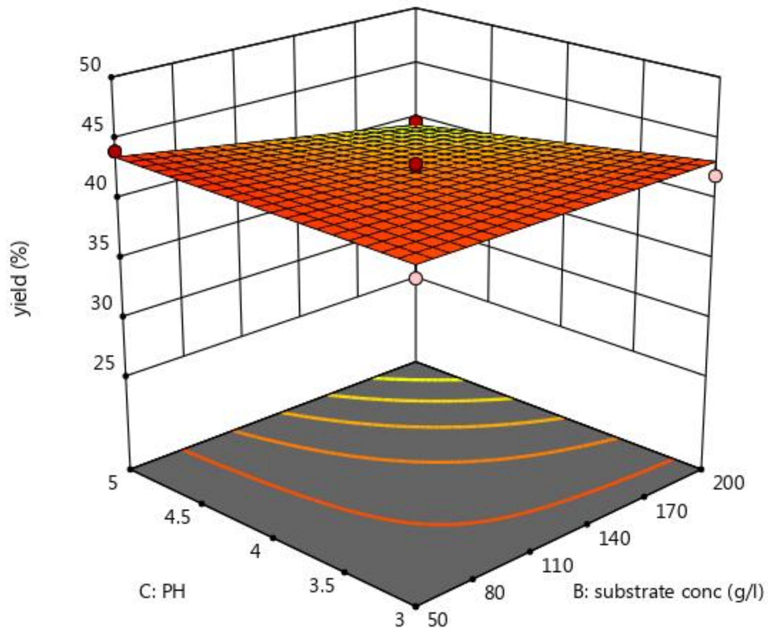

Figure 4.6: Effect of substrate conc. and PH on the yield of ethanol when temperature was at the center point

The effects of substrate concentration and PH on the yield of ethanol, temperature was selected at the center point, are shown in figure 4.4, 4.5 and 4.6 At the lower and higher levels of substrate concentration and PH, the production of ethanol yield level decrease since it has effect of the fermentation medium.

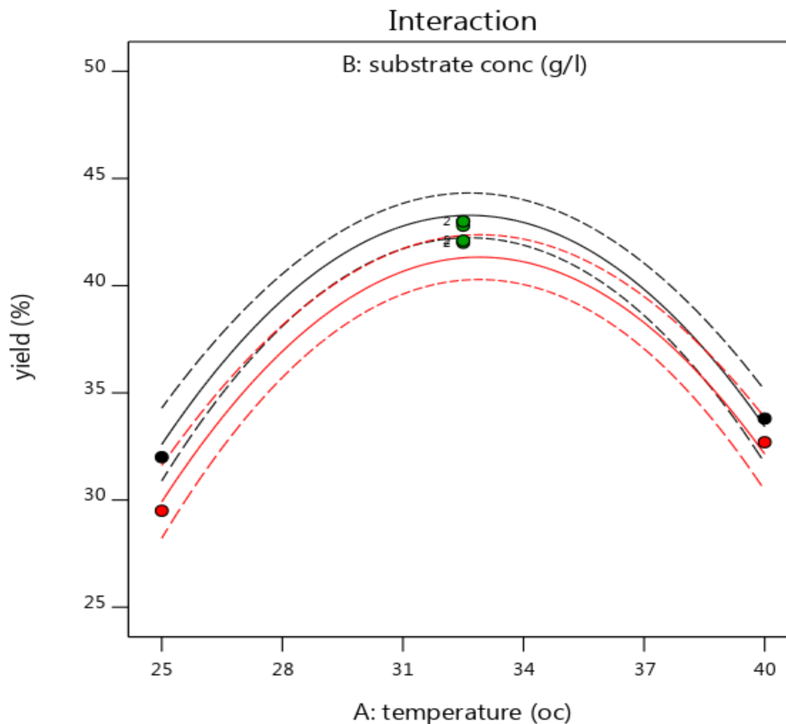

Figure 4.7: Effect of temperature and substrate on the yield of ethanol when PH was at the center point

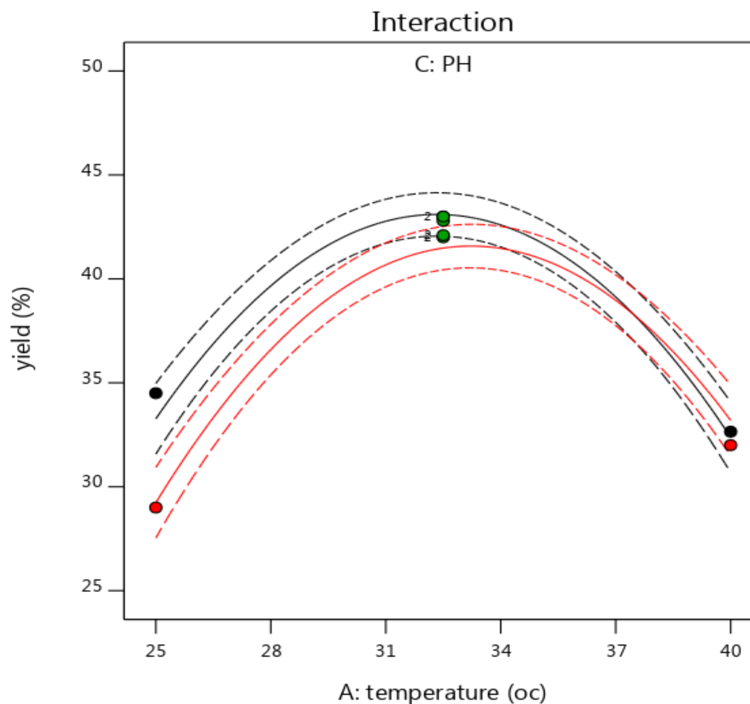

Figure 4.8: Effect of temperature and PH on the yield of ethanol when substrate conc. was at the center

At the lower and higher levels of temperature and substrate concentration, the production of ethanol yield decrease since it has effect of the fermentation medium. At lower temperature and substrate concentration the cellulose might not converted to ethanol and at higher substrate concentration and PH the cellulose might convert to other molecules which might not be fermentable. Hence both temperature and substrate concentration have strong relationship for the yield of ethanol production.

4.3.3 Individual effect of experimental variables on the yield of ethanol

Effect of temperature

Design-Expert® Software
Factor Coding: Actual

yield (%)
Design Points
95% CI Bands

X1 = A: temperature

Actual Factors
B: substrate conc = 125
C: PH = 4

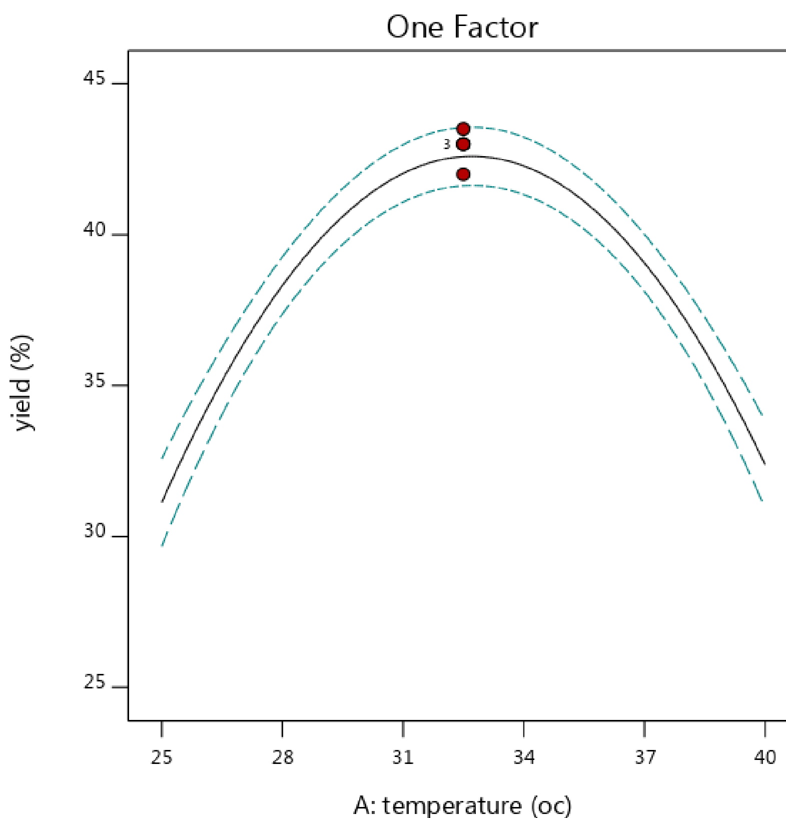

Figure 4.9: Effect of temperature on the yield of ethanol

Figure 4.9 represents the effect of temperature on the yield of ethanol at constant PH and substrate concentration at the center point. As shown on the figure 4.9 the yield of ethanol is very sensible to the temperature. Yield of ethanol was highly increased as temperature increase from 25°C to 32.5°C. Optimum yield of ethanol was obtained around 32.5 °C temperatures. Beyond 32.°C Temperature the yield of ethanol is slightly decreased.

Effect of substrate concentration

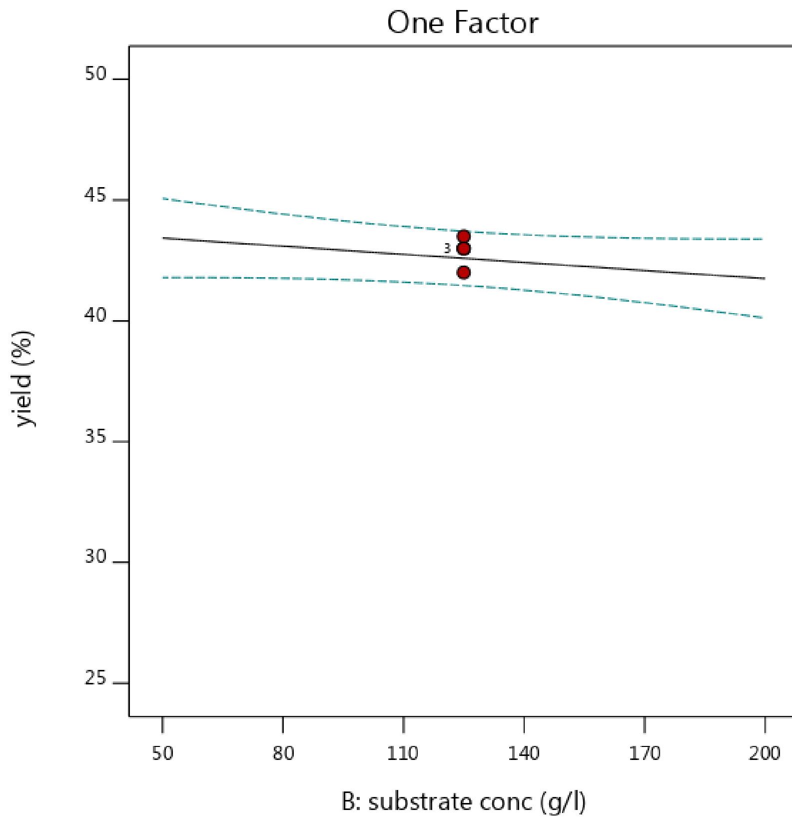

Figure 4.10: Effect of substrate conc. on the yield of ethanol

Figure 4.10 shows the effect of substrate concentration on the yield of ethanol at constant temperature and PH in the center point.

As shown in figure 4.10 above the yield of ethanol was affected slightly by substrate concentration, as the concentration of substrate increase from 50 to 126 the yield slightly increases, beyond 126 the yield of ethanol slightly decreased.

Effect of PH

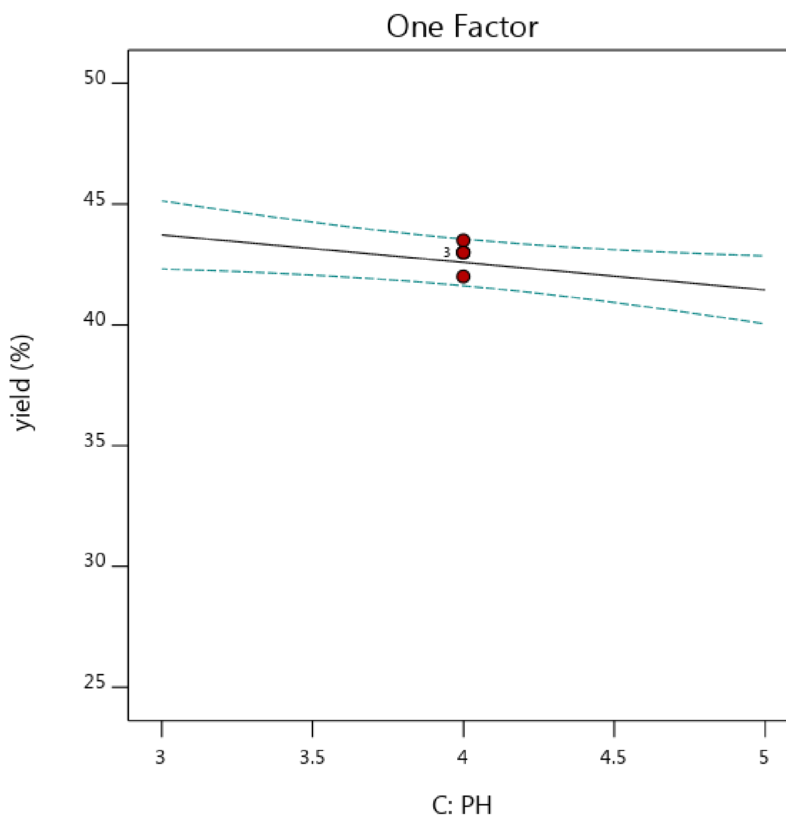

Figure 4.11: Effect of PH on ethanol yield

Figure 4.11 shows the effect of hydrolysis PH on the yield of ethanol at constant temperature and substrate concentration in the center point. As it observe from figure 4.11 above the yield of ethanol is slightly affect by PH, as the PH increase from 3 to 4 the yield slightly increase. Beyond PH 4 the yield of ethanol slightly decreases.

4.4 Optimization of operating process variables in fermentation process using RSM

Response surface methodology (RSM) is a collection of statistical and mathematical techniques useful for developing, improving, and optimizing processes. It also has important applications in the design, development, and formulation of new products, as well as in the improvement of existing product designs. The optimization of fermentation criteria for ethanol production from corn cob are summarized as follows:

Table 4.5: Goal of optimization and limits of process parameters

Parameter	Goal	Lower	Upper
Temperature ℃	In range	25	40
Substrate conc. g/ml	In range	50	200
PH	In range	3	5
Ethanol yield %	Maximum	29	44

Table 4.6: Optimum possible solution

Solution number	Temperature	Substrate conc.	PH	Yield	Desirability	
1	**32.718**	**125.000**	**4.000**	**42.598**	**0.907**	**Selected**
2	32.778	125.001	4.000	42.597	0.906	
3	32.649	125.002	4.000	42.597	0.906	
4	32.727	125.553	4.000	42.592	0.906	
5	32.536	125.000	4.000	42.592	0.906	
6	32.723	125.001	4.008	42.589	0.906	
7	32.760	125.942	4.000	42.587	0.906	
8	32.916	125.506	4.000	42.585	0.906	
9	33.024	125.001	4.000	42.579	0.905	
10	32.879	126.436	4.000	42.577	0.905	
11	32.797	125.002	4.019	42.576	0.905	
12	32.715	127.117	4.000	42.575	0.905	
13	32.709	125.002	4.022	42.573	0.905	
14	32.985	127.613	4.000	42.556	0.904	
15	32.767	130.639	4.000	42.536	0.902	
16	32.791	125.000	4.059	42.531	0.902	
17	32.740	131.337	4.000	42.529	0.902	
18	32.061	125.002	4.000	42.515	0.901	
19	32.737	125.001	4.080	42.509	0.901	

20	33.020	125.000	4.096	42.476	0.898	
21	32.342	125.002	4.086	42.471	0.898	
22	32.634	137.860	4.000	42.455	0.897	
23	32.758	125.001	4.162	42.418	0.895	
24	32.819	141.991	4.000	42.412	0.894	
25	32.370	125.001	4.147	42.406	0.894	
26	31.942	125.000	4.101	42.361	0.891	
27	32.405	148.532	4.000	42.314	0.888	
28	33.027	180.690	4.000	41.990	0.866	
29	34.089	125.000	4.387	41.866	0.858	
30	33.112	267.487	4.000	41.073	0.805	

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 42.592% was found at substrate concentration 125g/ml, temperature 324.536 ℃ and PH 4 and the value of desirability obtained was 90.6%

4.5 FTIR characterization of the produced bio-ethanol

FTIR- Analysis: The Fourier Transform Infrared (FTIR) spectroscopy also deals with quantitative and qualitative analysis of organic samples and recognizes chemical bonds in a molecule by generating an infrared retention range; the spectra generate a profile of the sample, a particular molecular fingerprint that can be utilized to screen and scan samples for a wide range of segments (Anukam et al. 2017). FTIR is an operative analytical instrument for distinguishing functional groups and characterizing covalent bonding data

Table 4.7: Functional groups and respective frequency

Frequency range (Cm^{-1})	Groups	Class of compound	Reference
3303	O-H Stretching	Alcohol ,phenols	(Anukam et al. 2017)
2844	C-H stretching	Alkanes	

1589	C=C bending	Aromatic compound	
1029	C-OH stretching	Alcohol ,phenols, esters	
582	C-H	Aromatic compound	

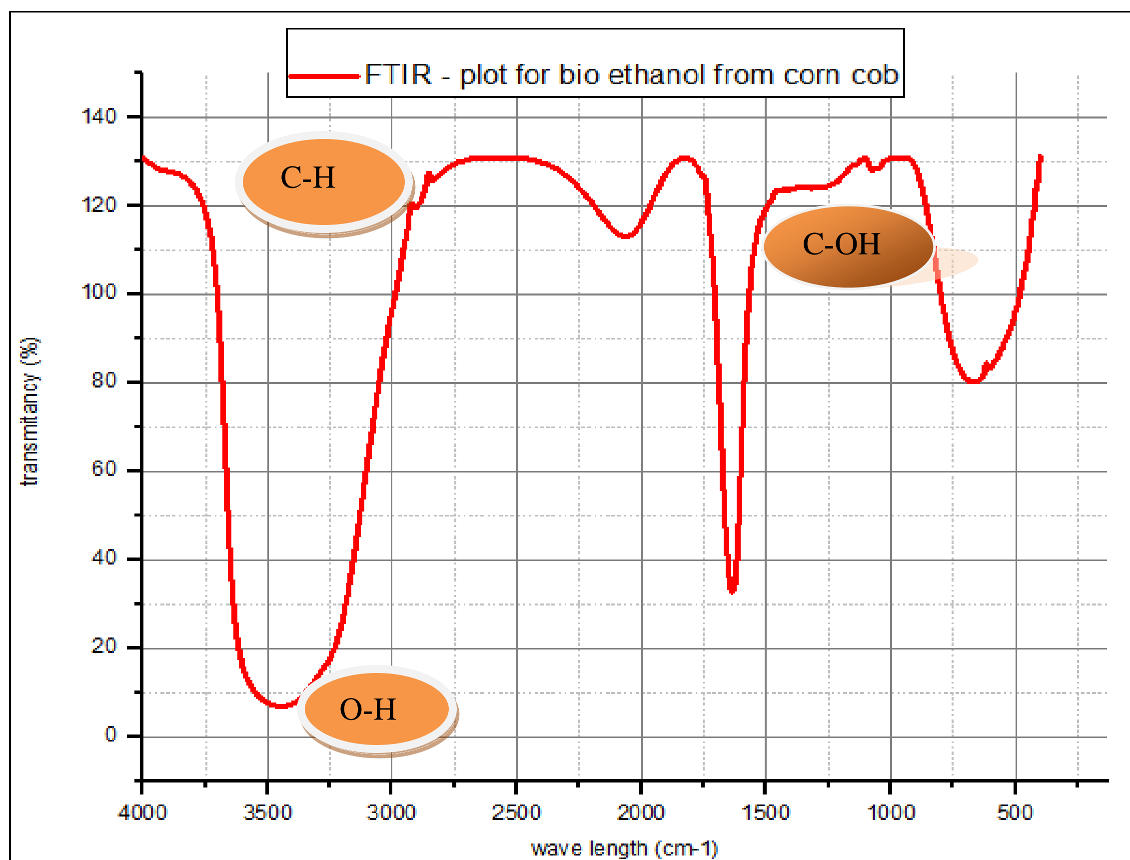

Figure 4.12: FTIR result of the ethanol yield at temperature of 32.718 ℃, for a substrate concentration of 125g/ml and PH of 4

It is quite obvious from Figure 4.12 that the peak at 3303 cm−1 corresponds to O–H stretching vibrations that indicates the presence of hydroxyl groups; while that near 2844 cm−1 depicts C–H stretching that corresponds to the presence of alkanes. 1000 cm−1 depicts C–O stretching, with the peak near 600 cm−1 showing characteristics of C–H bending.

4.6 Characterization of bio ethanol produced

In this study, viscosity, PH, density, flash point and functional group of bio ethanol, produced by separate hydrolysis and fermentation using the culture of *Saccharomyces cerevisiae* was estimated .

Density measurement

The density of bio ethanol, produced by separate hydrolysis and fermentation using the culture of *Saccharomyces cerevisiae* was estimated. The observation recorded was showed that the specific gravity of bio ethanol produced was 0.809 g/ml at temperature of 19.9 ℃

Density of water=1000kg/m^3

$\rho\ of\ ethanol$ =0.809*1000

=809 kg/m3

It is denser than ethanol at room temperature which is 785kg/m3. This due to the presence of water approximately 5% was found in the recovered ethanol due to the formation of an azeotrope, a condition by which the vapor and liquid phase of a mixture are at the same Composition at specific temperature, at 78.℃.

Density is an important parameter for ethanol fuel injection systems. The value of density must be maintained within the tolerable limits to allow optimal air to fuel ratios for complete combustion. High density bio ethanol can lead to incomplete combustion and particulate matter emission. It is important to state that the slight disparity in density observed can be strongly attributed to differences in feedstock used, fermentation process employed and presence of impurities.

Viscosity:

First clean the viscometer by water and dry it then put a certain amount of produced ethanol in the large bulge viscometer and pull it by pipette until the small bulge is full.

Let the ethanol to flow through the capillary tube with run time when the ethanol reaches the mark shown then the viscometer machine shows the result.

Figure 4.13: Viscosity measurement

The viscosity of a fuel must be given significant consideration for fuel injection combustion chambers system. This property is a measure of the resistance of a substance (mostly liquids) to flow.

Kinematic viscosity = = $1.2324*10^{6}$ $m^{2}s^{-1}$

There is small deviation in the value happened when compare with the value the standard value. This may due to personal and experimental error.

Fuels tends to flow with much ease when its viscosity is excessively low such situation usually have adverse effect as the lubricating film between moving and stationary parts in the carburetor or pump are not maintained. On the other hand very high fuel viscosity hinders the atomization the fuel into small droplets to facilitate good vaporization and combustion

Flash point

This is a key property in determining the flammability of a fuel. The flash point is the lowest temperature at which an applied ignition source causes the vapours of fuel to ignite. It is therefore the tendency of a sample to form flammable mixture. The flashpoint of ethanol produced was 17 °C which is shows close proximity to 12-13°C reported in literature. The difference may due to personal error.

5. CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

Corn cob (CC) is promising lignocellulosic feedstock's for production of bio ethanol fuel. It is the most abundant byproduct that available in farm land. This study investigated the potential of corn cob use for production of ethanol via anaerobic fermentation. Chemical characterization of the bio-ethanol produced was performed using FTIR. From the result obtained, it was observed that the ethanol produced from corn cob contains OH, CO, CH2, and CH3 functional groups; which confirm the product is ethanol.

In this study optimization of fermentation parameters were carried out and the effect of the fermentation process variable (temperature, PH and substrate concentration) in the yield of ethanol was investigated and optimized using response surface methodology. Based on analysis of variance (ANOVA) fermentation temperature, PH and interaction between temperature and substrate concentration have significant effect on the yield of ethanol. Positive yield of ethanol was obtained at a high substrate concentration and low temperature as well as at high temperature and low substrate concentration. As the result of RSM optimization at 32.718℃, 4 PH and 125 g/ml fermentation temperature, PH and substrate concentration, respectively resulted in 42.598 % ethanol.

5.2 Recommendations

Producing ethanol from renewable resources is becoming an important issue for the whole world. Therefore, the work needs to be continued for further development of ethanol production from Corn cob by optimizing different parameters in the process.

- ❖ It is , recommend that in this study fermentation variables are optimized; future studies Should include; optimization of pretreatment process, and optimization of distillation process variables to obtain maximum yield of ethanol from corn cob.

- ❖ Further researches have to be carried out to increase the yield of bio ethanol from corn cob by use other microorganisms which are capable of converting Xylose since it can’t be converted by Saccharomyces cerevisiae.

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REFERENCES

Ã, Carlo N Hamelinck, and Geertje Van Hooijdonk. 2005. “Ethanol from Lignocellulosic Biomass : Techno-Economic Performance in Short- , Middle- and Long-Term” 28: 384–410. https://doi.org/10.1016/j.biombioe.2004.09.002.

Aditiya, H B, T M I Mahlia, W T Chong, Hadi Nur, and A H Sebayang. 2016. “Second Generation Bioethanol Production : A Critical Review.” *Renewable and Sustainable Energy Reviews* 66: 631–53. https://doi.org/10.1016/j.rser.2016.07.015.

Anukam, Anthony I, Boniswa P Goso, Omobola O Okoh, and Sampson N Mamphweli. 2017. “Studies on Characterization of Corn Cob for Application in a Gasification Process for Energy Production” 2017.

Badger, P C. 2002. “Ethanol From Cellulose : A General Review,” 17–21.

Balat, Mustafa. 2011. “Production of Bioethanol from Lignocellulosic Materials via the Biochemical Pathway : A Review.” *Energy Conversion and Management* 52 (2): 858–75. https://doi.org/10.1016/j.enconman.2010.08.013.

Bharathiraja, B, J Jayamuthunagai, R Praveenkumar, J Vinotharulraj, and P Vinoshmuthukumar. 2014. “Bioethanol Production from Lignocellulosic Materials - An Overview” 1 (7).

Bhavsar, P A, M H Jagadale, Y P Khandetod, and A G Mohod. 2018. “Proximate Analysis of Selected Non Woody Biomass” 7 (9): 2846–49.

Biosci, Int J, Zulfiqar Ali, Maqsood Hussain, Muhammad Arshad, and Int J Biosci. 2014. “Saccharification of Corn Cobs an Agro-Industrial Waste by Sulphuric Acid for the Production of Monomeric Sugars” 6655: 204–13.

Ecology, Applied, Muhammad Tayyab, Fujian Agriculture, Ali Noman, Saquib Waheed, and Fujian Agriculture. 2017. “Bioethanol Production from Lignocellulosic Biomass by Environment-Friendly Pretreatment Methods : A Review BIOETHANOL PRODUCTION from lignocellulosic biomass by environment-friendly pretreatment methods : a review,” no. December. Https://doi.org/10.15666/aeer/1601.

Fiseha amare. 2016. “addis ababa university addis ababa institute of technology (aait) school of chemical and bio engineering process engineering stream bioethanol production from barley- spent grain (breweries spent grain) addis ababa university addis ababa institute of,” no. June.

Hettenhaus, James R. 1998. “ETHANOL FERMENTATION STRAINS by Sponsored by UNITED STATES DEPARTMENT OF ENERGY Office of Energy Efficiency & Renewable Energy Ethanol Program,” 1–25.

Kefale, Ayele, Mesfin Redi, and Araya Asfaw. 2012. “Potential of Bioethanol Production and Optimization Test from Agricultural Waste : The Case of Wet Coffee Processing Waste (Pulp)” 2 (3): 1–5.

Kumar, Jitendra, and Saini Reetu. 2015. “Lignocellulosic Agriculture Wastes as Biomass Feedstocks for Second-Generation Bioethanol Production : Concepts and Recent Developments,” 337–53. https://doi.org/10.1007/s13205-014-0246-5.

Limayem, Alya, and Steven C Ricke. 2012. “Lignocellulosic Biomass for Bioethanol Production : Current Perspectives , Potential Issues and Future Prospects.” *Progress in Energy and Combustion Science* 38 (4): 449–67. https://doi.org/10.1016/j.pecs.2012.03.002.

Mahapatra, Samhita, and Ramesh Pathy Manian. 2016. “Review Article Bioethanol from Lignocellulosic Feedstock : A Review” 8 (9): 31–41.

Mebrhit, G. 2016. “Production of Bioethanol from Corn Cob.”

Mtui, Godliving Y S. 2009. “Recent Advances in Pretreatment of Lignocellulosic Wastes and Production of Value Added Products” 8 (8): 1398–1415.

Mussatto, S I, and J A Teixeira. 2010. “Lignocellulose as Raw Material in Fermentation Processes,” 897–907.

Mussatto, Solange I, Giuliano Dragone, Pedro M R Guimarães, João Paulo, A Silva, Lívia M Carneiro, Inês C Roberto, António Vicente, Lucília Domingues, and Teixeira, José A. 2010. “Technological Trends , Global Market , and Challenges of Bio-Ethanol Production.” *Biotechnology Advances* 28 (6): 817–30. https://doi.org/10.1016/j.biotechadv.2010.07.001.

Nigatu, Aemiro Nigussie. 2017. “Large-Scale Sugarcane Ethanol Production and Its Implications to Ethiopia Aemiro Nigussie Nigatu.”

Onuki. 2016. “No Title.”

Potentials, Bioethanol, O F Corn, C O B Hydrolysed, and Cellulases Of. 2012. “Original Article : CELLULASES OF ASPERGILLUS NIGER AND,” 468–79.

Rutz, Dominik. 2008. “Biofuel Technology Handbook.”

Sarkar, Nibedita, Sumanta Kumar Ghosh, Satarupa Bannerjee, and Kaustav Aikat. 2012. “Bioethanol Production from Agricultural Wastes : An Overview.” *Renewable Energy* 37

(1): 19–27. https://doi.org/10.1016/j.renene.2011.06.045.

Schnepf, Randy. 2010. "Cellulosic Ethanol : Feedstocks , Conversion Technologies , Economics , and Policy Options."

Singh, Yengkhom Disco, and Kunja Bihari Satapathy. 2018. "Conversion of Lignocellulosic Biomass to Bioethanol : An Overview with a Focus on Pretreatment" 15: 17–43. https://doi.org/10.18052/www.scipress.com/IJET.15.17.

Sławik, L. 2014. "Bioethanol-Production and Utilization," no. October.

Sun, Ye, and Jiayang Cheng. 2002. "Hydrolysis of Lignocellulosic Materials for Ethanol Production : A Review Q" 83: 1–11.

Taylor, Publisher. 2006. "Bioethanol from Cellulosic Materials : A Renewable Motor Fuel from Biomass Bioethanol from Cellulosic Materials : A Renewable Motor Fuel from Biomass," no. June 2013: 37–41. https://doi.org/10.1080/00908310390266643.

Vohra, Mustafa, Shri Vinoba, Bhave Civil, Jagdish Manwar, Rahul Manmode, and Sanjay V Patil. 2013. "Bioethanol Production : Feedstock and Current Technologies," no. November. https://doi.org/10.1016/j.jece.2013.10.013.

Yah, Clarence S, Sunny E Iyuke, Emmanuel I Unuabonah, Odelia Pillay, Chetty Vishanta, and Samuel M Tessa. 2010. "Temperature Optimization for Bioethanol Production from Corn Cobs Using Mixed Yeast Strains Temperature Optimization for Bioethanol Production from Corn Cobs Using Mixed Yeast Strains," no. June. https://doi.org/10.3844/ojbsci.2010.103.108.

Zakpaa, H D, and F S Johnson. 2009. "Production of Bio-Ethanol from Corncobs Using Aspergillus Niger and Saccharomyces Cerevisae in Simultaneous Saccharification and Fermentation" 8 (13): 3018–22.

APPENDICES

Appendix A: Laboratory work pictures

Figure 1: corn cob

fig 2: size reduction equipment

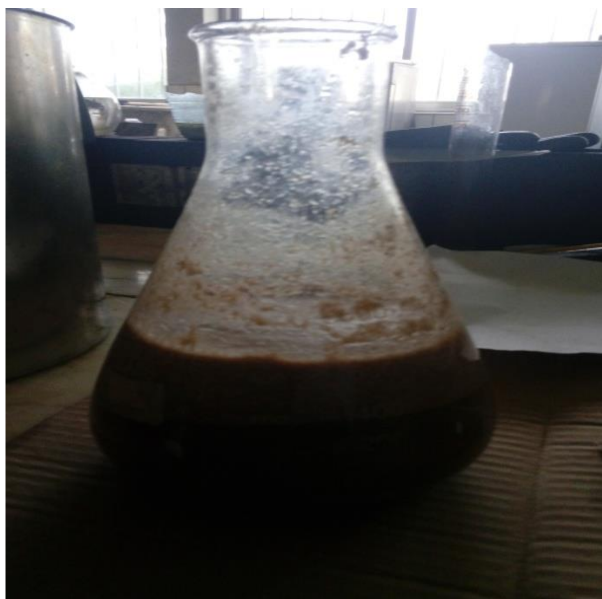

Fig 3 : sample after hydrolysis

Fig 4: sample after hydrolysis

Fig 5: filtration

Fig 6 : ph adjustement

fig 8:test tube for reducing sugar

Fig 7: Autoclave

Figure 8: labratory work

Appendix B: Properties of Ethanol

Density and phase	0.789 g/cm3, liquid
Solubility in water	Fully miscible
Melting point	−114.3 °C (158.8 K)
Boiling point	78.4 °C (351.6 K)
Acidity (pKa)	15.9 (H+ from OH group)
Viscosity	1.200 cP at 20 °C
Dipole moment	1.69 D (gas)

Appendix C: Design Summary of factorial designs

Design Summary of Design expert® 11 software	
Study type	Response surface
Initial design	Box-Behnken
Design model	Quadratic polynomial
Run	17
Block	No block