Addis Ababa University

Addis Ababa Institute of Technology

School of Chemical and Bio-Engineering

Production of Bioethanol from Corn Cob Hydrolysate

Tsegay Gebrejewergs Gebrekidan

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This is to certify that the thesis prepared by Tsegay Gebrejewergs Gebrekidan, entitled "Production of Bioethanol from Corn Cob Hydrolysate" and submitted in partial fulfillment of the requirements for the degree of Master of Science in Chemical Engineering (Process Engineering) complies with the regulations of the university and meets the accepted standards with respect to originality and quality.

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Advisor	Signature	Date
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Internal examiner	Signature	Date
Mr. Teshome Worku	___________________________	_________________
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Tsegay Gebrejewergs Gebrekidan	____________________	______________
Name	Signature	Submission date
Professor Eduardo Ojito Cespedes	____________________	______________
Advisor	Signature	Date

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List of Acronyms

ANOVA	Analysis of variance
CI	Confidence interval
BDFC	B&D Food Corporation
FTIR	Fourier transform infrared spectroscopy
CSA	Central statistical agency
GWh	Giga watt per hour
MSW	Municipal solid waste
NGO	Non-governmental organization
NRE	National Renewable Energy Laboratory
ODW	Oven dried weight
R^2	Regression coefficient
SNNP	South nation and nationalities and peoples
USA	United States of America

Abstract

All societal activities depend up on energy services to meet the daily. The environmental issues, the growing demand for energy, increasing petroleum-based oil prices and the depletion of petroleum created the need for the development of sustainable and safe alternative energy source. Bioethanol is one of the alternative energy sources. In this study of ethanol production from corn cob dilute sulfuric acid method of hydrolysis was employed because it is easy and productive process. The samples were mixed with distilled water in a ratio of 1:10 (w/v) and pretreated using 1% dilute sulfuric acid in autoclave at temperature of 120℃ for 30 minutes. It was hydrolyzed using diluted H_2SO_4 of 2%, 3% and 4% at a temperature of (100 ℃, 120 ℃ and 140 ℃) for (20, 30 and 40) minutes. Sugar concentrations were determined using phenol sulfuric acid method. The maximum, medium and minimum sugar yields were 53.56536, 47.46514 and 41.64815 g/l respectively. Fermentation was conducted using the hydrolysates(sugars) obtained in previous process, inoculum level of (5, 10, 15 %v/v) and temperature of (25, 30, 35 ℃) for 72 hours. Sugar concentration and inoculum level have a statistically significant effect on ethanol yield with p-value of 0.0021 and 0.0068 respectively. Ethanol yield was maximum at middle values of the fermentation parameters and it is negatively affected at their minimum and maximum values. From the statistical analysis maximum ethanol yield of 0.530 mL/g was found at sugar of 4.917 g, inoculum of 12.17 %v/v, and at a temperature of 29.977 ℃ with 89.8 % desirability. In FTIR, it was observed that the ethanol produced from corn cob contains O-H functional group, C-O, -CH_2, and CH_3 groups. Finally, a good yield of ethanol was obtained. So, corn cob is a potential feedstock for ethanol production.

Key words: Energy, Bioethanol, Corn cob, saccharomyces cerevisiae, glucose equivalent, Pretreatment, Hydrolysis, Fermentation, FTIR

1. Introduction

1.1. Background

The overall well-being of the world; industrial competitiveness and the functioning of society are dependent on safe, sustainable and affordable energy (Tesfaye & Gebru, 2011). Energy provides an essential power for almost all human activities: it provides services for cooking, heating, lighting, health, food production and storage, education, mineral extraction, industrial production and transportation(Anonym, 2001). The rapid growth of industries and technological advancement in the world call for development in the chemical sector. The production of industrial chemicals will enhance the economic progress of any nation. Ethanol, one of the important industrial chemicals, can be produced extensively from biomass such as corn cob(Chang et al., 1981).

The world energy consumption has been increasing steadily with population growth and industrialization processes. Since 1900 to the present fossil fuels, e.g., crude oil and natural gas are the predominant energy sources. However, crude oil and natural gas are limited resources that will be depleted sometime in the near future. Although there are debates about the exact year of peak oil production, it is generally believed that it will occur before 2025, after which a decline in worldwide crude oil production will begin(Campbell et al., 1998). Annual global oil production would decline from the current 25 billion barrels to approximately 5 billion barrels in 2050. An increasing demand for energy and inevitable depletion of fossil fuels has stimulated exploration for alternative energy sources (Campbell et al., 1998).

Furthermore, the dramatic increase in the price of petroleum, the finite nature of fossil fuels, increasing concerns regarding environmental impact, especially related to greenhouse gas (GHG) emissions, and health and safety considerations are forcing the search for new energy sources and alternative ways to power the world’s motor vehicles. An alternative fuel must be technically feasible, economically competitive, environmentally acceptable, and readily available.

Even though, world primary energy source nowadays is dominated by fossil fuels (coal, oil and natural gas), the acceleration of technological development will opened the door to continuous and rapid worldwide economic growth and will in fact allowed the world to achieve energy sustainability using many different energy sources called renewable energy sources, comprising mainly biofuel, hydropower, nuclear, geothermal, wind and solar energy, currently represent less percentage of the primary energy use(Anonym, 2001).

Biofuels are renewable, can substitute fossil fuels, reduce fossil greenhouse gas emissions and they can be produced, where they are needed, to reduce the dependence on oil producing countries. The biofuels that are currently in use, known as first generation biofuels, are mainly produced from sugarcane, maize or soy(Goldschmidt, 2008).

Unlike fossil fuels, bioethanol is a renewable energy source produced through fermentation of sugars, and it has been recognized as a potential alternative renewable energy source to petroleum derived transportation fuels. Developing bioethanol from renewable biomass would provide environmental and social benefits(Wyman, 1994).

No other sustainable option for production of transportation fuels can match ethanol made from lignocelluloses biomass with respect to its dramatic environmental, economic and infrastructure advantages. The lignocellulosic materials include agricultural residues, municipal solid wastes (MSW), pulp mill refuse, switch grass and lawn, garden wastes(Pimentel & Patzek, 2005).

Production of ethanol from lignocellulosic biomass seems very attractive and sustainable due to several reasons, among which the renewable and ubiquitous nature of biomass and its non-competitiveness with food crops are the major ones. Another significant factor which adds value as well as importance to lignocellulosic ethanol is the reduction in greenhouse gas emission. The utilization of lignocellulosic biomass for ethanol production necessitates the large-scale production technology to be cost effective and environmentally sustainable(Parameswaran et al, 2011).

Production of ethanol (bioethanol) from biomass is one way to reduce both consumption of crude oil and environmental pollution. Bioethanol is appropriate for the mixed fuel in the gasoline engine because of its high-octane number, and its low cetane number and high heat of vaporization impede self-ignition in the diesel engine. So, ignition improver, glow-plug, surface ignition, and pilot injection are applied to promote self-ignition by using diesel-bioethanol-blended fuel. Conversion technologies for producing bioethanol from cellulosic biomass resources such as forest materials, agricultural residues and urban wastes are under development and have not yet been demonstrated commercially(Mustafa et al., 2008).

Biofuels that are produced from bio-based materials are a good alternative to petroleum-based fuels. They offer several benefits to society and the environment. Producing second generation biofuels is even more challenging than producing first generation biofuels due the complexity of the biomass and issues related to producing, harvesting, and transporting less dense biomass to centralized biorefineries(Balan, 2014).

The Ethiopian Petroleum Supply Enterprise (EPSE) has procured 3.3 million tons of petroleum products with a cost of 37 billion Br during the recently ended fiscal year. The imported volume exceeds the amount during the preceding fiscal year by 10 percent and the cost is seven billion Birr higher than the 2016/17 financial year. The Enterprise, which is the mandated body to buy oil in the country, imported the fuel through the ports of Djibouti and Sudan. It distributes petroleum products to 17 oil companies, which have 800 stations in the country(Addis Fortune, 2017**)**.

Corn is the largest and most productive crop in Ethiopia. In 2007/08, corn production was 4.2 million tons, 40 percent higher than teff, 56 percent higher than sorghum, and 75 percent higher than wheat production. With an average yield of 1.74 tons per hectare (equal to 3.2 million tons grown over 1.8 million hectares) from 1995 to 2008, corn has been the leading cereal crop in Ethiopia since the mid-1990s in terms of both crop yield and production(Kindie et al., 2010).

In Ethiopia there is no proper handling or use of corn cob and there is no second-generation biofuel production. This is a motivation to conduct a research on converting it to bioethanol.

1.2. Problem statement

The effects of global warming are the ecological and social changes caused emissions of greenhouse gases during different activities and by naturally occurring conditions. Nowadays, there is an increasing cost of fuels at an alarming rate, pollution of the environment by the release of greenhouse gases such as CO_2, methane, nitrous oxide and sulfur containing compound and our main energy resource, petroleum oil, is being depleted. (Wongwatanapaiboon et al, 2012).

The commercial production of fuel ethanol in Ethiopia relies mainly on molasses in sugar industries. So, the production of bioethanol from corn cob will be expected to solve the problem associated with energy security.

Ethiopia’s corn production is increasing from year to year as a result of this, huge amount of corn cob is disposed as a waste. This waste has high amount of cellulose that is better than other agricultural wastes such as fruit wastes.

Bioethanol from agricultural waste was produced several times but they are always focused on hydrolysis parameters optimization and people who worked on this recommends to optimize the parameters of fermentation process (which is not adapted well) of bioethanol production. So, this work aims basically on investigating the effects and optimizing the fermentation process variables of bioethanol production from corn cob hydrolysate.

1.3. Objective

1.3.1. General objective

The general objective of this study is to produce bioethanol from corn cob hydrolysate.

1.3.2. Specific objectives

The specific objectives are,

1. Characterization of corn cob (proximate analysis and chemical constituents)
2. Determination of corn cob hydrolysate.
3. To investigate the effects of hydrolysate dose, inoculum and temperature in fermentation process for bioethanol production from corn cob hydrolysate.
4. Product characterization and optimization.

1.4 Significance of the study

This study will have a great significance in making sure of optimized production of bioethanol from non-edible agricultural waste (corn cob) which is abundantly available in Ethiopia. Bioethanol production from corn cob is considered a 2nd generation biofuel process since it has no direct conflict with human food, as the case of 1st generation biofuels produced from agricultural crops, such as corn and sugarcane. So that need to increase domestic ethanol production and to further develop the production from alternative feedstock rather than sugar cane molasses. It will also stimulate the high and modernized corn productivity of farmers because the waste (corn cob) will have economic and environmental advantage in addition to the corn they produce. As a result, a renewable, cheap and non-food competitive feedstock raw material is desirable for the production bioethanol, so corn cob is the best one.

2. Literature Review

2.1. Introduction to bioethanol

Bioethanol (ethyl alcohol, grain alcohol, CH3–CH2–OH or ETOH) is a liquid biofuel which can be produced from several different biomass feedstocks and conversion technologies. Bioethanol is an attractive alternative fuel because it is a renewable bio-based resource and it is oxygenated thereby provides the potential to reduce particulate emissions in compression–ignition engines. Bioethanol has a higher-octane number, broader flammability limits, higher flame speeds and higher heats of vaporization than gasoline. These properties allow for a higher compression ratio, shorter burn time and leaner burn engine, which lead to theoretical efficiency advantages over gasoline in an internal combustion engine (Mustafa et al., 2008).

Bioethanol is a renewable alcohol-based fuel that can be produced from starches, sugars, and cellulosic biomass. Traditional feedstock, which is used for ethanol production, includes crops such as corn, wheat, and sorghum. With recent advances in cellulosic technology, ethanol can also be produced from agricultural waste products like sugar cane bagasse, rice hulls, potato waste, and brewery waste; from forestry and paper wastes; and from municipal solid waste (Anonym, 2001).

2.2. Feedstocks for ethanol production

The raw materials for bioethanol production can broadly be classified as (i) sucrose-containing feedstock (sugarcane, sugar beet and sweet sorghum), (ii) starch-containing feedstock (wheat, corn and cassava) and (iii) cellulosic feedstock (straw, grasses, wood, agricultural wastes, paper, etc.)(Muktham, Bhargava, Bankupalli, & Ball, 2016). The use of ethanol as an alternative motor fuel has been steadily increasing around the world for a number of reasons. Domestic production and use of ethanol for fuel can decrease dependence on foreign oil, reduce trade deficits, create jobs in rural areas, reduce air pollution, and reduce global climate change carbon dioxide buildup. Ethanol, unlike gasoline, is an oxygenated fuel that contains 35% oxygen, which reduces particulate and NOx emissions from combustion(Singh et al., 2006).

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. 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 (Badger, 2002).

2.2.1. Sugar feedstocks

Ethanol is commonly derived from biological feedstocks utilizing fermentation processes. During these processes, monosaccharides are fermented to ethanol by yeast or bacteria. There are a variety of carbohydrate-containing feedstocks that yield monosaccharides for fermentation, such as corn grain, sugarcane, wheat, sugar beet and another biomass(Stefan, 2009).

Fermentation involves microorganisms that use the fermentable sugars for food and in the process produces ethanol and other byproducts. These microorganisms can typically use the 6-carbon sugars, one of the most common being glucose. Therefore, biomass materials containing high levels of glucose or precursors to glucose are the easiest to convert to ethanol. However, since sugar materials are in the human food chain, these materials are usually too expensive to use for ethanol production. One example of a sugar feedstock is sugarcane(Larissa, 2012).

Given expectations of rising oil prices, the significant potential for expansion of global sugarcane production as ethanol feedstock has resulted in a heightened global focus on sugar and ethanol as internationally traded commodities(Nyberg, 1998). 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 (Badger, 2002).

Although fungi, bacteria, and yeast microorganisms can be used for fermentation, a 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 (Badger, 2002).

2.2.2. Starchy feedstocks

Another potential ethanol feedstock is starch. Starch molecules are made up of long chains of glucose molecules. Thus, starchy materials can also be fermented after breaking starch molecules into simple glucose molecules. Examples of starchy materials commonly used around the world for ethanol production include cereal grains, potato, sweet potato, and cassava. Cereal grains commonly used in the US for ethanol production include maize and wheat. Approximately 475 million tons of maize were produced in the world in 1990 with about 200 million t produced in the US. Approximately 8 to 9 million t, or 4% of US maize grain went into ethanol in 1990. A bushel of maize grain (25.3 kg or 56 lb. at 15% moisture) can produce from 9.4 to 10.9 L (2.5 to 2.9 gallons) of pure ethanol, depending on the technology used. Starchy materials require a reaction of starch with water (hydrolysis) to break down the starch into fermentable sugars (saccharification) (Badger, 2002).

Starch is a high yield feedstock for ethanol production, but its hydrolysis is required to produce ethanol by fermentation. Starch was traditionally hydrolyzed by acids, but the specificity of the enzymes, their inherent mild reaction conditions and the absence of secondary reactions have made the amylases to be the catalysts generally used for this process. α-amylase obtained from thermoresistant bacteria like *Bacillus licheniformis* or from engineered strains of *Escherichia coli* or *Bacillus subtilis* is used during the first step of hydrolysis of starch suspensions. For amylases to attack starch, these suspensions should be brought to high temperatures (90–110 °C) for the breakdown of starch kernels (Harmsen, 2011).

2.2.3. Lignocellulosic feedstocks

Utilizing lignocellulosic biomass as sustainable material has lately become a compelling alternative among conversion technologies in the biofuels and bio-based industry. Widely distributed and largely untapped, lignocellulose can continuously provide low cost feedstock(Sanchez et al., 2007).

Cellulosic resources are in general very widespread and abundant. For example, forests comprise about 80% of the world’s biomass. Being abundant and outside the human food chain makes cellulosic materials relatively inexpensive feedstocks for ethanol production. Cellulosic materials are comprised of lignin, hemicellulose, and cellulose and are thus sometimes called lignocellulosic materials. One of the primary functions of lignin is to provide structural support for the plant. Thus, in general, trees have higher lignin contents then grasses. Unfortunately, lignin which contains no sugars, encloses the cellulose and hemicellulose molecules, making them difficult to reach. Cellulose molecules consist of long chains of glucose molecules as do starch molecules, but have a different structural configuration. These structural characteristics plus the encapsulation by lignin makes cellulosic materials more difficult to hydrolyze than starchy materials. Hemicellulose is also comprised of long chains of sugar molecules; but contains, in addition to glucose (a 6-carbon or hexose sugar), contains pentoses (5-carbon sugars). To complicate matters, the exact sugar composition of hemicellulose can vary depending on the type of plant(Badger, 2002).

Cellulosic biomass the fibrous, non-edible part of plants is an abundant domestic resource that can potentially provide a renewable feedstock for next generation biofuels and bioproducts(Bioenergy technologies office, 2013). In addition, lignocellulosic is a resource that can be processed in different ways for production of many other products like synthesis gas, methanol, hydrogen and electricity(Chung et al., 2001).

Lignocelluloses is the primary building block of plant cell walls. It is a renewable organic material contained by all plants. Lignocellulosic biomass is composed of cellulose, hemicellulose and lignin, as well as other minor components(Harmsen et al., 2011).

Depending on the conversion process used, the concentration of these components in a particular feedstock will affect the efficacy of biofuel production. Cellulose is a sugar polymer chain of glucose, or six carbon sugars. Hemicellulose is a sugar polymer chain of xylose, or five carbon sugars. Lignin forms the hard plant cell walls and cannot be fermented into liquid fuels in a biochemical conversion process, as can cellulose and hemicellulose. Lignin, however, can be utilized in the thermochemical conversion process and serves as a byproduct in the biochemical conversion process useful for providing energy to power the plant and even for generating electricity(Schnepf, 2010). Both the cellulose and hemicellulose fractions are polymers of sugars, and thereby a potential source of fermentable sugar(Harmsen et al., 2011).

Lignocellulosic biomass has a complex internal structure. It is comprised of a number of major components that have, in their turn, also complex structures Cellulose is the β-1, 4-polyacetal of cellobiose (4-O-β-D-glucopyranosyl-D-glucose). Cellulose is more commonly considered as a polymer of glucose because cellobiose consists of two molecules of glucose. The chemical formula of cellulose is (C6H10O5)n and the structure of one chain of the polymer is presented in figure below(Harmsen et al., 2011).

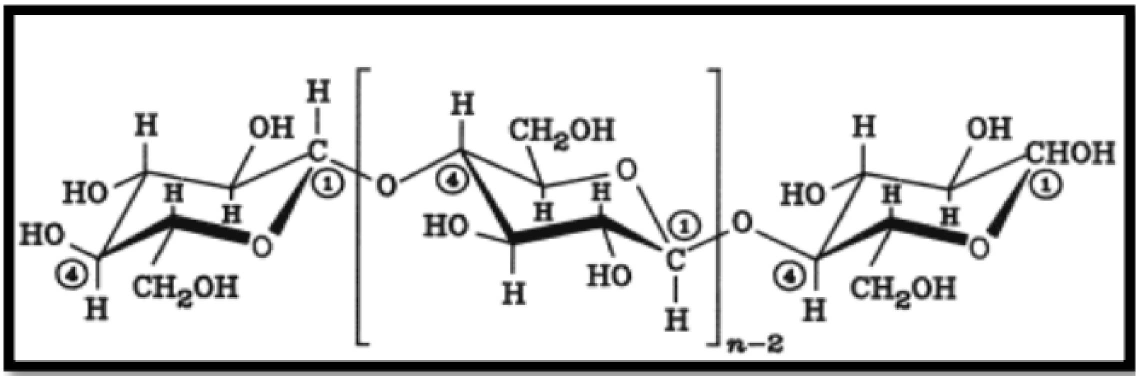


Figure 2.1: Structure of single cellulose molecule

Table 2.1. The contents of cellulose, hemicellulose, and lignin in common agricultural residues and wastes: Source: (Sun, Y. and Cheng, J. 2002)

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

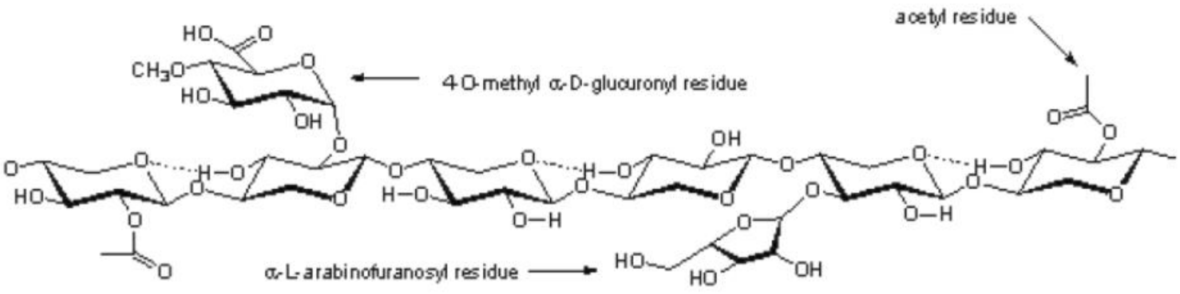


Figure 2.2: Schematic representation of the hemicellulose backbone of arborescent plants

2.3. Corn cobs as a feedstock

The maize plant comprises of the stalks, husks, shanks, silks, leaf blades, leaf sheaths, tassels and cobs. The corn cob carries the grain and together with associating husks, shanks and silks are harvested from the farm(Kudirat, 2012).

Corn cob is the material remaining after removing the grains and is traditionally used as feedstock or fuel. It contains hemicelluloses, cellulose and lignin. Despite polymeric fibers consist of monomer molecules. Amidst cellulose is composed of C6 sugars and hemicelluloses are C5 sugars (xylose and arabinose). In most of the plant derived biomass cellulose, hemicellulose and lignin are wrapped in a complex matrix which is very resistant to enzymatic degradation. Hence, the identification of suitable technique for the utilization of available cellulose and hemicelluloses matter in the corncob biomass is still the biggest task(Anandakumar, 2016).

Maize is critical to smallholder livelihoods in Ethiopia. In addition, maize is the staple crop with the greatest production at 4.2 million tons in 2007/08(Shahidur et al., 2013).

The corn cob, stalks, and leaves can be converted to fermentable sugars with cellulose processing technology that consists of pretreatment, hydrolysis, and fermentation using yeast or other microorganisms. In contrast to grain-based feedstocks, cellulose-based ethanol production requires microorganisms that are capable of producing ethanol from both glucose and xylose(Stefan, 2009).

The agricultural residues from maize production are potential sources of sugar for ethanol production, in addition to starch and by-products. When maize is harvested in the field, the corn grain is separated from the cobs, stalks, and leaves. While the grain is transported for storing and processing, the Stover is currently not widely collected. However, this biomass could be used for lignocellulosic ethanol production. Corn Stover includes stalks, leaves, and corn cobs. Unlike the corn grains, of which the major component is starch, the main components of corn Stover are cellulose, hemicellulose, and lignin(Stefan et al, 2009).

Corn cob hydrolysates possess rich in sugars like glucose and xylose. Depending on the type of fermentation and end product requirement the fermentation process has to be carried out. For example, the xylose fermentation the hydrolysate is called non detoxified hydrolysate which can be fermented by using yeast under aerobic condition at optimum temperature at 30^oC. Since, the microbes consume the sugar and produce ethanol end product(Anandakumar, 2016).

Table 2.2. Chemical composition of corn cobs

Component	Content (%)
Cellulose	32.3 – 45.6
Hemicellulose	39.8
Starch	0
Lignin	6.7 – 15
Volatile matter	78.7
Ash	0.9
Extractive	3

Source (Kudirat, 2012)

2.3.1. Corn (maize) production in Ethiopia

Ethiopia is considered a success story for maize production as, apart from South Africa, it is the only country in Sub-Saharan Africa that has shown substantial progress in maize productivity and input use. After a period of limited growth, yield more than doubled from around 1.5 ton/ha in 2000 to over 3 ton/ha in 2013(EEA, 2017).

Maize is widely grown in Ethiopia; only three regional states contribute to 94% of the total annual production. These regions are Oromia, Amhara and SNNP. According to five years (2003/04 - 2007/08) CSA data, the share of Oromia region was on the average, 60% of the total Maize production in the country. This was followed by Amhara with 21.67% and SNNP with 12.55%.

Thus, the trend of the National maize production was totally dependent on the production field of the three regions. Accordingly, 16 zones from Oromia, 5 zones from Amhara and 7 zones from SNNP region are found to be producers of more than 100,000 quintals per year in all the years from 2003/04 - 2007/08. In Oromia region, 11 of the 16 zones on average produce more than 1,000,000 quintals annually. The major maize producing zones of Ethiopia and their relative share of the national maize production is shown table 2.2 below(G/Mariam, 2016).

Table 2.3: Major Maize Producing Administrative Zones of Ethiopia

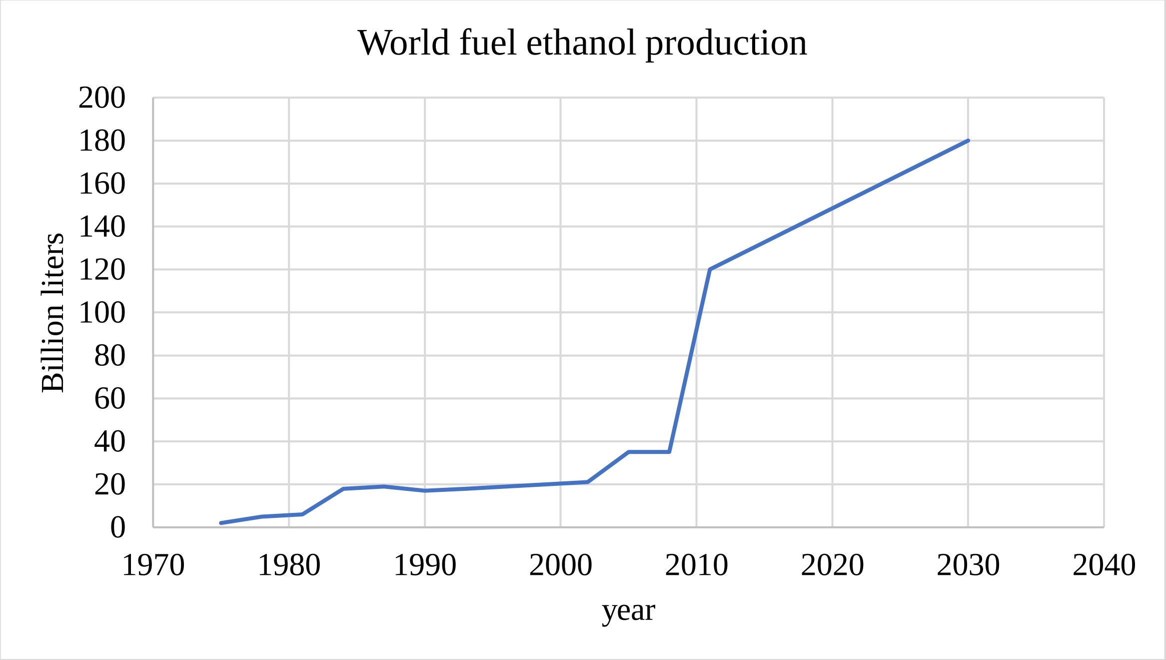
Zone	Average production (in Quintals)
West Gojam	3,209,274
Jimma	2,128,619
West Welega	1,795,239
East Welega	1,790,953
South Gonder	1,561,297
Arsi	1,460,934
Illubabor	1,171,244
Total	16,090,758

Source: (CSA, 2003/04 – 2007/08)

2.4. Global trends of bioethanol production

Today, bio-ethanol is the most dominant bio-fuel and its global production showed an upward trend over the last 25 years with a sharp increase from 2000. As of 2005, worldwide production capacity for bio-ethanol fuel was about 45 billion liters per year, with approximately 15% annual growth between 2000 and 2005. This value increased to 49 billion liters in 2006, when the Americans produced 75% of the total world ethanol output, followed by Asia/Pacific and Europe/Africa with respective values of 15 and 10% (Licht, 2006). The industrial alcohol market showed a rather modest rate of growth similar to the increase in Gross Domestic Product in many countries. The market for beverage alcohol in most developed countries is stagnating, due to increased health awareness. Fuel ethanol production is predicted to have the strongest increase in the Americans, where the production is expected to rise to around 75 billion liters by 2015, representing about 42 billion liters increase in the projection period. In Asia this value is anticipated to increase to 8 billion liters during the same period, and in Europe, with the policy of increasing the share of bio-fuels in the transportation sector, the production will rise strongly. Therefore, total output in 2015 is forecast to reach over 115 billion liters(Licht, 2006).

The production and use of bioethanol is increasing worldwide due to various driving factors explained in the preceding sections. The production in 1990 was only 4 billion gallons and it took 15 years for the quantity to double to around 8 billion gallons in 2005. But in recent years it only took three years to double again to around 17 billion gallons in 2008 and in 2011 around 23 billion gallons of ethanol is produced annually worldwide. Moreover, predictions indicate that the production would reach 34 and 48 billion gallons by the year 2020 and 2030 respectively as shown in Figure 2:1. With this the global future demand of bioethanol is expected to surpass the supply which signifies that there will be market opportunities for low cost producer in developing countries, especially for tropical countries with low labor and land costs(Yacob, 2013).



Source(Yacob, 2013)

Figure 2.3: World fuel ethanol production trend

2.5. Bioethanol production in Ethiopia

There are apparent reasons for promoting biofuels in a country like Ethiopia that depends heavily on imported fossil fuels. The current plan is to produce 1.8 billion liters of liquid biofuels by

2015, consisting of 195 million liters of ethanol and 1.6 billion liters of biodiesel. Biofuel is considered as an opportunity for providing domestic energy security, rapid economic development and creation of wealth. There is great expectations that biofuel could contribute to solve main development challenges the country is facing today(Tesfaye et al., 2011).

Almost all feedstock needed for bioethanol production (sugarcane, sugar-beat, cereals, and corn) grow in Ethiopia. In light of the national policies that discourage the use of food crops as feedstock for food security reasons, current production of bioethanol is only as by-product from sugar estates. Currently, Ethiopia produces about 8 million liters of bioethanol annually from Fincha, one of the four state-owned sugar estates. With the upgrading and expansion of existing sugar estates and new ones in the pipeline, annual production of bioethanol is expected to reach over 100 million liters over the next five years. For instance, the upgrading of Metehara Sugar Estate which is expected to be completed in 2010 would raise annual ethanol production to about 16 million liters per annum, also with the newly integrated cogeneration unit, the sugar estate is expected to generate 435 GWh of electricity during the same time horizon(Shanko, 2009).

So far, there are six sugar industries in Ethiopia. Four of them are state-owned and two of them are private. At the moment, only one of the public sugar estates is producing ethanol from molasses, a byproduct of sugar production, with an annual production of 8 million liters. One of the two private sugar estates is an Indian company. It has started plantation but information could not be obtained as to the current status of development. The other one is a subsidiary of the US based B&D Food Corporation (BDFC). It has received 18,000 ha of land from the Awi Zone in Amhara Regional State to grow sugarcane with the intention of producing sugar and ethanol. It has also a plan for an additional sugarcane supply from out-growers, which is expected to reach up to 30,000 ha. This project was previously planned for development by the Tana Beles Project that was based mainly on the Beles River. The company has already received land and has a plan to produce 70,000 tons of sugarcane and 30,000 tons of ethanol per year.

Since the end of 2008, five percent ethanol blending in gasoline (E-5) has been introduced. This takes much of the current production of ethanol in the country. There is a plan to increase the percentage of ethanol in gasoline as the production capacity of the sugar estates increases. An NGO, Gaia Association, is also working to promote ethanol fuel for household cooking. So far, a couple of thousands of cook stoves suitable for burning ethanol have been disseminated to households in refugee areas in Eastern parts of the country. Depending on availability of ethanol, further promotion of its use in major cities is planned to commence(Shanko, 2009).

Ethanol production in Ethiopia is linked with sugar factories and aimed for import substitute of petroleum products, enhance agricultural development and agro processing, job creation, and export earnings. However, only a small fraction of the potentials is utilized yet and an alternate 5% and 10% ethanol blend has accessed in the capital city of the country. Moreover, at present only two of the sugar factories, Finchaa and Metehara, are producing bioethanol(Gebreegziabher Z., Mekonnen A., Ferede T., 2014).

Given rising world prices of fossil oil, the biofuels industry has developed a very significant national presence. Accordingly, there are biofuels investment activities in different regions of Ethiopia with a focus on bioethanol and biodiesel production. Besides, Ethiopia embarked on a 5% blend of bioethanol in transport fuel in 2008, which was doubled to 10% a few years later. Official reports also indicate that, by blending more than 38.2 million liters of bioethanol with gasoline, the country has been able to save 30.9 million US dollars on oil imports since 2008(Gebreegziabher et al., 2014).

2.6. Process overview of bioethanol production

The production of bioethanol from lignocellulosic material consists of a pretreatment, a hydrolysis and a fermentation step. In these processes, lignin is discharged as a by-product and can be used to generate electricity to supply the process with energy, or to export electricity to the grid. Lignin is composed of phenolic components, which are not fermentable under anaerobic conditions. Pretreatment is necessary to break open the lignocellulosic structures and to facilitate the separation of the main carbohydrate fractions hemicellulose and cellulose from lignin, in order to make these better accessible for hydrolysis, the next step in the process. Hydrolysis is the process to convert the carbohydrate polymers cellulose and hemicellulose into fermentable sugars. Hydrolysis can be performed either chemically in a process involving the use of concentrated acids, or enzymatically by using enzymes. Most pathways developed today are based on enzymatic hydrolysis, by using cellulases and hemicelluloses(Chung et al., 2001).

Ethanol is produced either synthetically from petrochemical feedstock (gasoline) or biomass by microbial fermentation. In comparison to first generation bioethanol from sugar and starch materials, second generation bioethanol production from lignocellulosic materials requires additional processing steps, as the crystalline structure of cellulose makes it highly insoluble and resistant to enzymatic attack. It is important to mention that efficient depolymerization of cellulose (source of hexose sugars such as glucose) and hemicellulose (mainly source of pentose sugars such as xylose) is not accessible to microorganisms producing first generation bioethanol. The combination of hemicellulose and lignin provides a protective sheath around the cellulose, which has to be modified or removed before efficient hydrolysis of cellulose can occur(Rita Mergner et al., 2013).

Polysaccharides - long carbohydrate molecules - are very stable and pentose sugars are difficult to ferment. First of all, polysaccharides need to be hydrolyzed or broken down into simple sugars using acid treatment or enzymes. Therefore, to economically hydrolyze cellulose, more advanced pretreatment technologies are required than for processing sugar or starch crops(Rita Mergner et al , 2013).

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

2.6.1. Biochemical conversion process

Compared with other conversion technologies, biomass biochemical conversion technologies are moderate, pure, clean, and efficient(Hongzhang Chen, 2017).

A (bio-)chemical conversion system for lignocellulosic biomass will generally consist of a pretreatment step to release the C6 and C5 sugars followed by conversion of the polysaccharides into products like fuels or chemicals. Second-generation cellulosic ethanol production is the best known example(Elbersen, 2017).

The technology of ethanol production from biomass feedstocks consists of several steps, and varies depending on the type of raw materials used. It becomes more sophisticated as the raw materials turn from sugars to starches and cellulosic materials. Unlike starch, the specific structure of cellulose favors the ordering of the polymer chains into tightly packed, highly crystalline structures those are water-insoluble and resistant to depolymerization. For production of ethanol from cellulosic feedstocks, four major unit operations are required: pretreatment, hydrolysis, fermentation, and separation purification(Solomon, B. D., 2007).

2.6.1.1. Pretreatment

The effect of pretreatment of lignocellulosic materials has been recognized for a long time. The purpose of the pretreatment is to remove lignin, reduce cellulose crystalline and increase the porosity of the materials. Pretreatment must meet the following requirements: Improve the formation of sugars or the ability to subsequently form sugars by acidic or enzymatic hydrolysis; avoid the degradation or loss of carbohydrate; avoid the formation of byproducts inhibitory to the subsequent hydrolysis and fermentation processes. Physical, chemical, physico-chemical, and biological processes have been used for pretreatment of lignocellulosic materials(Gupta, 2009).

In another words, the purpose of the pretreatment step is to further increase the surface area of the lignocellulosic material, disrupt the structure of the lignocellulose such that the cellulose component is accessible to hydrolyzing agents and reduce the crystallinity of the cellulose to further facilitate hydrolysis. Depending on the nature of the pretreatment technology selected, this step can also include solubilization of the lignin or the hemicellulose component. Various pretreatment options are available now to fractionate, solubilize, hydrolyze and separate cellulose, hemicellulose, and lignin components. These include physical, physicochemical, chemical and biological pretreatment(Avira et al., 2010).

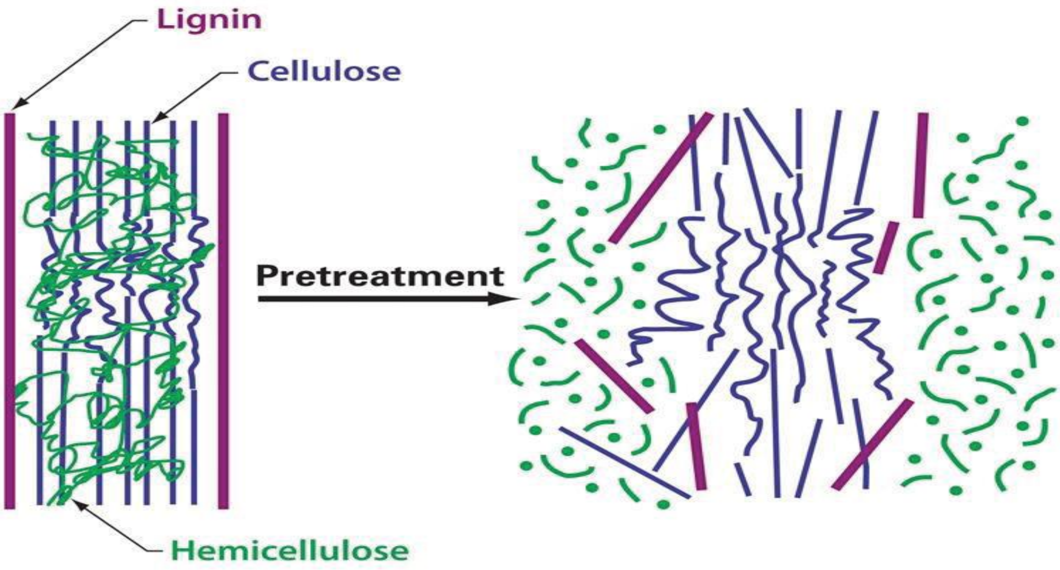


Figure 2.4: Schematic representation on biomass pretreatment

Physical pretreatment

Physical pretreatments aim to degrade the cellulose crystallinity to improve biomass digestibility by changing the physical character of materials using pyrolysis and mechanical comminution including dry, wet and vibratory ball mills(Millet et al., 1976). There are several ways under this category.

Mechanical comminution: is a combination process involving chipping, grinding and milling which reduces the material size down to 0.2 - 2 mm(Cheng, J., & Sun, 2001).

Ozonolysis: Ozone can be used to degrade lignin and hemicellulose in many lignocellulosic materials such as wheat straw, bagasse, green hay, peanut, pine, cotton straw, and poplar sawdust. The degradation was essentially limited to lignin and hemicellulose was slightly attacked, but cellulose was hardly affected. Ozonolysis pretreatment has the following advantages: it effectively removes lignin; it does not produce toxic residues for the downstream processes; and the reactions are carried out at room temperature and pressure. However, a large amount of ozone is required, making the process expensive(Gupta, 2009).

Pyrolysis*:* Pyrolysis has also been used for pretreatment of lignocellulosic materials. When the materials are treated at temperatures greater than 300°C, cellulose rapidly decomposes to produce gaseous products and residual char. The decomposition is much slower and less volatile products are formed at lower temperatures.(Gupta, 2009)

Chemical Pretreatment

Dilute-acid pretreatment: is one the major pretreatment employed in lignocellulosic biomass processing. The acid pretreatment is highly effective for bioethanol production. It involves exposure of biomass to 0.1–1% sulfuric acid at elevated temperatures of 140–200 °C for 5–30 min or longer. This method yields highly digestible biomass solids plus a liquid fraction that contains most of the hemicellulose sugars as monomeric or oligomeric (small chains of sugars) molecules(Xu, 2015).

Alkaline pretreatments: are carried out under milder conditions, some of them even at ambient temperature, as evidenced by soaking in sodium hydroxide, or in ammonium hydroxide. Such methods can eliminate the need for expensive materials and special designs to cope with corrosion and severe reaction conditions. It is also possible to recover and reuse chemical reagents in some of the alkaline pretreatment methods(Hyun Kim, 2016).

In ethanol production from grass as (Cotta, 2007) Alkaline peroxide pretreatment was performed. The milled grasses were suspended in 7.5% (v/v) H_2O_2 and NaOH was then added to adjust the pH to 11.5. The pretreated samples were then incubated at 35^{o}C, shaken at 250 rpm for 24 hours. Finally, conc. HCl was added to adjust the pH to 4.8 before enzymatic hydrolysis.

Oxidative Delignification: Lignin biodegradation could be catalyzed by the peroxidase enzyme with the presence of H2O2. The pretreatment of cellulosic biomass with hydrogen peroxide greatly enhanced its susceptibility to enzymatic hydrolysis. About 50% lignin and most hemicellulose were solubilized by 2% H2O2 at 30 °C within 8 h, and 95% efficiency of glucose production from cellulose was achieved in the subsequent saccharification by cellulase at 45 °C for 24 h(Gupta, 2009).

Physico-Chemical Pretreatment

Steam Explosion (Auto hydrolysis): Steam explosion is the most commonly used method for the pretreatment of lignocellulosic materials. In this method, chipped biomass is treated with high-pressure saturated steam and then pressure is swiftly reduced, which makes the materials undergo an explosive decompression. Steam explosion is typically initiated at a temperature of 90 - 160 ºC for several minutes to a few hours the material is exposed to atmospheric pressure. The process causes hemicellulose degradation and lignin transformation due to high temperature, thus increasing the potential of cellulose hydrolysis and steam treatment with dilute acid of 0.5 to 1%, temperature of 90 to 140o and residence time of 10 to 40 minutes gives highest yield of ethanol(Solomon, 2007).

The advantages of steam explosion pretreatment include the low energy requirement compared to mechanical comminution and no recycling or environmental costs. The environmental mechanical methods require 70 % more energy than steam explosion to achieve the same size reduction. Steam explosion is recognized as one of the most cost-effective pretreatment processes for hardwoods and agricultural residues, but it is less effective for softwoods. Limitations of steam explosion include destruction of a portion xylan fraction, incomplete disruption of the lignin-carbohydrate matrix and generation of compounds that may be inhibitory to microorganisms used in downstream processes(Cheng, 2009).

Biological Pretreatment

In biological pretreatment processes, microorganisms such as brown, white and soft-rot fungi are used to degrade lignin and some hemicellulose in waste materials. Brown rots mainly attack cellulose, while white and soft rots attack both cellulose and lignin. White-rot fungi are the most effective basidiomycetes for biological pretreatment of lignocellulosic materials(Gupta, 2009).

2.6.1.2. Hydrolysis

In the hydrolysis reaction, the complex chains of sugars that make up the hemicellulose are broken, releasing simple sugars. The complex hemi-cellulose sugars are converted to a mix of soluble five-carbon sugars, xylose and arabinose, and soluble six-carbon sugars, mannose and galactose. The rest of hemicelluloses are degraded to weak acids, furan derivates, and phenolics. These compounds, however, are potential fermentation inhibitors. By the action of acids and/or enzymes, the glucose yields of cellulose hydrolysis often exceed 90%, but hydrolysis without preceding pretreatment yields typically less than 20% only. The pretreated feedstock can be hydrolyzed by two methods (Gupta, 2009), chemical or enzymatic hydrolysis.

In the past various acid hydrolysis technologies have been developed. The different acid hydrolysis technologies can be divided into two broad categories: i) hydrolysis with concentrated acid at low temperatures; ii) hydrolysis with dilute acid at high temperatures (Brodeur, G., Yau, E., Badal, K. & Ramachandran, K.B. and Ramakrishnan, 2011).

Another basic method of hydrolysis is enzymatic hydrolysis. Enzymes are naturally occurring plant proteins that cause certain chemical reactions to occur. Enzymatic hydrolysis is not commercialized yet but is recognized to be the most promising hydrolysis technology. A reduction of the cost of ethanol production can be achieved by reducing the cost of either the raw materials or the cellulase enzymes. Reducing the cost of cellulase enzyme production is a key issue in the enzymatic hydrolysis of lignocellulosic materials (Kuila et al., 2011).

As (Akpan, Kovo, Abdullahi, & Ijah, 2005) different concentrations of sulfuric acid (H_2SO_4) were used to determine the acid concentration that could produce an optimal yield of glucose. The results revealed that 4.5M H_2SO_4 produced the optimal yield of glucose and ethanol. This acid concentration was then used for the study of temperature effects on yield of glucose. The results indicated that glucose yield increased with temperature within the experimental set-up. The maize cobs and groundnut shells were mixed at various ratios and pretreated to remove all extractives. The ratio of 3:1 of maize cobs to groundnut shells and at 4.5M acid gave a better glucose yield than those obtained from individual biomass. The ultimate product (glucose) was hydrolyzed and 8% ethanol was obtained within three hours.

In order to obtain the various concentration of metabolite in the hydrolysate, size of the particle can be adjusted accordingly. This helps in the simple extraction of metabolites like glucose, xylose, acetate, furfural and hydroxyl methyl furfural from ground material. Further, those ground matter will be acid hydrolyzed with H_2SO_4 in the ratio of 1:20 W/V (Solid and liquid ratio), which is ideal for the breakdown of inner core of corncob embedded with combination of metabolites. In addition, numerous researchers studied to release/extract all the available composition of metabolite in acid hydrolysate material by vigorous agitation, which represents the level of metabolite release considerably higher amount (Anandakumar, 2016).

2.6.1.3. Fermentation

Fermentation is the process involving the biochemical activity of microorganisms, which converts carbohydrates to alcohols, organic acids or gases. Fermentation process is carried out in a container called the fermenter or bioreactor. The design and nature of the fermenter varies depending upon the type of fermentation carried out.

Types of Fermentations

1. Batch Fermentation:

A batch fermentation is a closed culture system, because initial and limited amount of sterilized nutrient medium is introduced into the fermenter. The medium is inoculated with a suitable microorganism and incubated for a definite period for fermentation to proceed under optimal physiological conditions. Oxygen in the form of air, an antifoam agent and acid or base, to control the pH, are being added during the course of fermentation process

During the course of incubation, the cells of the microorganism undergo multiplication and pass through different phases of growth and metabolism due to which there will be change in the composition of culture medium, the biomass and metabolites. The fermentation is run for a definite period or until the nutrients are exhausted. The culture broth is harvested and the product is separated. Merits are:

(a) The possibility of contamination and mutation is very less.

(b) Simplicity of operation and reduced risk of contamination.

2. Continuous Fermentation:

It is a closed system of fermentation, run for indefinite period. In this method, fresh nutrient medium is added continuously or intermittently to the fermenter and equivalent amount of used medium with microorganisms is withdrawn continuously or intermittently for the recovery of cells or fermentation products

3. Fed Batch Fermentation:

It is a modification to the batch fermentation. In this process substrate is added periodically in instalments as the fermentation progresses, due to which the substratum is always at an optimal concentration.

4. Anaerobic Fermentation:

A fermentation process carried out in the absence of oxygen is called as anaerobic fermentation. There are two types of anaerobic microorganisms viz, obligate anaerobic microorganisms and facultative anaerobic microorganisms. The former like Clostridium sp. cannot withstand oxygen or remain active only in the absence of oxygen.

5. Aerobic Fermentation:

A fermentation process carried out in the presence of oxygen is called as aerobic fermentation. In most of the commercial processes and majority of the products of human utility are produced by this type of fermentation.

The fermenting of the biomass is conducted under standard fermenting conditions and will utilize all the major biomass. Yeast is the most commonly used microorganism in fermentation processes. Yeasts are minute, often unicellular, fungi. The yeasts used are typically brewers' yeasts. Examples of yeast capable of fermenting the decaying biomass include, but are not limited to, *Saccharomyces cerevisiae* and *Saccharomyces uvarum*(Sanchez et al., 2007).

Non-Sacharomyces yeasts, also known as non-conventional yeasts, are also used to make a number of commercial products. Some examples of non-conventional yeasts include *Kuyberomyces lactis, Yarrowia lipolytica, Hansenula polymorpha and Pichia pastoris*. Microorganisms other than yeast can also be useful in making fermentation products. For example, cellulosic ethanol production also utilizes fungi and bacteria(Cheng & Sun, 2001).

$$3C_5H_{10}O_5 \longrightarrow 5C_2H_5OH + 5CO_2, \qquad \Delta H = -74.986\ \text{kJ/mol} \qquad (2.1)$$

$$C_6H_{12}O_6 \longrightarrow 2C_2H_5OH + 2CO_2 \qquad \Delta H = -84.394\ \text{kJ/mol} \qquad (2.2)$$

(Syawala & Wardiyati, 2013) Fermentation takes place in anaerobic conditions. The added yeast Saccharomyces cerevisiae culture as much as 10% (v / v). The fermentation process lasts for seven days. During the fermentation process the pH value, total sugar and ethanol content were analyzed. After eight days, filtered fermented, then distillation to separate the ethanol from the water fraction of the substrate fermentation at a temperature 78-80 °C using Rotary vacuum evaporator.

As (Zakpaa & Johnson, 2009) Maize is the most abundant cereal grown in Ghana and is accompanied by enormous amount of agrowastes of which corncobs form 30%. This agrowaste which is currently underutilized was used to produce bio-ethanol. *Aspergillus niger* isolated from soil sampled from Ejura farms was used to hydrolyze the corncobs into simple sugars. Filtrate obtained from corncobs broth fermented by *A. niger* was used as crude enzyme in optimization tests on corncobs powder suspended in 50 mM citrate buffer pH 5.0. Optimum temperature, pH and substrate concentration for saccharification were 40^{o}C, 4.0 and 6% respectively. *Saccharomyces cerevisae* was added to *A. niger* filtrate to cause fermentation of the corncobs. The highest ethanol concentration of 0.64 g/l was recorded over the 24 h fermentation period.

2.6.1.4. Distillation

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

Whatever method of preparation is used, the ethanol is initially obtained in a mixture with water. The ethanol is then extracted from this solution by fractional distillation. Although the boiling point of ethanol, 78.3 ^{0}C, is significantly lower than the boiling point of water, 100 ^{0}C, these materials cannot be separated completely by distillation. Instead, an azeotrope mixture (i.e. a mixture of 95% ethanol and 5% water) is obtained, and the boiling point of the azeotrope is 78.15 ^{0}C. In a distillation, the most volatile material (i.e. The material that has the lowest boiling point) is the first material to distill from the distillation flask, and this material is the azeotrope of 95% ethanol which has the lowest boiling point. If an efficient fractionating column is used, 95% alcohol could be obtained first and then a small intermediate fraction of lower concentration, and then water. But no matter how efficient the fractionating column used, 95% alcohol cannot be further concentrated by distillation because the vapor has exactly the same composition as the liquid; towards distillation, then, 95% alcohol behaves exactly like a pure compound(Acevedo et al., 2003).

2.6.1.5. Dehydration

After distillation, about 5% of water remains in ethanol. Especially, this water is a big problem for fuel ethanol because the presence of this amount of water enhances the molecular polarity of ethanol when it is mixed with gasoline. Consequently, they separate into two phases, ethanol phase and gasoline phase. It is easy to imagine that this inhomogeneous fuel is not acceptable. Thus, dehydration can be another issue. For the ethanol to be usable as a fuel, water must be removed. Most of the water is removed by distillation, but the purity is limited to 95-96% due to the formation of a low boiling water-ethanol azeotrope. (Krishnan et al., 1999).

2.6.2. Thermo-chemical conversion process

In addition to the biological conversion of cellulosic biomass to ethanol, biofuels may also be produced through thermochemical conversion. Thermochemical conversion processes can yield a wide variety of fuel types, including hydrogen, diesel, gasoline, ethanol, methanol and mixed alcohols. Two of the most widely examined and mature thermochemical conversion methods are gasification of biomass to produce syngas and pyrolysis to produce bio-oil. In both cases, additional steps are required to upgrade or convert the end product into a fuel that can be used by the transportation sector(MacLean, 2016).

This process route includes production of synagas using gasification of the biomass, and fermentation or catalytic synthesis of the synagas to produce ethanol. The process begins with biomass gasification where, under a controlled oxygen supply, cellulose, hemicellulose, and lignin are converted to synthesis gas (synagas), primarily CO, CO_2 and H_2. This synagas can be converted into ethanol either through catalytic synthesis; biomass gasification offers an attractive 32 alternative system for producing cellulosic ethanol. Although gasification reactions can take many forms, these processes are defined by cranking up the temperature to between 650 and 1,400^{o}C. Although fermentation; convert the synagas mixture into ethanol using organisms and separate ethanol from water(Mekonen, 2012).

2.7. Analysis of ethanol content

A highly accurate Digital Density Meter such as the DMA4100M provides the easiest means to measure the density of an alcohol/water mixture and automatically converting this measurement into an alcohol concentration. The measurement is fast, accurate, and highly reproducible. The DMA4100M Plus is loaded for measurements by injecting a small 3ml to 5ml sample with a syringe(Tsgehiwet Gebremichal, 2017).

3. Materials and Methods

The experimental works of this research was done in Addis Ababa Institute of Technology, School of Chemical and Bio Engineering labs.

3.1. Materials

Table 3.1. Major Equipment and chemicals

Items	**Purpose**
Equipment	
Hammer	Reduce the size of corn cob
Oven	Dry sample
Grinder (WRB80)	Reduce the size of corn cob
Sieve (AS200)	Sieving/screening the sample
Weighing balance (EP214C)	Weighing
Digital PH meter (JEWWAY 3505)	PH measurement
Flasks of different volume	Hydrolysis, fermentations…
Graduated cylinders of different volumes	Volume measurement
Vacuum filtration	Separation of the soluble liquid from non-soluble
Autoclave (LaMCS204)	Sterilization, pretreatment and hydrolysis
Safety cabinet (Flow FAST V15P)	Safety area for inoculation
Electric furnace (VF2)	Heating(drying)
Shaking incubator (Memer GF3017)	Fermentation
Distillation set ups	Distillation
Spectro UV-VS (UVD-3200)	Absorbance measurement
Density meter (DMA 4100 M)	Density and ethanol percent measurement
Fourier Transform Infrared spectroscopy	Determine the functional group of ethanol
Chemicals	
98% sulfuric acid (H2SO4)	Pretreatment and hydrolysis of corn cob
Acetone	Determination of corn cob composition
Sodium hydroxide (NaOH)	PH adjusting
Distilled water	Pretreatment and media preparation
Microorganism	
Saccharomyces cerevisiae	To be refreshed and used for fermentation

3.2. Methods

Experimental framework

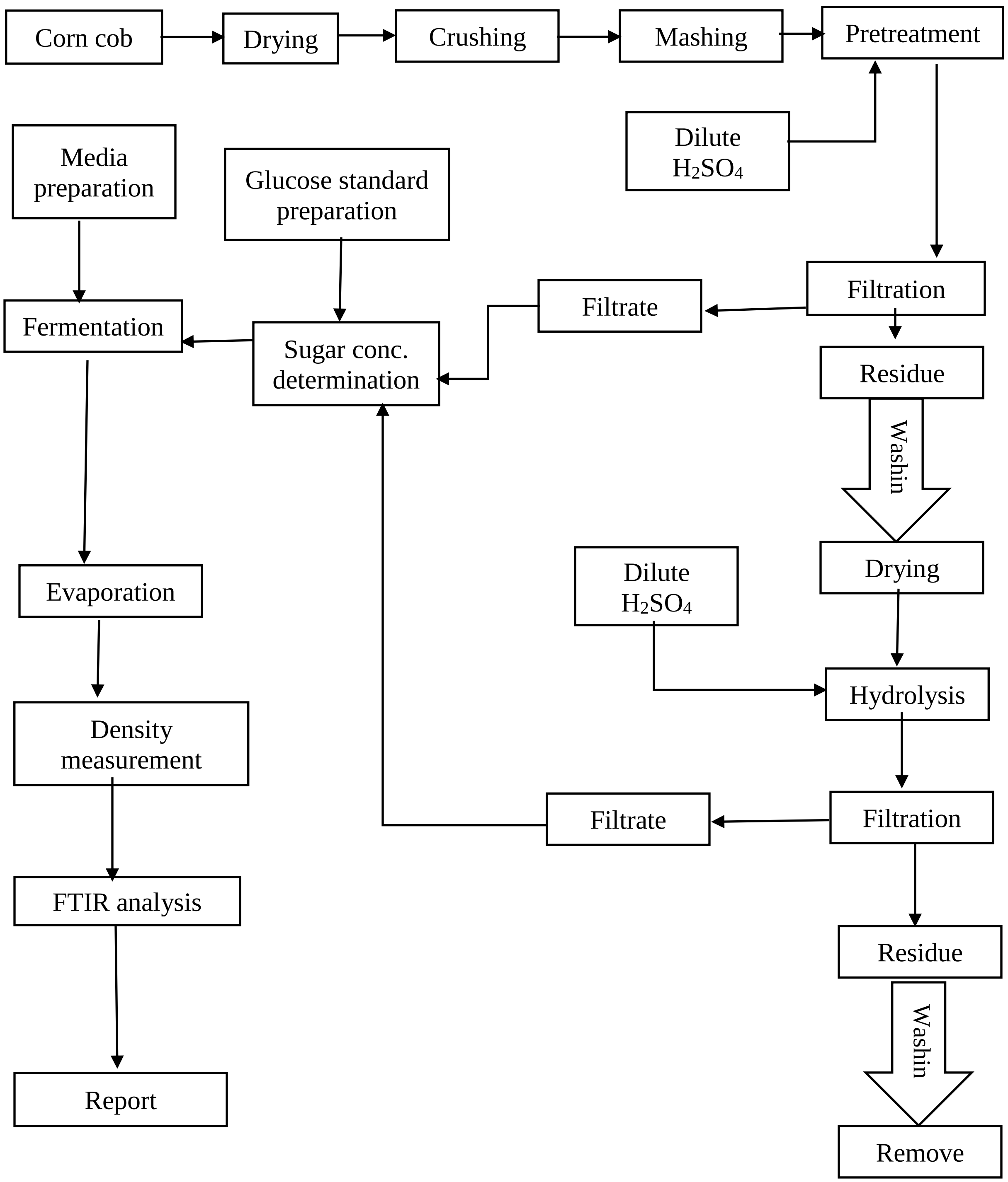


Figure 3.1: Experimental framework for production of ethanol from corn cob hydrolysate

3.2.1. Sample preparation and characterization

Corn was bought from market and the corn cob was separated from the corn by hands. The corn cob was cut by knife into pieces. of about 3-5 cm length and sun dried for 11 days. The sun-dried corn cob was crushed/ reduced in size to below 1.5 mm diameter with electrical grinder to increase surface area to volume ratio. The sample was partitioned in to 17 samples of 80 gram each and packed on polyethylene plastic for pretreatment.

3.2.1.1. Characterization of corncob

Proximate analysis

Proximate analysis including moisture content, volatile matter content, fixed carbon content, and ash content of biomass were characterized based on NREL procedure(Sluiter et al., 2012).

Moisture Content

Three samples of 2 grams each were weighed in clean oven-dried crucible of known weight. The samples and crucibles were kept in an oven 105℃ for three hours. The crucibles with samples were covered and transferred to desiccators, and weighed after reaching room temperature. The crucibles were heated in the oven for another one hour and was re-weighed. it was repeated until constant weight was obtained. The loss of weight percent expresses the moisture content of corn cob which is calculated as;

$$\text{Moisture content } (\%) = \frac{W_1 - W_2}{W_1} * 100 \qquad (3.1)$$

Where: W_1 = original weight

W_2 = weight after oven dried

Volatile Matter Content

Oven dried samples were put in a known weight of dry crucibles. The samples and the crucibles were placed in a furnace for 30 min at 600 ℃. The crucible was removed from furnace and placed in a desiccator for cooling, then was reweighed. The process was repeated until constant weight was obtained. Then,

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

Where: W_1 = oven dried weight of sample

W_2 = weight after heating in furnace

Ash Content

Crucibles were weighed empty, and then the oven dried samples were put in them. The samples and the crucibles were placed in as furnace for 2 hours at 550 °C. The crucibles were removed from furnace and placed in a desiccator to cool, then was reweighed. Then

$$\text{Ash content (\%)} = \frac{\text{Weight}_{\text{crucible plus ash}} - W\text{eight}_{\text{crucible}}}{\text{ODW}_{\text{sample}}} * 100 \qquad (3.3)$$

Where: ODW= oven dried sample

Fixed Carbon Content

Carbon content can also be estimated using the formula,

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

Chemical composition

Extractive Content

To determine the extractive content of the wood residues, 5 grams of the oven dried raw biomass was placed in to the thimble. 300 mL acetone was measured in 500 mL conical flask as solvent and the Soxhlet extractor was set up with the boiling flask positioned on the water bath set at 70 °C for 4 hrs. After extraction the sample was air dried at room temperature for few minutes and it was placed in an oven at 105 °C until the moisture content was removed completely so that the weight of dried biomass is constant. This experiment was repeated three times and finally, the percentage extractives content was calculated as the difference in weight between the raw extractive-laden biomass and extractive-free biomass using the formula(Blasi et al., 2010):

$$\text{Extractive content}, \% = \frac{W_1 - W_2}{W_1} * 100 \qquad (3.5)$$

Where W_1=weight of dry biomass before extraction in grams

W_2= weight of dry biomass after extraction in grams

The results of this calculation and the findings are discussed on result and discussion part of this paper.

Hemicellulose Content

To determine the hemicellulose content,1g of dried biomass from the extractive analysis was transferred into a 250 mL conical flask and then 150 mL of 0.5 mole/L NaOH solution was added (three replicates). The mixture was boiled for 3.5 hrs with recycled distilled water so as to increase the heating effect. After boiling, the mixture was cooled and filtered by using vacuum filtration and washed until neutral pH was achieved. The residue was then dried at 105 ℃ in oven and cooled in a desiccator and finally weighed until constant weight was obtained(Ayeni et al., 2013). The difference between the sample weight before and after this process is the hemicellulose and it was calculated as:

$$\text{Hemicellulose content}, \% = \frac{W_1 - W_2}{W_1} * 100 \tag{3.6}$$

Where W_1=weight of dry biomass before analysis in grams

W_2= weight of dried biomass after analysis in grams

Lignin Content

0.3 mg of extracted dried biomass (that was found from extractive determination) was weighed in glass test tubes and 3 mL of 72% H_2SO_4 was added and the sample was kept at room temperature for 2 hrs by carefully shaking at 30 min intervals to allow for complete hydrolysis. After the initial hydrolysis, 84 ml of distilled water was added to sample to dilute the sample to 4% and placed in an autoclave for 1 h at 121 °C. The slurry was then cooled at room temperature and the hydrolysates were filtered through vacuum using a filtering crucible. The acid insoluble lignin was then determined by drying the residues at 105 ℃ and accounting for ash by incinerating the hydrolyzed samples at 575 ℃ in a muffle furnace.

Finally, the result was calculated using the formula(Sluiter et al., 2012):

$$\text{Lignin}, \% = \frac{W_l}{W_s} * 100 \tag{3.7}$$

Where: W_l= lignin in g and

W_s= weight of air-dried sample in g

Determination of Cellulose Content

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

$$\%\mathrm{W\ cellulose} = 100\% - (\%\mathrm{W\ ash} + \%\mathrm{W\ hemicellulose} + \%\mathrm{w\ lignin} + \%\mathrm{W\ extractives}) \quad (3.8)$$

3.2.2. Sample pretreatment

Dilute acid hydrolysis pretreatment for biofuel production was applied from 0.05 to 2% H_2SO_4 (w/v) at between 120 and 220 ℃) for 2 to 90 minutes (Lei et al., 2011). Thus, in this study, dilute sulfuric acid of 1% concentration was used. Each of the 80-gram samples were mixed with distilled water in a ratio of 1:10 (w/v) in one-liter volume flask. 80 grams corn cob powder was mixed with 800 mL of distilled water and 1% sulfuric acid solution of 80 mL was added. Each sample of corn cob powder was pretreated in autoclave at temperature of 120 ℃, for 30 minutes.

Then the pretreated samples were allowed to cool and was filtered using vacuum filter. The filtrate was kept in another flask for fermentation and the residue was washed by plenty of distilled water two times to remove the residual acid and then sun dried for hydrolysis.

3.2.3. Hydrolysis

The cellulose molecules which are composed of long chains simple sugars are broken down to simple sugar, before it is fermented for alcohol production. Even though there are many types of hydrolysis types, dilute acid hydrolysis is an easy and productive process(Mekonen, 2012).

The residue from pretreatment was sun dried and weighed for hydrolysis. 1:10 w/v of residue from pretreatment to distilled water was used. The hydrolysis step was experimented in autoclave using the parameters; diluted H_2SO_4 of 2%, 3% and 4%, at temperature of (100 ℃, 120 ℃ and 140 ℃) and time (20, 30 and 40) minutes. The hydrolysis runs were done by design expert 11.0 (response surface – Box-Behnken). 50-gram sun dried residue was found from pretreatment.

Table 3.2 represents the experimental runs for hydrolysis randomized by response surface – Box-Behnken design.

Table 3.2: Hydrolysis of pretreated corn cob at different parameter variations

Run №	Acid Conc. (%)	Temperature (℃)	Time (min)	Sugar yield (conc. unit)
1	2	100	30	
2	4	100	30	
3	2	140	30	
4	4	140	30	
5	2	120	20	
6	4	120	20	
7	2	120	40	
8	4	120	40	
9	3	100	20	
10	3	140	20	
11	3	100	40	
12	3	140	40	
13	3	120	30	
14	3	120	30	
15	3	120	30	
16	3	120	30	
17	3	120	30	

The solid part of the hydrolysis was separated by vacuum filter and the filtrate was mixed into the pretreatment filtrate while the residue was washed and removed.

(a) (b)

Figure 3.2: (a) dry corn cob, (b) acid pretreated corn cob

3.2.4. Determination of total carbohydrate

Total sugar measurement using phenol sulfuric acid method

100 g/l of standard stock glucose solution was prepared by dissolving 10 g of glucose in 100 mL distilled water. From the stock solution; 0, 1, 3, 5, and 7 g/L were pipetted into 50 ml separate flasks. They were diluted by distilled water to constant volume. Five test tubes were prepared for standard preparation; one tube for blank, the four tubes for glucose standard. To each tube 4 mL of sample containing glucose standard was pipetted and 1 mL of 5% phenol was add to all tubes and mixed. Then 5 mL of 96% concentrated sulfuric acid was added, simultaneously the tubes were shacked to effect fast and complete mixing. They were placed in water bath at 30℃ for 10 minutes for color display. Blank solutions were prepared in the same way, except that the 4 mL of the standard solution was replaced by distilled water. The absorbance was measured at 490 nm in UV spectrophotometry.

The amount of total reducing sugar present in the sample was calculated using the standard graph of sugar concentration and absorbance.

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

Where: y is absorbance

x is concentration

m is the slop and b is the intercept

so,

$$\text{Sugar conc. of sample} = (\text{absorbance of the sample} - \text{intercept})/\text{slope} \tag{3.10}$$

$$\text{Ethanol yield} = \text{ethanol produced} / \text{sugar concentration} \tag{3.11}$$

pH adjustment

The pH of the hydrolysate was acidic which is very harsh environment for saccharomyces cerevisiae to ferment sugar. Before addition of any micro-organism to the hydrolysates, pH of the hydrolysates must be adjusted. So, the pH of the hydrolysate was measured by digital pH meter and 10 M of NaOH solution was pipetted to it until the pH becomes 5.

3.2.5. Fermentation

The fermentation studies were carried out using Saccharomyces cerevisiae in the hydrolysates obtained from pretreated and acid hydrolyzed.

The fermentation process was done anaerobically with 180 rpm steering rate and at different levels of sugar concentration, volume of inoculum and temperature. A volume of 100 mL hydrolysate of sugar concentration (41.65 g/L, 47.47 g/L and 53.57 g/L) was fermented at inoculum level of (5, 10, 15 mL or %v/v) and temperature of (25,30,35 ℃) for 72 hours. The inoculum was prepared by preparing growth media and inoculating saccharomyces cerevisiae brought from Ethiopian Biodiversity Institute.

Media and inoculum preparation

The media was prepared in 400 mL distilled water using 500 mL flask. The media content was potato dextrose broth (40g) as carbon source, urea (4g) for nitrogen source and for fast growth, $MgSO_4 . 7H_2O$ (4g) as source of magnesium, and yeast extract agar (1g) as source of vitamins and minerals (Mebrahtom et al., 2014). The pH was adjusted to 5. Then it was sterilized at 121 ℃ for 15 minutes. To this media yeast (Saccharomyces cerevisiae) was added. The culture was covered by cotton and aluminum foil to prevent oxygen entry then placed in a shaker incubator for 24 hrs at a temperature of 30 ℃ and 200 rpm for growth. The cells were measured for their density in UV spectrophotometry and was increasing with time.

The materials used for fermentation was sterilized in autoclave at 121 ℃ for 15 minutes. Table 3.3 shows the experimental runs of fermentation process randomized by response surface – Box-Behnken design.

Table 3.3: Different level of parameters for fermentation of corn cob hydrolysate

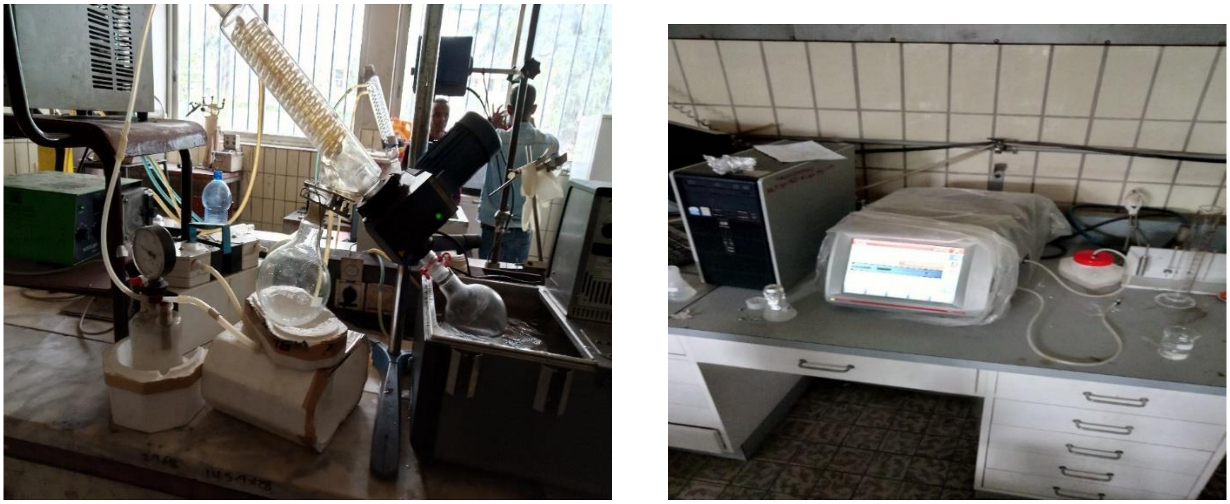
No	Sugar (g)	Inoculum (%)	Temperature (℃)	Ethanol yield (mL/g)
1	4.17	5	30	
2	5.36	5	30	
3	4.17	15	30	
4	5.36	15	30	
5	4.17	10	25	
6	5.36	10	25	
7	4.17	10	35	
8	5.36	10	35	
9	4.75	5	25	
10	4.75	15	25	
11	4.75	5	35	
12	4.75	15	35	
13	4.75	10	30	
14	4.75	10	30	
15	4.75	10	30	
16	4.75	10	30	
17	4.75	10	30	

3.2.4. Distillation

Distillation is the method used to separate two liquid based on their different boiling points. So, it is the last step in production of ethanol from corn cob. The fermented solution was distilled using rotary evaporator at 80 °C for 3 hrs. Then the alcohol (ethanol percentage) and their density was measured using density meter at room temperature.

3.2.6. Density and ethanol percentage measurement

The concentration of ethanol was measured by density meter at room temperature. A 7 ml syringe was used for injection of ethanol to be measured and distilled water for washing the instrument. So, density and percent of ethanol was determined using density meter at around 20 ℃ (its working temperature).



(a) (b)

Figure 3.3: (a) Rotary evaporator for distillation process, (b) Density meter

3.3. Experimental design

Design expert 11 software experimental method was used to investigate the effect of three operating variables of the fermentation process in ethanol production from corn cob. These were sugar level, inoculum level, and temperature. The response variable is ethanol yield. Significance of the variables/parameters was identified from analysis of variance (ANOVA).

Table 3.4 shows the experimental design of fermentation process which includes the independent variables (sugar level, inoculum level and temperature) and ethanol yield as dependent or response variable. The values of the independent variables were fed to design expert software for randomization of the experimental runs and analysis.

Table 3.4: Experimental design for fermentation process

Variable name	Unit	Factor (X)	levels		
			Minimum	Middle	Maximum
Sugar level	g	A	4.17	4.75	5.36
Inoculum level	% v/v	B	5	10	15
Temperature	℃	C	25	30	35

4. Result and Discussion

4.1. Characterization of corn cob

4.1.1. Proximate analysis

Moisture content is a measure of the amount of water in the corncob. Moisture content analysis used for the determination of proportionality of solid to liquid ratio in the pretreatment and hydrolysis method with increasing moisture content it affects the product quality. The analysis of total moisture is used to determine other properties such as volatile matter, ash content and fixed carbon. The sample of corncob with higher moisture content needs more heat for moisture vaporization. Ash is a measure of inorganic impurities in the corncob. In this study low ash content of corncob constituents, so decreasing sludge formation in the ethanol production. Finally, fixed carbon (FC) it is the carbon found in the material which is left after volatile materials are driven off this is used for the determination of carbon in the corncob(G/Mariam, 2016).

Volatile mater and fixed carbon are the organic content of the corn cob. The high value of organic matter leads to high amount of carbohydrate so that high amount of ethanol can be produced.

Table 4.1: Results of proximate analyses (physical composition) of the corncob sample

#	Physical content	Weight percentage (%wt. dry basis)
1	Moisture	3.46
2	Volatile matter	76.5
3	Ash	1.6
4	Fixed carbon	18.44

4.1.2. Chemical composition

Corn cob was characterized for its chemical content as shown in figure 4.2. The cellulose and hemicellulose content of corn cob both account about 81% which indicated the presence of high amount of fermentable sugar.

Table 4.2: Results of percent of chemical content of the corncob sample

#	Chemical content	Weight percentage (%wt. dry basis)
1	Extractive	2.5
2	Hemicellulose	35.4
3	Lignin	14.2
4	Cellulose	46.3

Literature(Kudirat, 2012)data for corncob of chemical composition analysis range from 32.5 to 45.6% of cellulose, 39.8% hemicellulose, and 3% extractive. (Samuel, 2010)also reported, 59.4 % cellulose, 6.5% hemicellulose, and 22.2% lignin. The results from this study are not comparable range with literature values as reported by the aforementioned researchers. This may be due to two reasons; the variety of the corn or the experimental works are different.

4.2. Total carbohydrates

Total reduced sugar content of the corn cob hydrolysate was determined using phenol sulfuric acid method. Thus, sugar concentrations and their absorbances in UV spectrophotometry were discussed in table 4.3.

Table 4.3: Standard glucose concentration and its absorbance

Glucose concentration (g/L)	Absorbance
1	0.12
3	0.203
5	0.298
7	0.394

Using excel, the calibration curve of glucose standard was sketched and the linear equation produced from the sketch was used for determination of unknown glucose concentration is as follows,

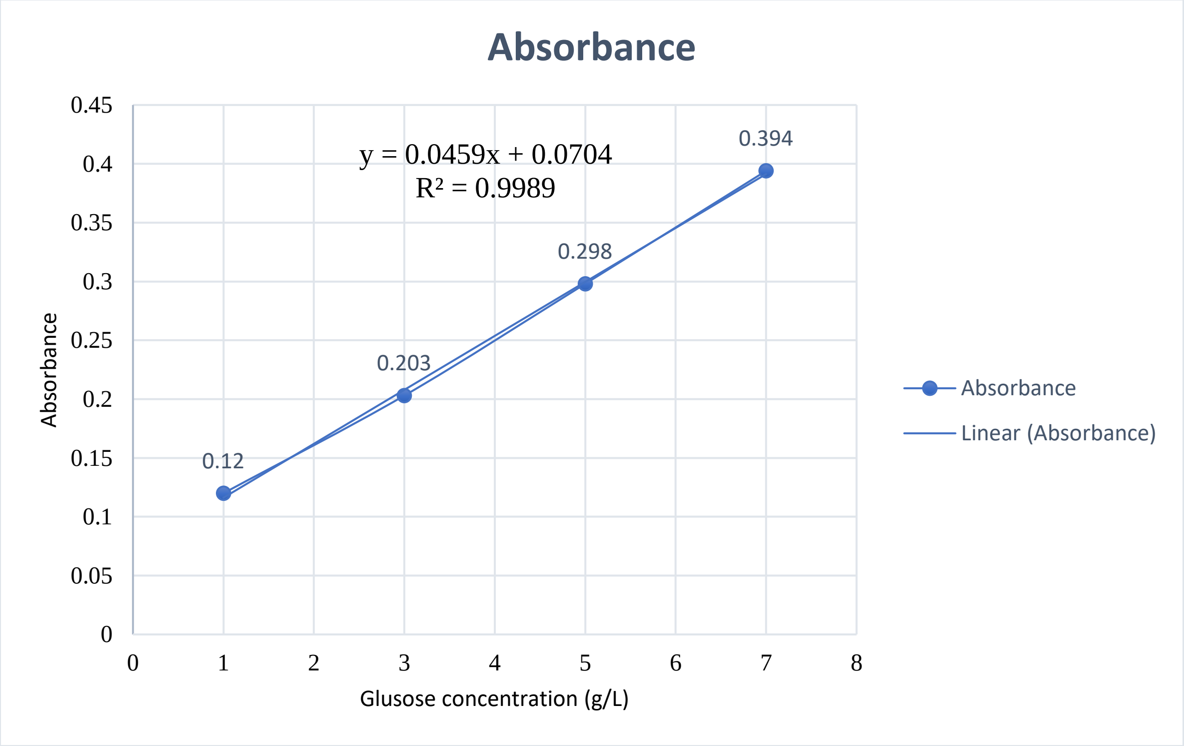


Figure 4.1: Calibration curve of glucose standard for determination of glucose content

$$y = mx + b \qquad (4.1)$$

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

From the graph above intercept is 0.07035 and slope is 0.0459

Then the equation becomes Y= 0.0459*X + 0.0704 with R-square of 0.99828.

From the equation x is the unknown variable, so rearranging it,

$$X = (Y - 0.0704)/0.0459 \qquad (4.2)$$

According to the above equation of a standard curve of glucose the concentrations of unknown sugar samples were determined. The maximum, medium and minimum sugar yield from the hydrolysis became 53.56, 47.47 and 41.65 g/L respectively. The products are a little bit high and this is because there was effective hydrolysis was undertaken or the standard equation has an effect on calculating the hydrolysate yield. (Mebrahtom, 2014) has obtained 45.088 g/L of sugar yield at 1.5% of acid, 91℃ and 22 minutes. It is possible to say that as acid concentration increases sugar yield could also increase. Generally, table 4.4 is the sugar yield from the hydrolysis of different experimental runs which are calculated using equation (4.2).

Table 4.4: Sugar concentration of the hydrolysate of corn cob at different conditions

Run Nº	Acid Conc. (%)	Temperature (°C)	Time (min)	Sugar concentration (g/L)
1	2	100	30	41.89
2	4	100	30	47.93
3	2	140	30	43.67
4	4	140	30	53.56
5	2	120	20	42.57
6	4	120	20	47.37
7	2	120	40	41.65
8	4	120	40	50.1
9	3	100	20	47.26
10	3	140	20	48.53
11	3	100	40	48.25
12	3	140	40	51.04
13	3	120	30	47.65
14	3	120	30	48.91
15	3	120	30	47.26
16	3	120	30	47.47
17	3	120	30	48.39

From the above table 4.4, it is observed that, as the temperature and acid concentration increase the sugar yield also increases. In addition to this with respect to time of hydrolysis the glucose yield is generally high at 40 minutes. Based on this, the maximum yield of glucose concentration was noted at 4% acid concentration, at a temperature of $120°C$ and hydrolysis time of 30 minutes. Because the 4% acid concentration is sufficient than other concentrations for hydrolysis. The medium yield of glucose concentration at 3% of acid concentration, at a temperature of $120°C$ and hydrolysis time of 30 minutes and the minimum yield of glucose concentration noted at 2% of acid concentration, at a temperature of $120°C$ and hydrolysis time of 40 minutes. The model of hydrolysis step is valid and all the variables are significant. See Appendix C for some detail of statistical analysis.

Fermentation process was done from the above results of minimum, medium and maximum sugar concentrations of 4.17, 4.747 and 5.36 g respectively for ethanol production using inoculum dose of 5, 10 and 15 (%v/v) at 25, 30 and 35 ℃.

4.3. Statistical analysis of the experimental results

4.3.1. Analysis of variance

In order to investigate the effect of different fermentation variables in ethanol production from corn cob, Box-Behnken design was used for analysis of variance (ANOVA). The inputs for design expert are sugar, inoculum levels and temperature as independent variables and ethanol yield as dependent variable. Ethanol yield was calculated using the formula:

$$\text{Ethanol yield (mL/g)} = \text{(Actual volume of ethanol (mL))}/\text{amount of sugar (g)} \quad (4.3)$$

Table 4.5: Experimental data of fermentation parameters and ethanol yield

No	Sugar (g)	Inoculum (%v/v)	Temp. (℃)	Ethanol yield (g)	Ethanol yield mL/g)
1	4.17	5	30	1.1	0.33
2	5.36	5	30	1.5	0.36
3	4.17	15	30	1.21	0.37
4	5.36	15	30	2.01	0.48
5	4.17	10	25	1.05	0.32
6	5.36	10	25	1.80	0.43
7	4.17	10	35	1.05	0.32
8	5.36	10	35	1.66	0.39
9	4.75	5	25	1.54	0.41
10	4.75	15	25	1.59	0.42
11	4.75	5	35	1.4	0.37
12	4.75	15	35	1.7	0.45
13	4.75	10	30	1.89	0.51
14	4.75	10	30	2.08	0.55
15	4.75	10	30	1.83	0.49
16	4.75	10	30	1.87	0.5
17	4.75	10	30	2.01	0.54

The maximum yield is 0.55 mL/g or 43.6%. In a research conducted by (Luis et al., 2016) maximum ethanol yield from corn cob achieved was 0.49%. When compared there is visible difference. This could be due to difference in process effectiveness.

The data in table 4.5 were fed into design expert software and generated the build information of the experimental analysis of the fermentation process as shown in table 4.6.

Table 4.6: Build Information of experimental analysis of fermentation process

File Version	11.1.0.1		
Study Type	Response Surface	Subtype	Randomized
Design Type	Box-Behnken	Runs	17
Design Model	Quadratic	Blocks	No Blocks

The probability, P-values was used to check the significance of each coefficient of regression model. equation. The P-values of corresponding coefficient should be less than or equals to 0.05.

Table 4.7: ANOVA for quadratic model of ethanol yield

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0871	9	0.0097	17.93	0.0005	significant
A-Sugar	0.0122	1	0.0122	22.67	0.0021	
B-Inoculum	0.0078	1	0.0078	14.36	0.0068	
C-Temperature	0.0002	1	0.0002	0.4240	0.5357	
AB	0.0019	1	0.0019	3.48	0.1043	
AC	0.0003	1	0.0003	0.5129	0.4971	
BC	0.0011	1	0.0011	1.96	0.2047	
A^2	0.0359	1	0.0359	66.40	< 0.0001	
B^2	0.0076	1	0.0076	13.98	0.0073	
C^2	0.0152	1	0.0152	28.16	0.0011	
Residual	0.0038	7	0.0005			
Lack of Fit	0.0008	3	0.0003	0.3321	0.8040	not significant
Pure Error	0.0030	4	0.0008			
Cor Total	0.0909	16				

F- Value is a test for comparing model variance with residual (error) variance. If the variances are close to the same, the ratio will be close to one and it is less likely that any of the 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 17.93 implies the model is significant. There is only a 0.05% chance that an F-value this could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case A, B, A^2, B^2, C^2 are significant model terms.

The Lack of Fit F-value of 0.33 implies the Lack of Fit is not significant relative to the pure error. There is a 80.40% chance that a Lack of Fit F-value this large could occur due to noise. Non-significant lack of fit is good -- we want the model to fit.

Coefficient of Variation, the standard deviation expressed as a percentage of the mean; Predicted Residual Error Sum of Squares, which is a measure of how the model fits each point in the design; the R-Squared, measure of the amount of variation around the mean explained by the model; Adjusted R^2 that is a measure of the amount of variation around the mean explained by the model, Predicted R^2, a measure of the amount of variation in new data explained by the model, and Adequate Precision, this is a signal to disturbance ratio due to random error, presented in the table below, are used to decide whether the model can be used or not.

Table 4.8: Fit Statistics measures

Std. Dev.	0.0232	R^2	0.9584
Mean	0.4260	Adjusted R^2	0.9050
C.V. %	5.46	Predicted R^2	0.8153
		Adeq Precision	10.8583

The Predicted R^2 of 81.53% is in reasonable agreement with the Adjusted R^2 of 90.5%; i.e. the difference is less than 0.2%.

Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. the ratio of 10.858 indicates an adequate signal. This model can be used to navigate the design space.

Regression model equation

Table 4.9: Regression coefficients in terms of coded factors

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	0.5182	1	0.0104	0.4936	0.5428	
A-Sugar	0.0391	1	0.0082	0.0197	0.0586	1.00
B-Inoculum	0.0311	1	0.0082	0.0117	0.0506	1.00
C-Temperature	-0.0054	1	0.0082	-0.0248	0.0141	1.00
AB	0.0217	1	0.0116	-0.0058	0.0492	1.00
AC	-0.0083	1	0.0116	-0.0358	0.0192	1.00
BC	0.0163	1	0.0116	-0.0112	0.0437	1.00
A^2	-0.0924	1	0.0113	-0.1192	-0.0656	1.01
B^2	-0.0424	1	0.0113	-0.0691	-0.0156	1.01
C^2	-0.0601	1	0.0113	-0.0869	-0.0333	1.01

The coefficient estimate represents the expected change in response per unit change in factor value when all remaining factors are held constant. The intercept in an orthogonal design is the overall average response of all the runs. The coefficients are adjustments around that average based on the factor settings. When the factors are orthogonal the VIFs are 1; VIFs greater than 1 indicate multi-collinearity, the higher the VIF the more severe the correlation of factors. As a rough rule, VIFs less than 10 are tolerable.

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

The model equation, which relates the response (ethanol yield) to the independent variables (factors) in terms of the coded and actual factors is given below.

Final equation in terms of coded factors:

$$\begin{aligned}\text{Ethanol yield} = {} & 0.5182 + 0.0391 \times \mathrm{A} + 0.0311 \times \mathrm{B} - 0.0054 \times \mathrm{C} + 0.0217 \times \mathrm{AB} \\ & - 0.0083 \times \mathrm{AC} + 0.0163 \times \mathrm{BC} - 0.0924 \times \mathrm{A}^2 - 0.0424 \times \mathrm{B}^2 \\ & - 0.0601 \\ & \times \mathrm{C}^2 \qquad (4.4)\end{aligned}$$

Final equation in terms of actual factors

$$\begin{aligned}\text{Ethanol Yield} = {} & -7.9174 + 2.5564 \times \text{sugar} - 0.01405 \times \text{Inoculum} \\ & + 0.149973 \times \text{Temperature} + 0.007281 \times \text{Sugar} \times \text{Inoculum} \\ & - 0.002794 \times \text{Sugar} \times \text{Temperature} \\ & + 0.00065 \times \text{Inoculum} \times \text{Temperature} - 0.0026044 \times \text{Sugar}^2 \\ & - 0.001694 \times \text{Inoculum}^2 \\ & - 0.0022404 \times \text{Temperature}^2 \qquad (4.5)\end{aligned}$$

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.

4.3.2. Graphical analysis (diagnostic plot)

The following graphical plots were used for checking the adequacy of the model for ethanol production from corn cob hydrolysate.

Normal probability plot

A normal probability plot of the raw data is used to check the assumption of normality. In the analysis of variance, it is usually more effective and straight forward to do this with the residuals. If the underlying error distribution is normal, this plot will resemble a straight line. In visualizing the straight line, place more emphasis on the central values of the plot than on the extremes. In addition, the normal probability plot indicates the residuals following a normal distribution. In the case of this experiment the points in the plots shows fit to a straight line, this shows that the quadratic polynomial model satisfies the assumptions of analysis of variance (ANOVA) i.e. the error distribution is approximately normal. The points are coded by color to the level of response and they represent going from cool blue for lowest values to hot red for the highest.

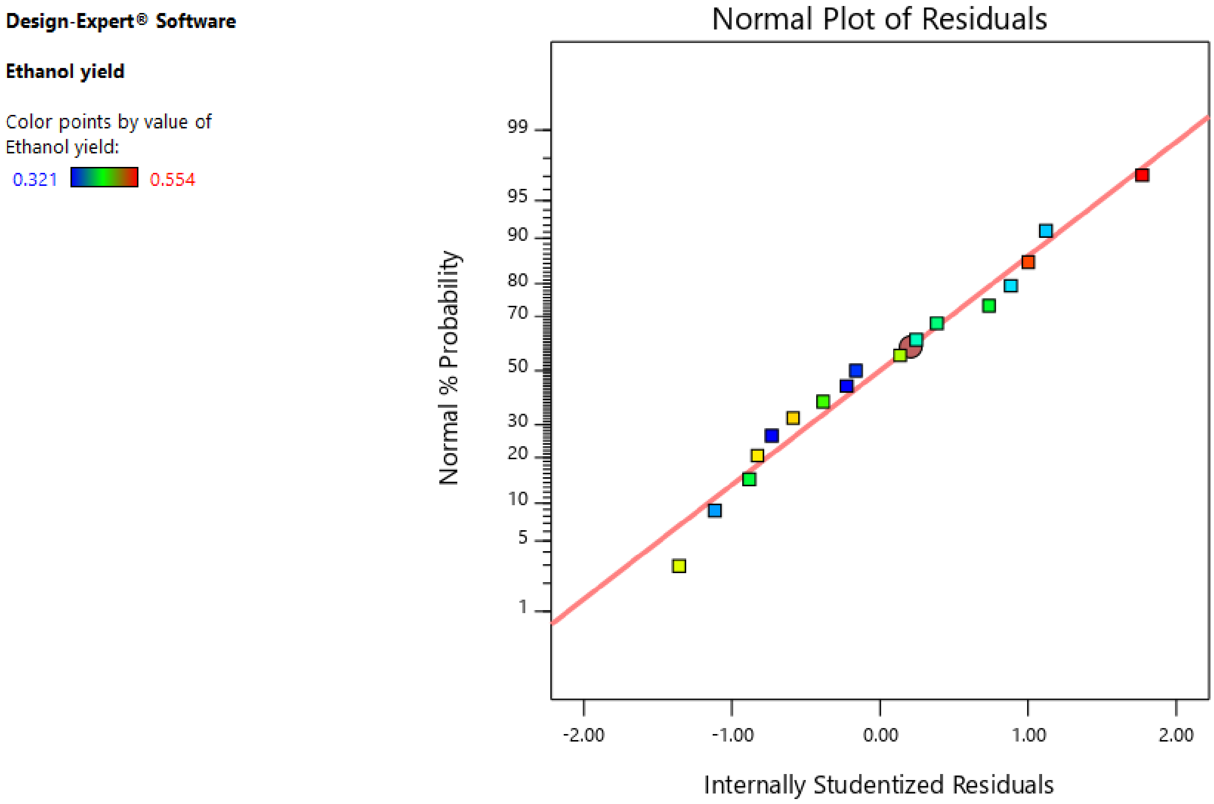


Figure 4.2: Normal plots of residuals for ethanol yield

Residual versus predicted plot

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. This is a plot of the residuals versus the ascending predicted response values. It tests the assumption of constant variance. The plot should be a random scatter (constant range of residuals across the graph). The plot shows random scatter which justifying no need for an alteration to minimize personal error. Look at the figure below,

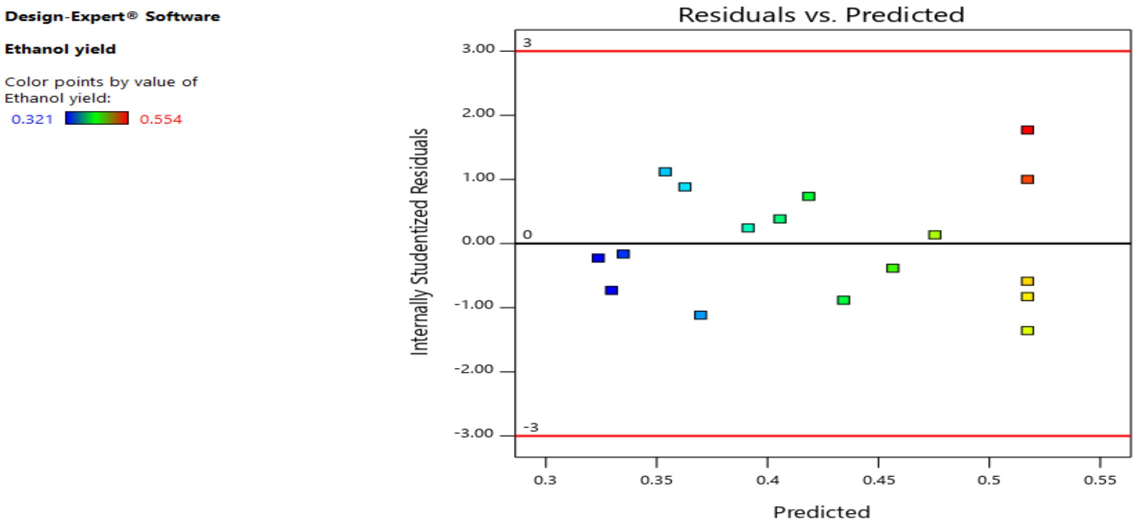


Figure 4.3: Residual versus predicted values

Residual versus run number plot

A plot of the residuals versus the experimental run order checks for lurking variables that may have influenced the response during the experiment. The plot should show a random scatter. Trends indicate a time-related variable lurking in the background. Blocking and randomization provide insurance against trends ruining the analysis. The random scatter makes zigzag along the origin line. The figure below shows the residual versus run.

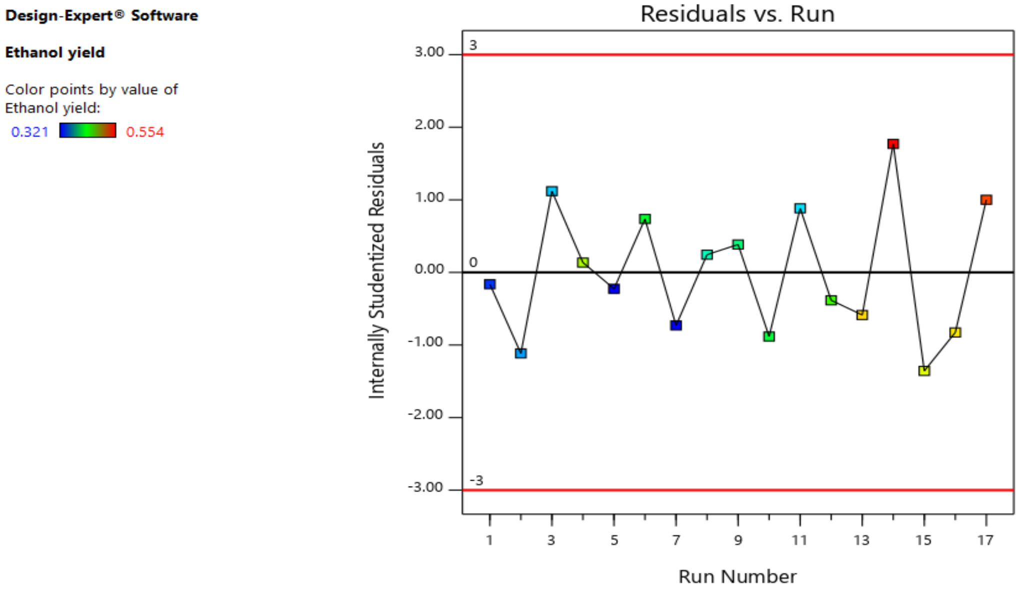


Figure 4.4: Residuals vs. experimental run number

Actual versus predicted plot

In the figure below predicted versus actual value of ethanol yield was plotted. The plot shows how precisely the model modeled. The purpose is to detect a value, or group of values, that are not easily predicted by the model. The points show how the predicted value and actual values of each run approach the straight line. The straight line shows how the predicted and actual values are closer to each other. When the point is above the straight-line predicted value is greater than the actual value and the reverse is also true. If the scatter of the plot lies almost on the diagonal line, this shows that the model is designed very well i.e. the experimental data is closely related to the data predicted from the model.

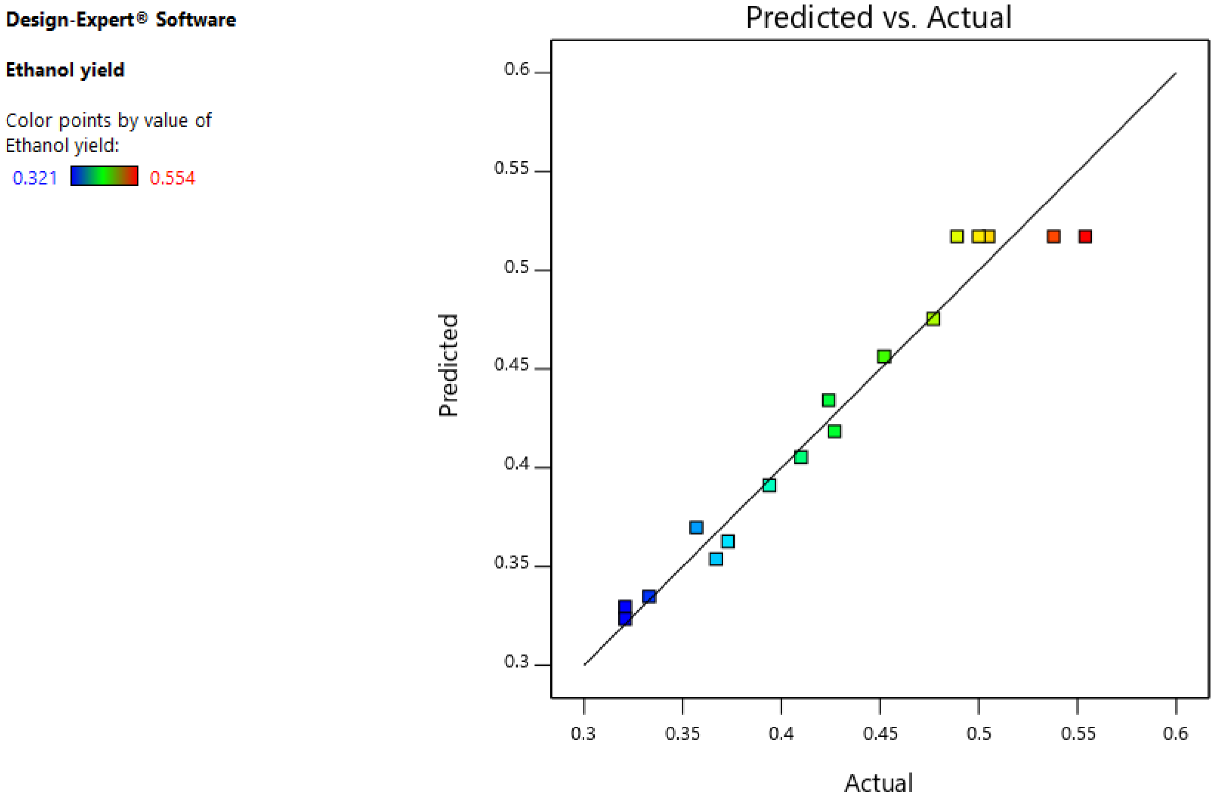


Figure 4.5: Actual versus predicted values of ethanol yield

Diagnostics report

This section contains descriptions of each case statistic. The values in the report Table 4.10 are used to produce the diagnostics graphs. The actual values are the measured response data for the particular run. Predicted value**s** are the value predicted from the model, generated using the prediction equation. Residual**s** are difference between actual and predicted values for each point. The residual values are close to each other. This shows there is small difference between actual and predicted values and normal error distribution.

Table 4.10: Diagnostics report of ethanol yield

Run Order	Actual Value	Predicted Value	Residual	Leverage	Internally Studentized Residuals	Externally Studentized Residuals	Cook's Distance	Influence on Fitted Value DFFITS	Standard Order
1	0.3330	0.3349	-0.0019	0.744	-0.163	-0.151	0.008	-0.257	4
2	0.3570	0.3698	-0.0128	0.756	-1.116	-1.139	0.386	-2.005	2
3	0.3670	0.3538	0.0132	0.744	1.119	1.144	0.364	1.949	15
4	0.4770	0.4754	0.0016	0.756	0.136	0.126	0.006	0.221	1
5	0.3210	0.3237	-0.0027	0.744	-0.226	-0.210	0.015	-0.358	17
6	0.4270	0.4185	0.0085	0.756	0.736	0.710	0.168	1.250	14
7	0.3210	0.3296	-0.0086	0.744	-0.731	-0.704	0.155	-1.200	11
8	0.3940	0.3912	0.0028	0.756	0.244	0.227	0.018	0.399	6
9	0.4100	0.4055	0.0045	0.750	0.384	0.359	0.044	0.623	10
10	0.4240	0.4343	-0.0103	0.750	-0.883	-0.867	0.234	-1.502	7
11	0.3730	0.3627	0.0103	0.750	0.883	0.867	0.234	1.502	8
12	0.4520	0.4565	-0.0045	0.750	-0.384	-0.359	0.044	-0.623	16
13	0.5050	0.5172	-0.0122	0.200	-0.587	-0.557	0.009	-0.279	3
14	0.5540	0.5172	0.0368	0.200	1.770	2.206	0.078	1.103	5
15	0.4890	0.5172	-0.0282	0.200	-1.357	-1.463	0.046	-0.731	12
16	0.5000	0.5172	-0.0172	0.200	-0.827	-0.807	0.017	-0.403	13
17	0.5380	0.5172	0.0208	0.200	1.001	1.001	0.025	0.500	9

4.4. Effects of fermentation parameters on ethanol yield

4.4.1. Effect of sugar level on the ethanol yield

From the plot of sugar level versus ethanol yield, as shown in figure 4.6 below as increasing sugar amount from the minimum to the middle and the yield of ethanol rapidly increased then it is slowly decreased when the amount sugar goes high. The ethanol yield at low sugar level is too low. This is because of the low concentration of fermentable sugars(Mutepe, 2012). The ethanol yield decrement was also due to the reason that the hydrolysate could include furfural, acetic acid, and other compounds. Furfural is a characteristic compound present in dilute acid hydrolysates(Gunnar et al., 2003). Here, the maximum ethanol yield is at a sugar of 4.85 g.

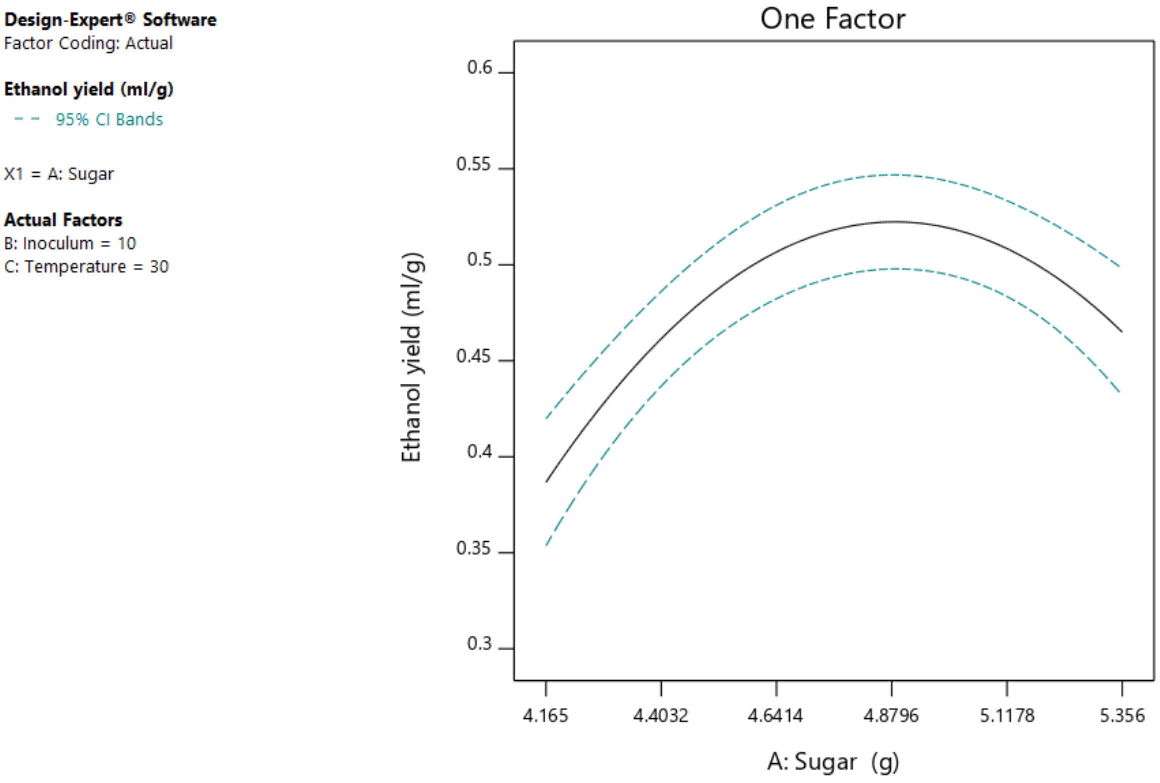


Figure 4.6: Effect of sugar level on ethanol yield

4.4.2. Effect of inoculum on the ethanol yield

The probability value of less than 0.05 in case of the inoculum size shows that yield is greatly affected by it. The effect of inoculum dose on the ethanol yield is drowned as in Figure 4.7 and the curve generally describes when inoculum level increased ethanol yield also slightly increased and after the maximum yield it gradually and slowly decreased due to substrate(sugar) depletion. When carefully looked at the figure the highest yield of ethanol is obtained at inoculum dose of 11.6 (%v/v).

(Adelabu et al., 2018)showed that highest volume of ethanol was produced with 7.5 % inoculum concentration, followed by 5 % inoculum concentration while 15 % produced the least bioethanol.

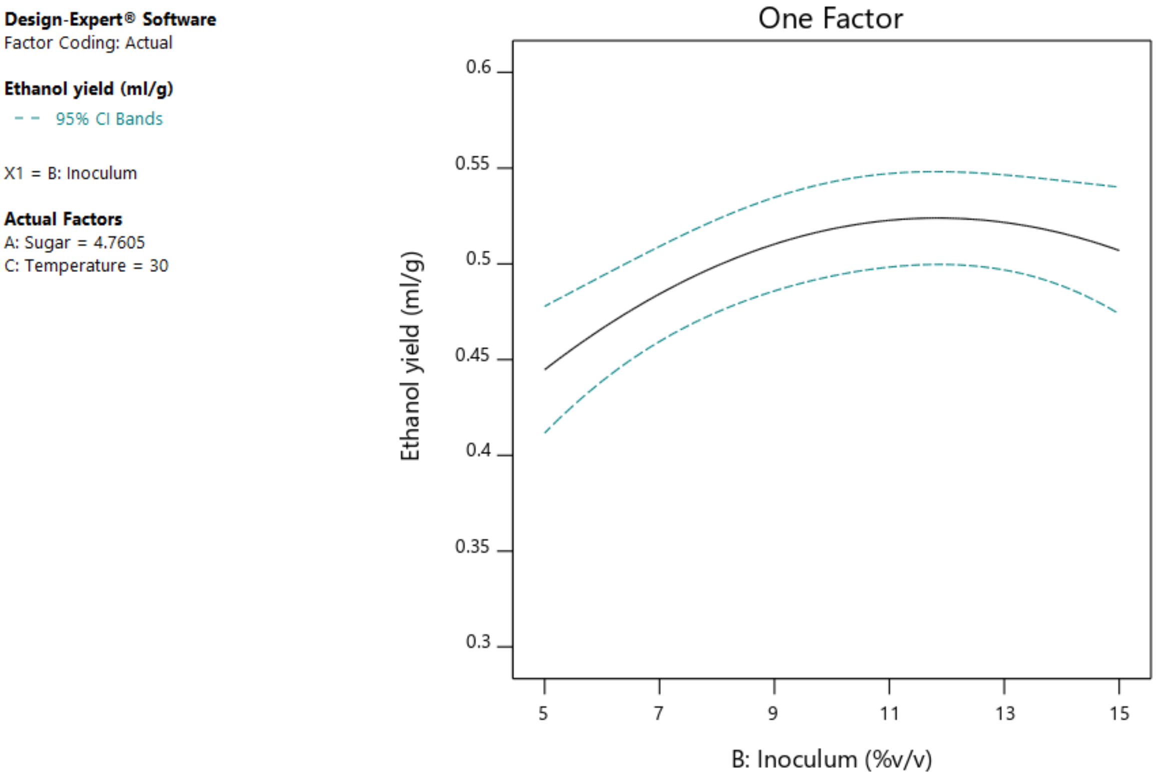


Figure 4.7: Effect of inoculum dose on ethanol yield

4.4.3. Effect of Temperature on the ethanol yield

From the figure below, the maximum ethanol yield was obtained at around 30 ℃ of temperature. The yield slightly increased with temperature until it reaches the maximum value and showed a significant decrement with increase in temperature beyond 30 ℃ of the fermentation process.

Generally the selected temperature range has less effect on fermentation process of ethanol production(Chacha et al., 2016).

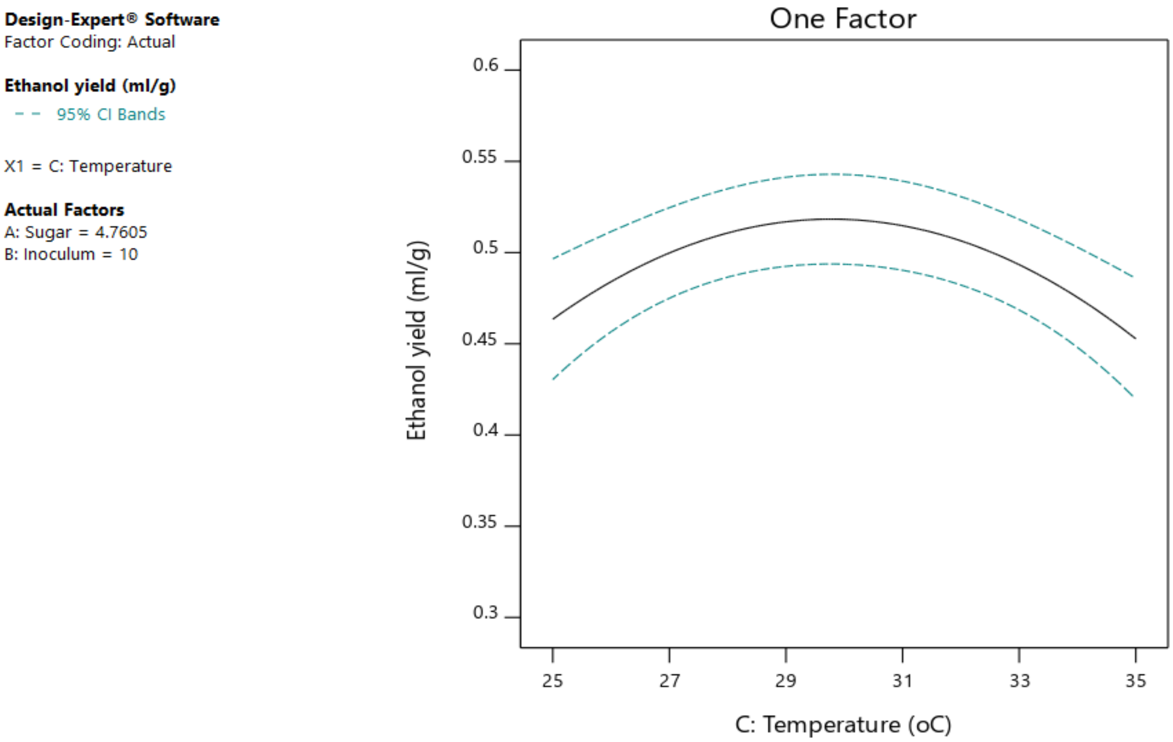


Figure 4.8: Effect of temperature on ethanol yield

4.4.4. Effect of sugar and inoculum on the ethanol yield

The figure below shows sugar level and inoculum effect on ethanol yield when temperature is constant at 30 °C. As it observed from figure the sugar amount and inoculum have not interaction effect on the ethanol yield. The red and the black curves created a conical shape together; thus, it is possible to say there is no significant interaction effect of both parameters on ethanol yield. Figure 4.9 shows high ethanol yield is obtained at high sugar concentration and high inoculum level than at high sugar concentration and lower level of inoculum. Even though the yield showed slight decrement after its maximum value, higher decrement is observed at low inoculum level than at high level of the same sugar concentration.

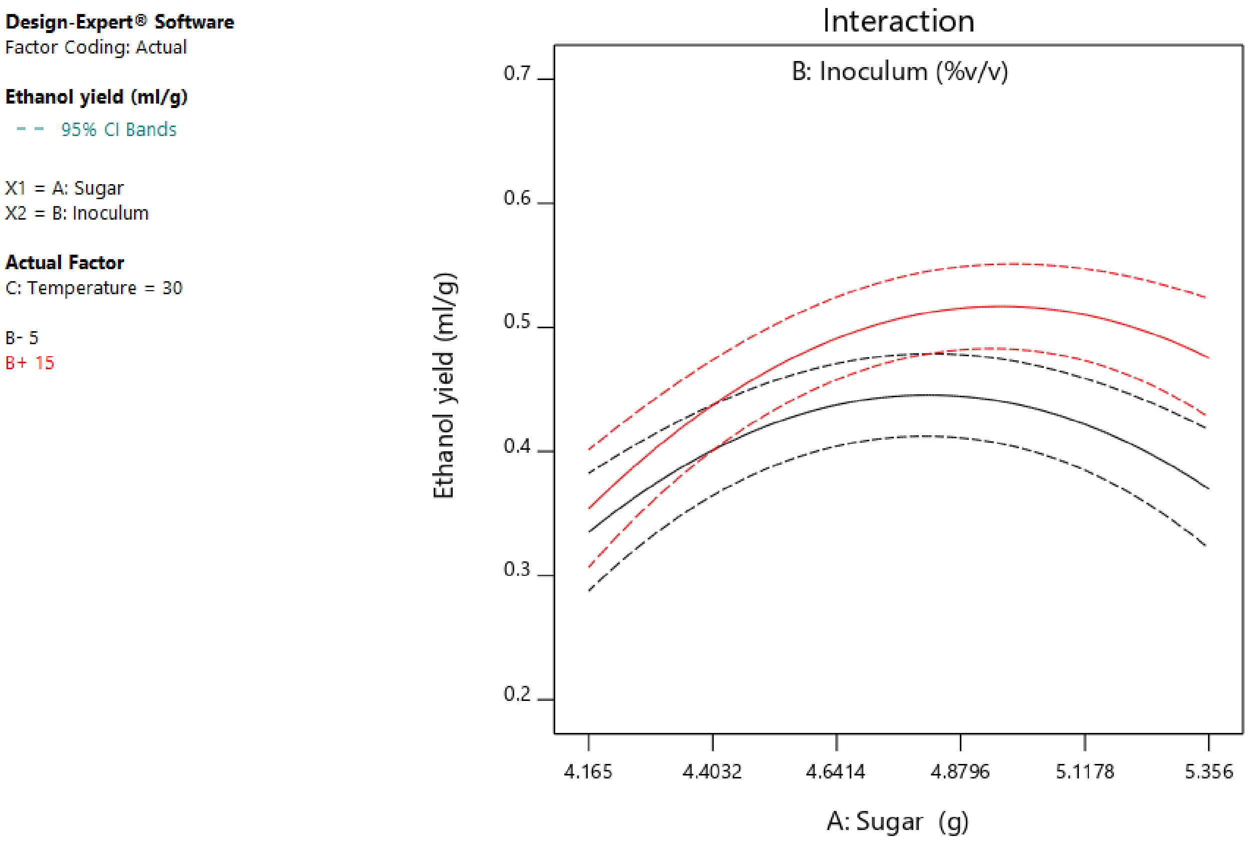


Figure 4.9: Interaction effect of sugar level and inoculum on ethanol yield at fixed (middle) temperature

Contour and response surface plot for the effect of sugar level and inoculum

In order to analyze the regression equation of the model, 2D contour and three-dimensional surface plots were obtained by plotting the response on the Z axis against any two variables while keeping the other variable at center level. The contour plot is a two-dimensional (2D) representation of the response plotted against combinations of numeric factors and/or mixture components. It can show the relationship between the responses, mixture components and/or numeric factors. Conical shape response surface plot indicates optimum operating conditions. The response optimized value for the production of bioethanol was based on the two process variables described on the response surface plot.

There is color change on the graph and the response variable is increasing from green color to red. The graph suggests operating at the center point where the response variable shows maximum amount. Operating in the red region is good to have high amount of ethanol yield.

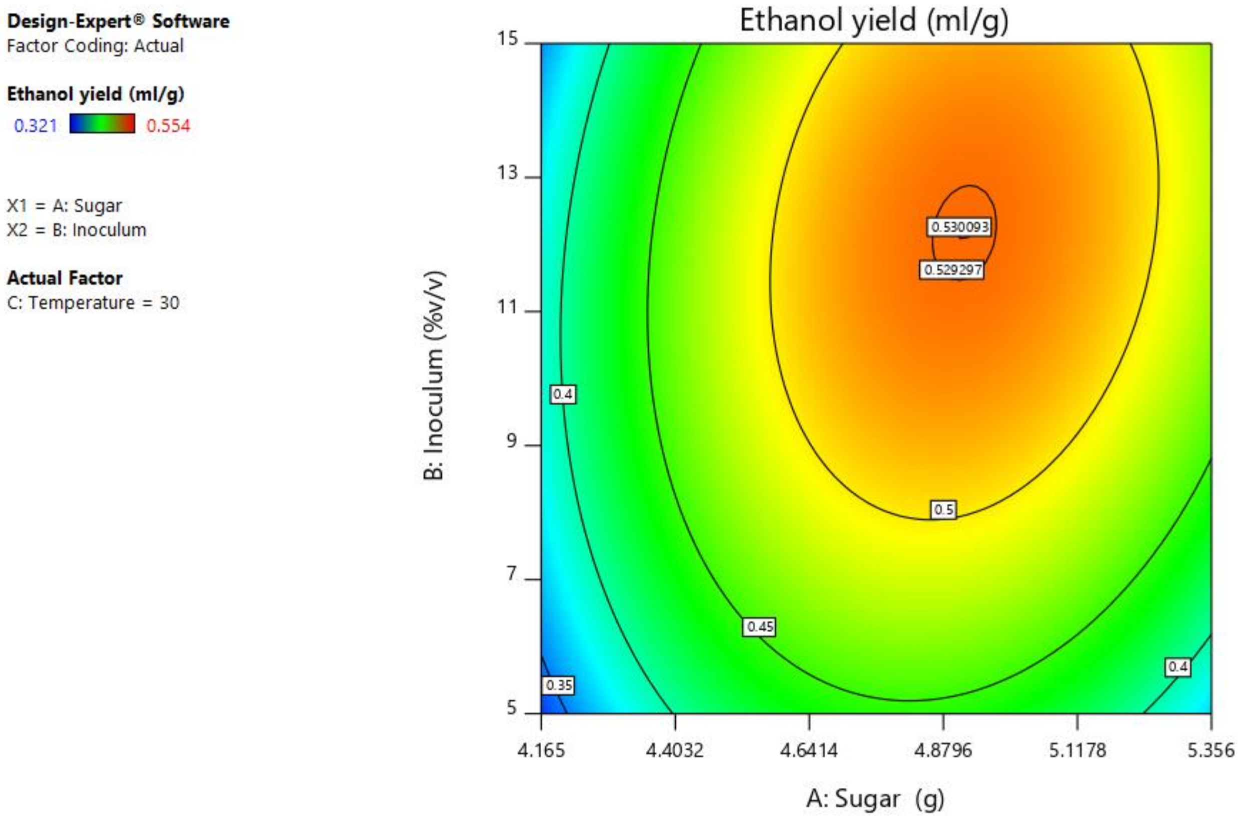


Figure 4.10: Contour plot of the effect of sugar level and inoculum at constant temperature (middle value)

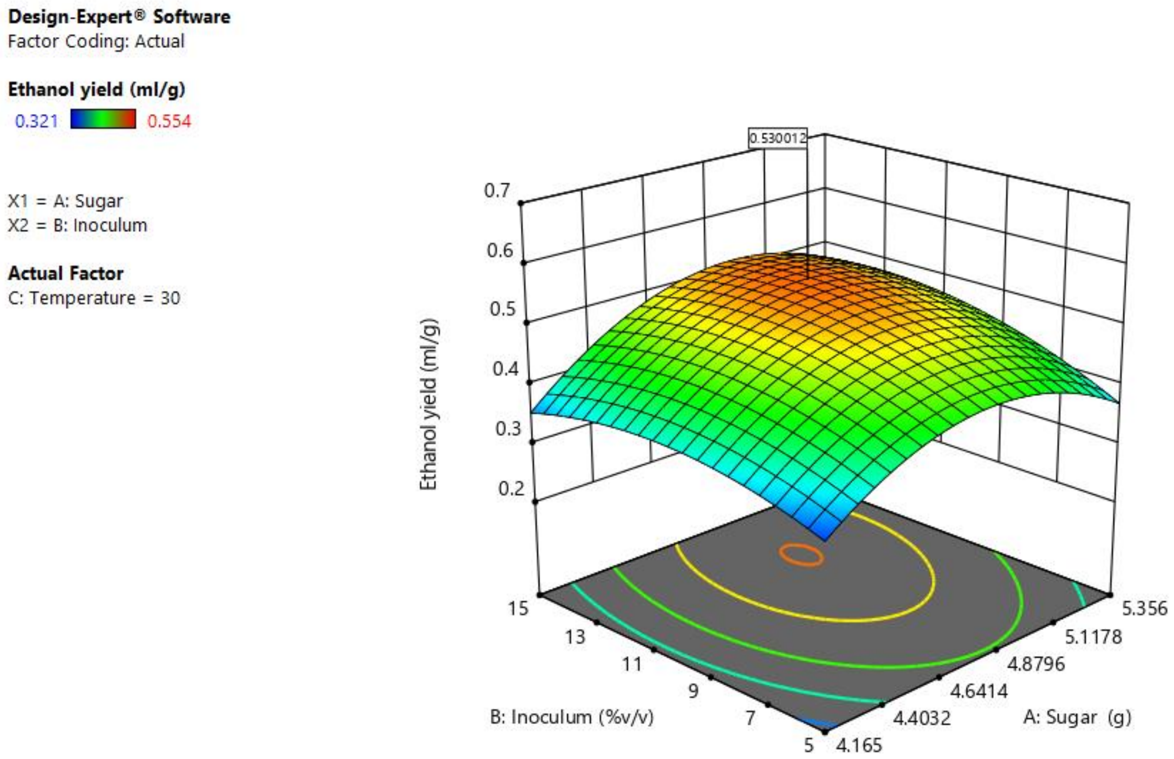


Figure 4.11: 3D surface plot of the effect of sugar level and inoculum at constant temperature (middle value)

From Figure 4.10, it is shown that a maximum ethanol yield of 0.53 ml/g is obtained at 4.9 g of sugar and inoculum of 12.13 %v/v. Similarly, in Figure 4.11 above the maximum ethanol yield is 0.53 mL/g which is obtained at sugar of 4.902 g and inoculum of 12.12 %v/v. So, both figures show similar quantitative meaning.

At constant temperature the highest ethanol yield is obtained from the nearly middle values of sugar concentration and inoculum level, which is similar to the actual observations and measurements.

4.4.5. Effect of sugar level and temperature on the ethanol yield

Figure 4.12 below shows the interaction effect of sugar level and temperature on ethanol yield when inoculum volume is constant at 10%(v/v). As it observed from figure the sugar level and temperature have very less interaction effect on the ethanol yield. As shown in the figure the red the black curves met each other, thus very less interaction effect was seen. Although the ethanol yield is rapidly increased with increase in sugar and temperature until it reaches the maximum value, the yield started to decrease fast at high sugar level and high temperature. Ethanol yield is at lower temperature is higher than at high temperature when the sugar level is high. This is because at high temperature the saccharomyces cerevisiae may not work well.

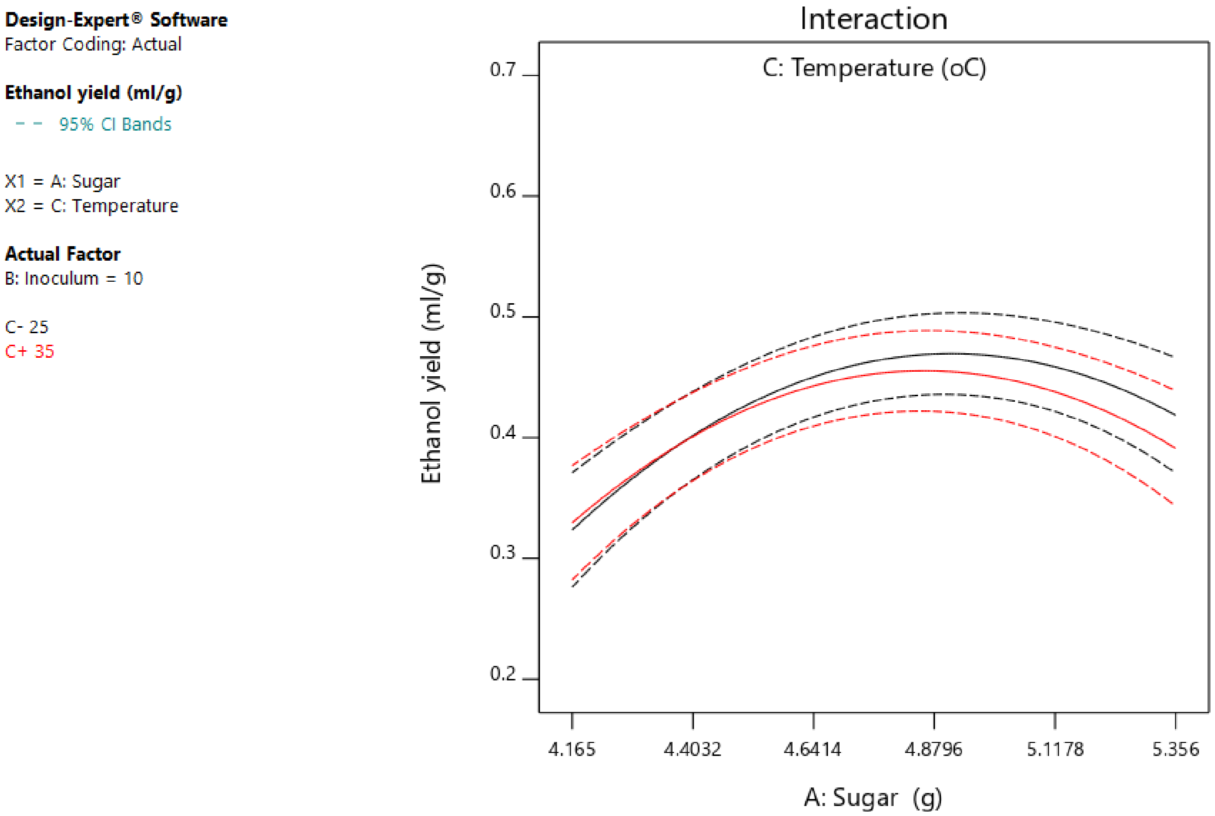


Figure 4.12: Interaction effect of sugar level and temperature on the ethanol yield at fixed inoculum level

Contour and response surface plot for the effect of sugar level and temperature

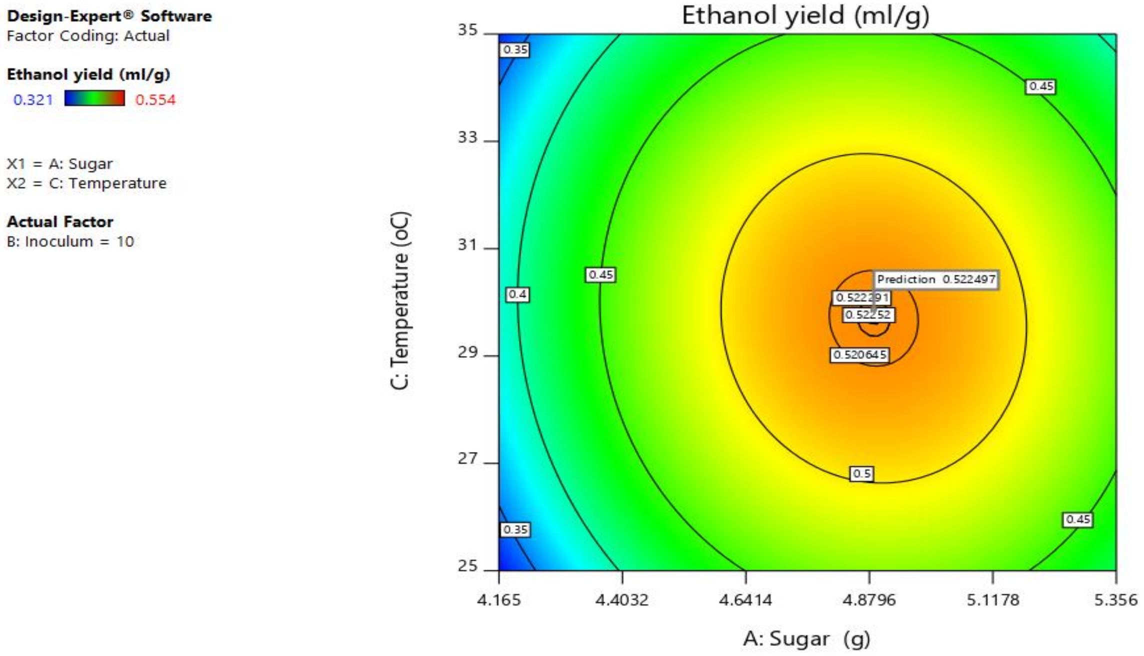


Figure 4.13: Contour plot of the effect of sugar level and temperature at constant inoculum (middle value)

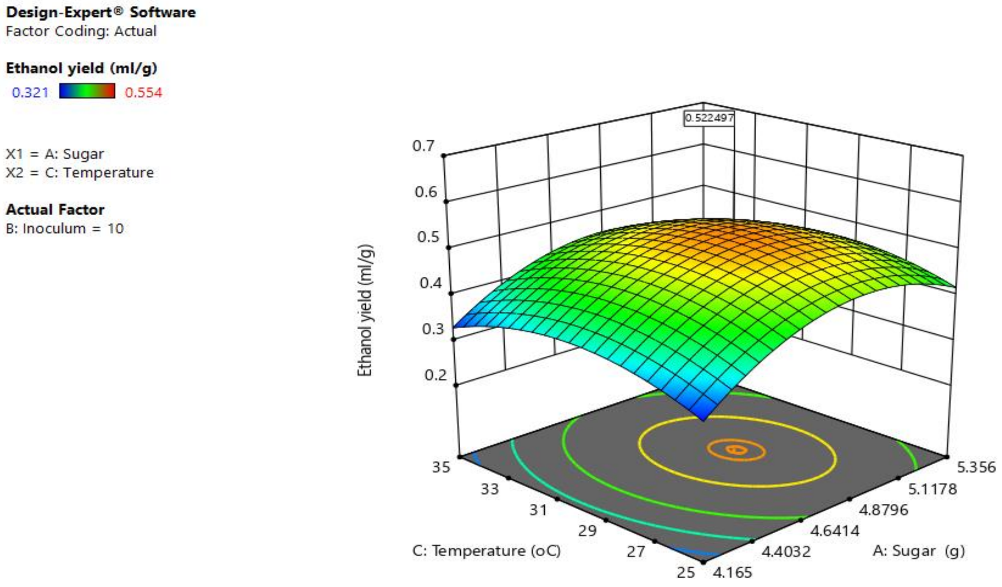


Figure 4.14: 3D surface plot of the effect of sugar level and temperature at constant inoculum (middle value)

From the figure, 4.13, maximum ethanol yield of 0.52 mL/g is obtained at 4.89 g of sugar and 29.85 ℃ of temperature and from figure 4.14, maximum yield is showed at 4.89 g of sugar and 29.86 ℃ of temperature. It indicates that both figures show similar quantitative meaning because both plots are only ways to show the effects of parameters over the dependent variable which is ethanol yield.

4.4.6. Effect of inoculum and temperature on the ethanol yield

The interaction effect of inoculum and temperature on the ethanol yield is given below in the following figure. Until it reaches maximum value, the yield is fairly increased with temperature. Ethanol yield is comparably high at low inoculum level and low temperature than at low inoculum level and high temperature. Then after its maximum value the yield is higher at high inoculum level and at high temperature than at low temperature. But there is a slight decrease in ethanol yield at high values of both factors.

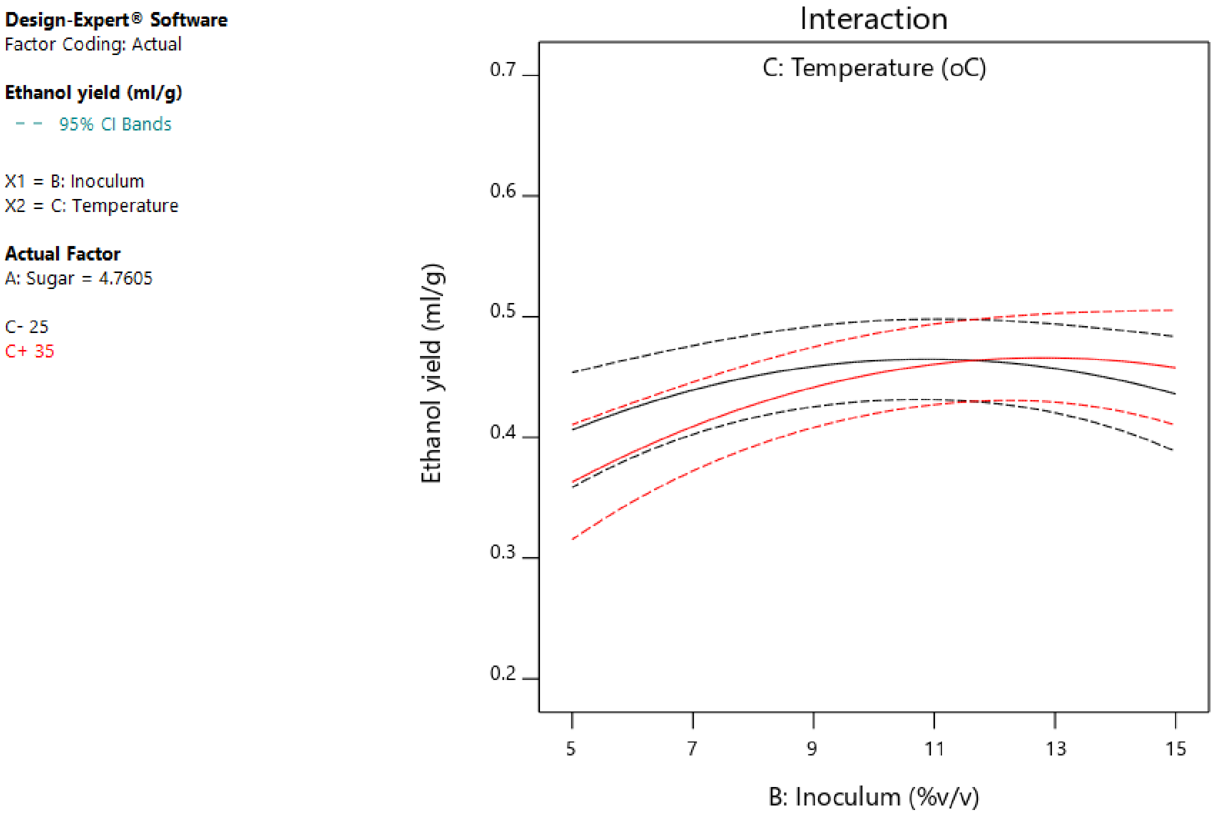


Figure 4.15: Interaction effect of inoculum and temperature on the ethanol yield at fixed (center point) sugar level

Contour and response surface plot for the effect of inoculum and temperature

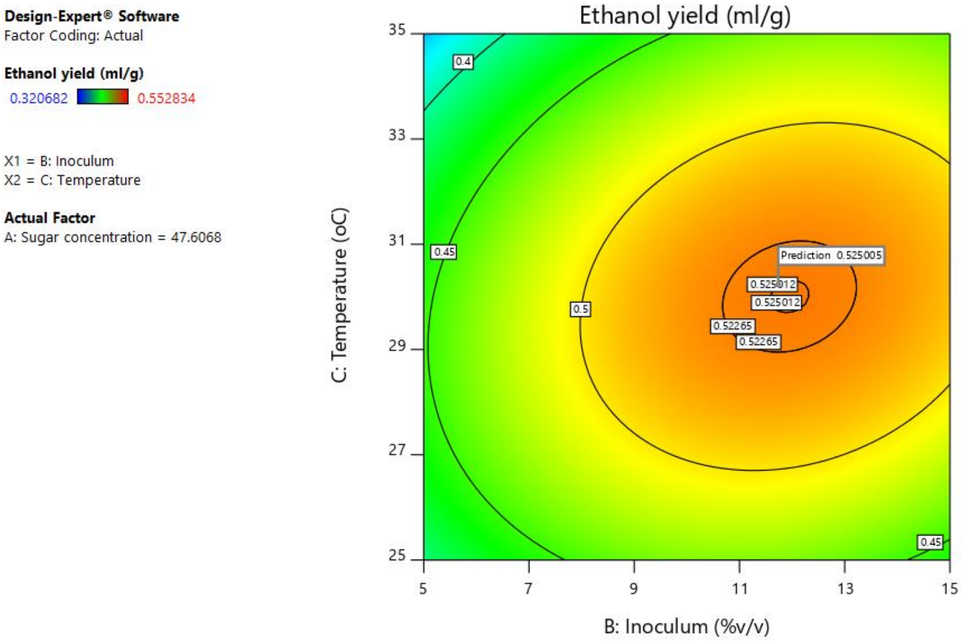


Figure 4.16: Contour plot of the effect of inoculum and temperature at constant sugar (middle value)

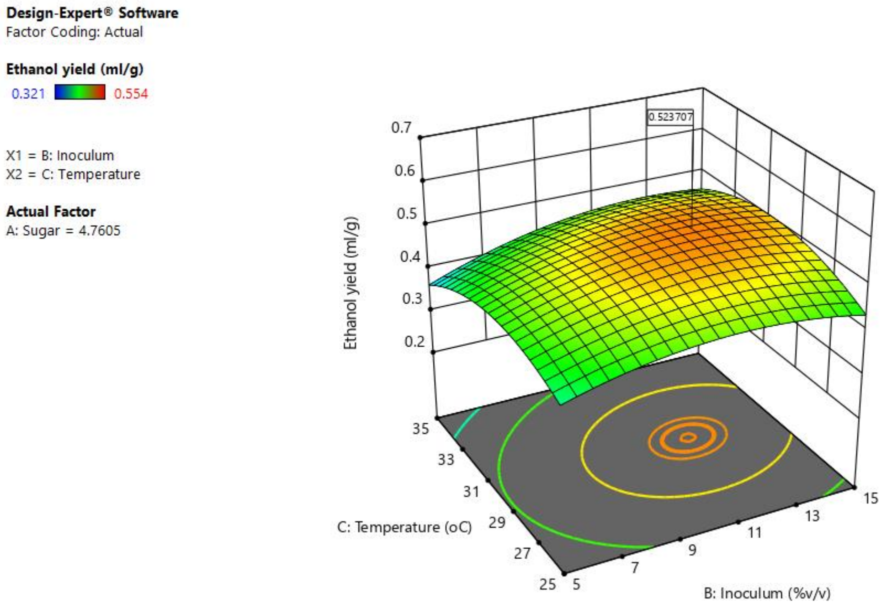


Figure 4.17: 3D surface plot of the effect of inoculum and temperature at constant sugar (middle value)

From the figure 4.16, it is shown that a maximum ethanol yield of 0.52 mL/g is obtained at inoculum of 11.99 %(v/v) and at a temperature of 30.29 ℃. Similarly, in figure 4.17, the maximum ethanol yield of 0.524 mL/g is obtained at inoculum of 12.8 %(v/v) and temperature of 30.36 ℃. Both figures show similar quantitative meaning.

4.5. Optimization of fermentation variables

The better ethanol yield came from the hydrolysate which is hydrolyzed using 3% acid at 120 ℃ and by fermenting the middle level of hydrolysate. Economically this is better solution. So, in the design expert the criteria of the sugar concentration, inoculum level and temperature were set in range and ethanol was set maximum as in Table 4.11. This arrangement (decision) gave the best of all possible solutions.

Table 4.11: Constraints for optimization of ethanol yield

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
A: Sugar	is in range	4.17	5.36	1	1	3
B: Inoculum	is in range	5	15	1	1	3
C: Temperature	is in range	25	35	1	1	3
Ethanol yield	maximize	0.32	0.55	1	1	3

From the 100 possible solutions the following table is the optimized solution. (appendix E for all the solutions)

Table 4.12: Optimized solution of ethanol yield from corn cob hydrolysate

Number	Sugar (g)	Inoculum (%v/v)	Temperature (℃)	Ethanol yield (mL/g)	Desirability	
1	4.92	12.2	29.98	0.53	0.898	Selected

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. From

Table 4.12, the maximum ethanol yield of 0.53 mL/g or 41.8 % was found at sugar level of 4.92 g, inoculum of 12.2 %v/v, and at a temperature of 29.98 ℃ with 89.8 % desirability. 3D response surface and couture plots of optimum possible solutions are as follows:

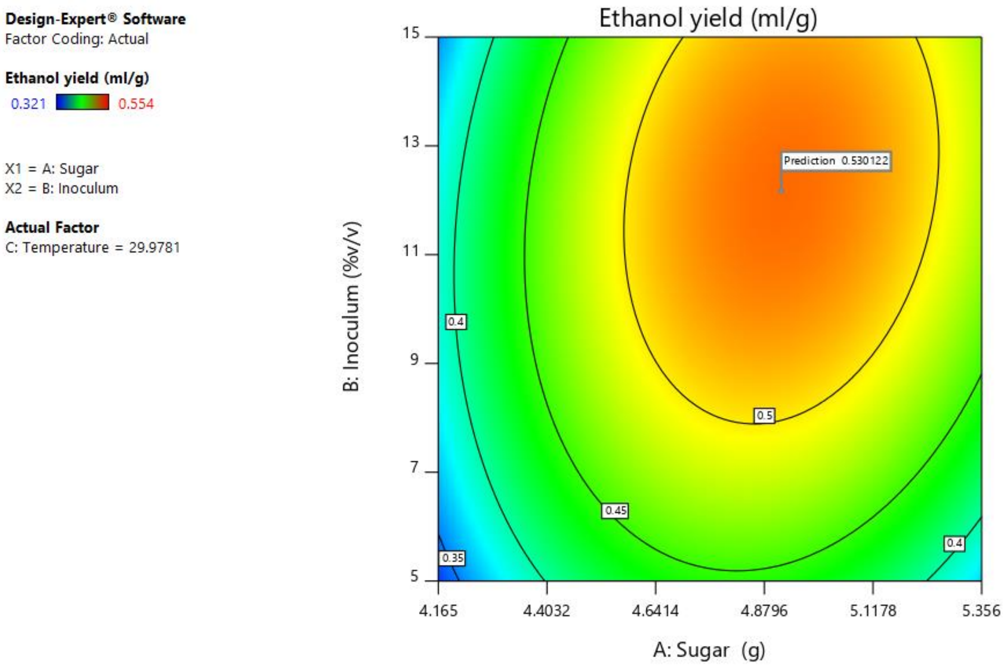


Figure 4.18: Contour plot of predicted ethanol yield at a constant temperature

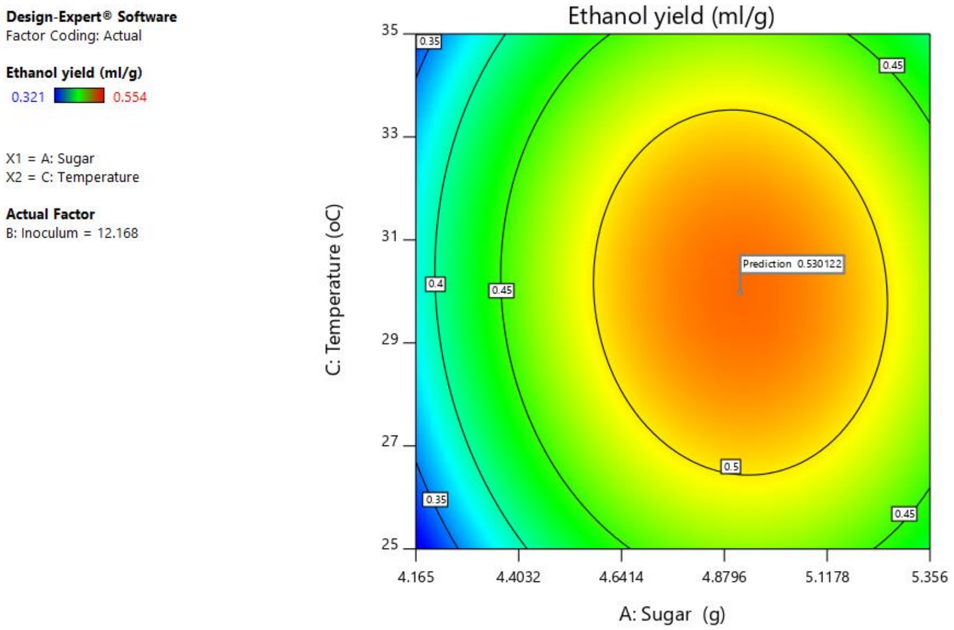


Figure 4.19: Contour plot of predicted ethanol yield at a constant inoculum

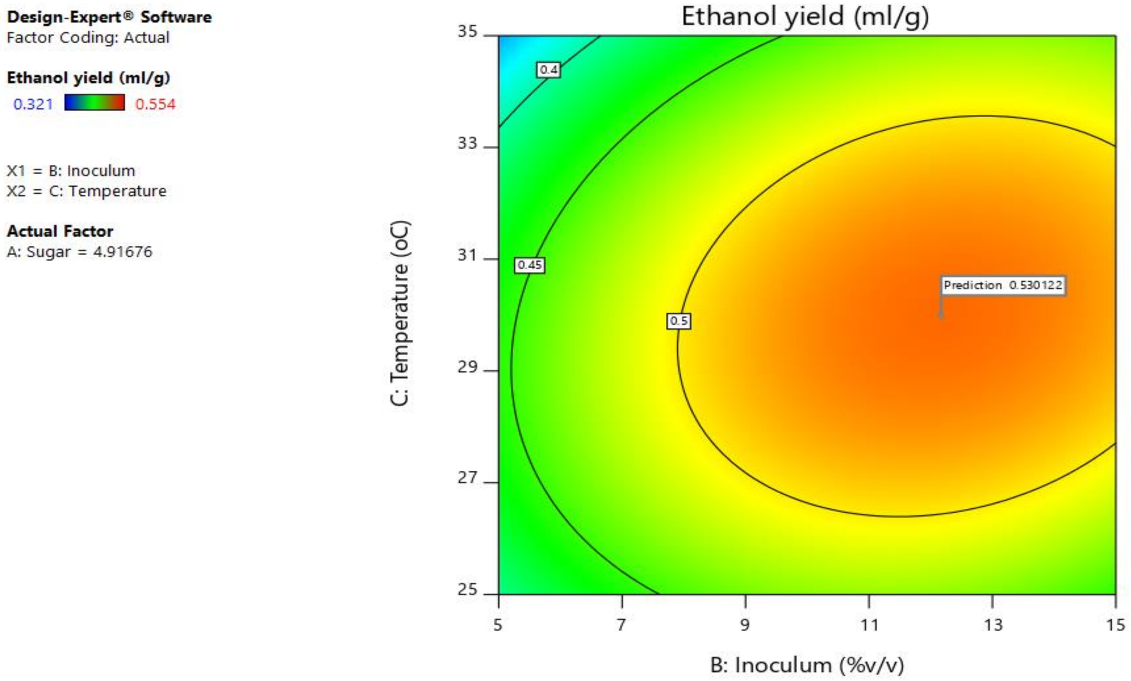


Figure 4.20: Contour plot of predicted ethanol yield at a constant sugar level

4.6. Model validation

Using the optimized solution obtained from Box Behnken design, in Table 4.13, experiment was conducted. Ethanol yield of 0.52 mL/g or 41.0 % was obtained. So, it is possible to say that this is in a good agreement with the predicted one. Therefore, the model is considered to be accurate and reliable for predicting the yield of ethanol.

4.7. Ethanol characterization using FTIR

Alcohols have characteristic IR absorptions associated with the O-H, C-O and the C-H stretching vibrations. When run as a liquid film the region 3500-3200 cm^{-1} with a very intense and broad band indicated the O-H stretch of alcohols, while the region 1260-1050 cm^{-1} confirms the C–O stretch(R. Bodirlau & Petru, 2009). The bands at around 2880 and 2930 cm^{-1} are assigned as the symmetric stretching modes of the –CH2 and –CH3 groups, respectively(Coates, Ed, & Coates, 2000).

The figure below shows FT-IR spectrum analysis of ethanol from corn cob. The O-H, C-H, and C-O stretch looks like the same as standard ethanol (appendix F). According to these references, it assures that the product obtained from corn cob hydrolysate is exactly ethanol.

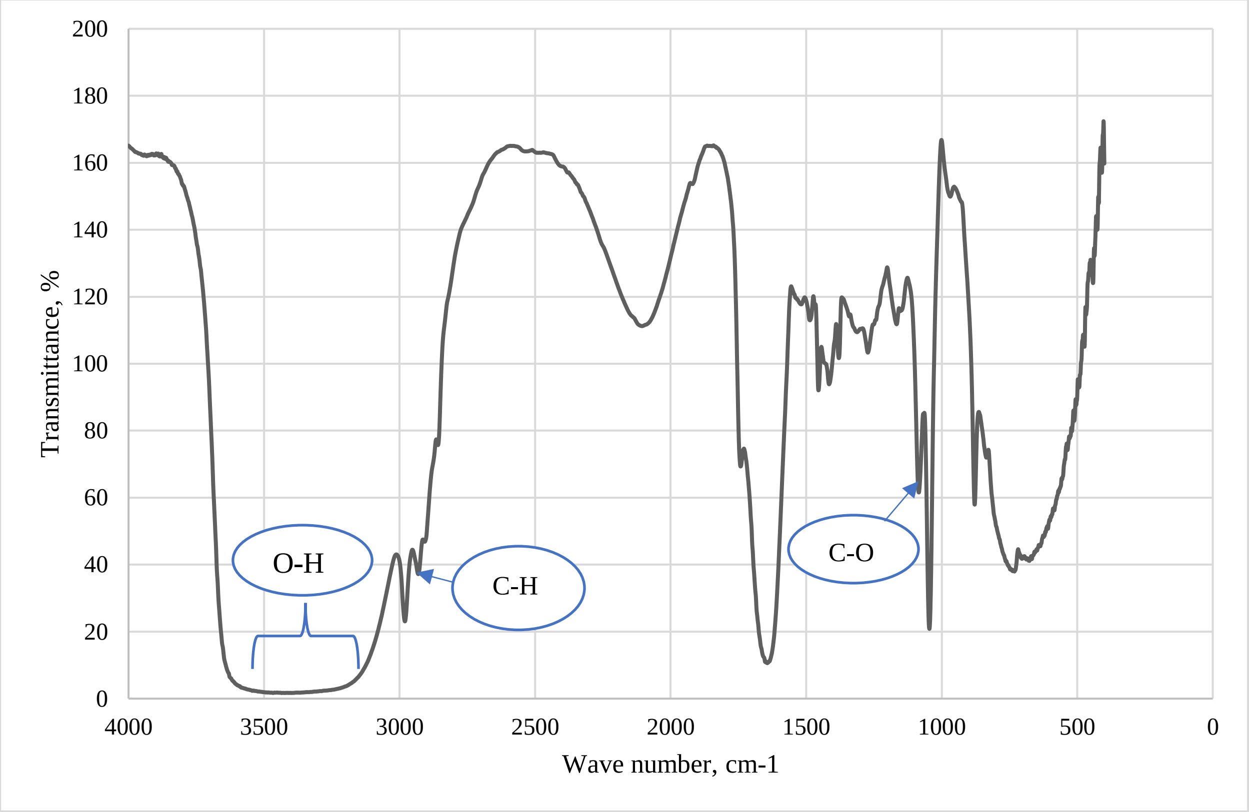


Figure 4.21: Result of FTIR analysis of corn cob ethanol

5. Conclusion and Recommendations

5.1 Conclusion

Bioethanol production from corn cob is one way to reduce consumption of crude oil and, to manage use of resources and to reduce environmental pollution. Sugar concentration of hydrolysate was estimated using phenol sulfuric acid method and UV spectrophotometry. The maximum, medium and minimum sugar yield from the hydrolysis were 5.36, 4.75 and 4.17 g respectively.

Because of its simplicity Box-Behnken design of response surface was used in this study to distinguish the experimental runs of hydrolysis and fermentation process. The single and interaction effect of sugar concentration, inoculum level and temperature of fermentation process in production of ethanol from corn cab was investigated. Hydrolysate dose and inoculum have significant effect on ethanol yield. Maximum ethanol yield of 0.530 mL/g or 41.82 % was obtained at sugar level of 4.92 g, inoculum of 12.2 %v/v, and at a temperature of 29.98 °C. The selected model was adequate to fit the data. In FTIR, it was confirmed that the presence of ethanol in the product.

5.2. Recommendations

The following recommendations are suggested;

1. Researchers should conduct a research on pretreatment step of bioethanol production from corn cob because there are different pretreatment methods, combination of them may give better efficiency of the process.
2. Other researchers should do optimization of distillation process parameters for production of fuel grade bioethanol.
3. Enzymatic methods of hydrolysis should be done to investigate the amount of sugar yield from corn cob.
4. Further research should be done to investigate the potential of bioethanol production from corn cob using combination of yeasts because saccharomyces cerevisiae can’t convert sugars other than glucose equivalent because corn cob hydrolysate also contains C-5 sugars such as xylose.

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Appendices

Appendix A: Properties of ethanol

Molecular formula	C_2H_5OH
Molar mass	46.07g/mol
Appearance	Colorless clear liquid
Density	0.789g/ml
Melting point	−114.3 °C, 159 K, -174 °F
Boiling point	78.4 °C, 352 K, 173 °F
Solubility in water	Fully miscible
Acidity	15.9
Viscosity	1.200 mPa·s (cP) at 20.0 °C
Dipole moment	5.64 fC·fm (1.69 D) (gas)

Appendix B: Calculation of ethanol yield

$$\text{Sugar concentration} = (\text{Absorbance} - 0.0704)/0.0459 \qquad (B.1)$$

$$\text{Amount of sugar in grams} = (\text{Sugar concentration})(0.1\text{L}) \qquad (B.2)$$

$$\text{Ethanol yield (mL/g)} = (\text{Actual volume of ethanol (mL)})/\text{amount of sugar (g)} \qquad (B.3)$$

$$\text{Ethanol yield (\%)} = \left(\text{Ethanol yield } \left(\frac{\text{mL}}{\text{g}}\right)\right)\left(0.789 \left(\frac{\text{g}}{\text{mL}}\right)\right) \qquad (B.4)$$

No	Sugar Conc. (g/L)	Inoculum (%v/v)	Temp.(^{o}c)	amount of sugar (g)	Actual volume of ethanol (mL)	Ethanol yield (mL/g)	Ethanol yield (%)
1	41.64815	5	30	4.168	1.3878	0.333	26.29485
2	53.56536	5	30	5.356	1.9152	0.357	28.13108
3	41.64815	15	30	4.165	1.5284	0.367	28.95464
4	53.56536	15	30	5.356	2.5529	0.477	37.90553
5	41.64815	10	25	4.165	1.33558	0.321	25.30181
6	53.56536	10	25	5.356	2.28536	0.427	33.77701
7	41.64815	10	35	4.168	1.33684	0.321	25.32564
8	53.56536	10	35	5.356	2.10904	0.394	31.06545
9	47.46514	5	25	4.746	1.9458	0.41	32.67809
10	47.46514	15	25	4.746	2.014	0.424	33.8425
11	47.46514	5	35	4.746	1.7703	0.373	29.42504
12	47.46514	15	35	4.746	2.14708	0.452	35.84632
13	47.46514	10	30	4.746	2.399225	0.505	39.98999
14	47.46514	10	30	4.746	2.63188	0.554	43.6186
15	47.46514	10	30	4.746	2.32068	0.489	38.57137
16	47.46514	10	30	4.746	2.375	0.5	39.58626
17	47.46514	10	30	4.746	2.55348	0.538	42.5847

Appendix C: Statistical analysis of hydrolysis

ANOVA for Quadratic model

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	166.71	9	18.52	34.10	< 0.0001	significant
A-Acid	113.17	1	113.17	208.38	< 0.0001	
B-Temp	16.46	1	16.46	30.31	0.0009	
C-time	4.83	1	4.83	8.89	0.0204	
AB	3.71	1	3.71	6.82	0.0348	
AC	5.17	1	5.17	9.52	0.0177	
BC	0.5763	1	0.5763	1.06	0.3372	
A^2	19.44	1	19.44	35.79	0.0006	
B^2	4.04	1	4.04	7.44	0.0295	
C^2	0.0836	1	0.0836	0.1540	0.7065	
Residual	3.80	7	0.5431			
Lack of Fit	1.88	3	0.6280	1.31	0.3871	not significant
Pure Error	1.92	4	0.4794			
Cor Total	170.51	16				

The Model F-value of 34.10 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case A, B, C, AB, AC, A^2, B^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant.

The Lack of Fit F-value of 1.31 implies the Lack of Fit is not significant relative to the pure error. There is a 38.71% chance that a Lack of Fit F-value this large could occur due to noise. Non-significant lack of fit is good -- we want the model to fit.

Fit Statistics

Std. Dev.	0.7370		R^2	0.9777
Mean	47.32		Adjusted R^2	0.9490
C.V. %	1.56		Predicted R^2	0.8056
			Adeq Precision	20.1683

The Predicted R^2 of 0.8056 is in reasonable agreement with the Adjusted R^2 of 0.9490; 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 20.168 indicates an adequate signal. This model can be used to navigate the design space.

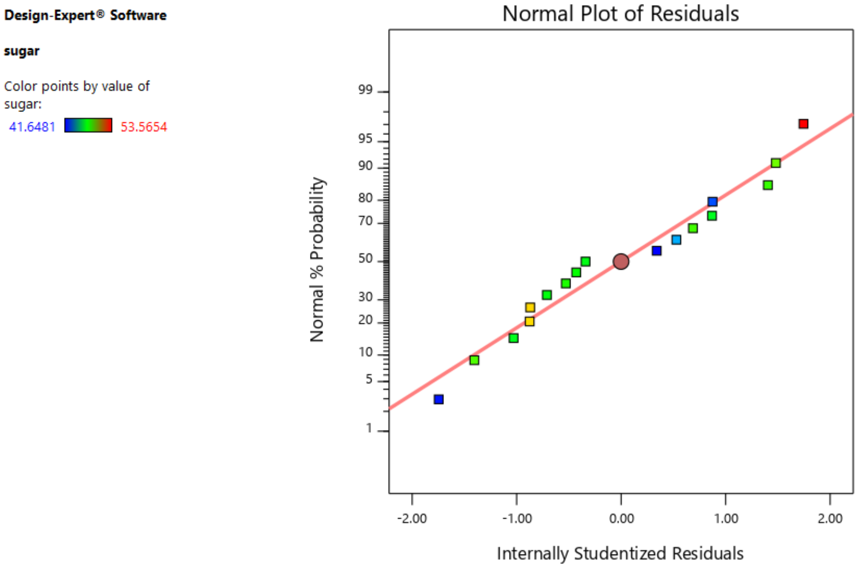


Figure C.1. Normal plot of residuals for model validity

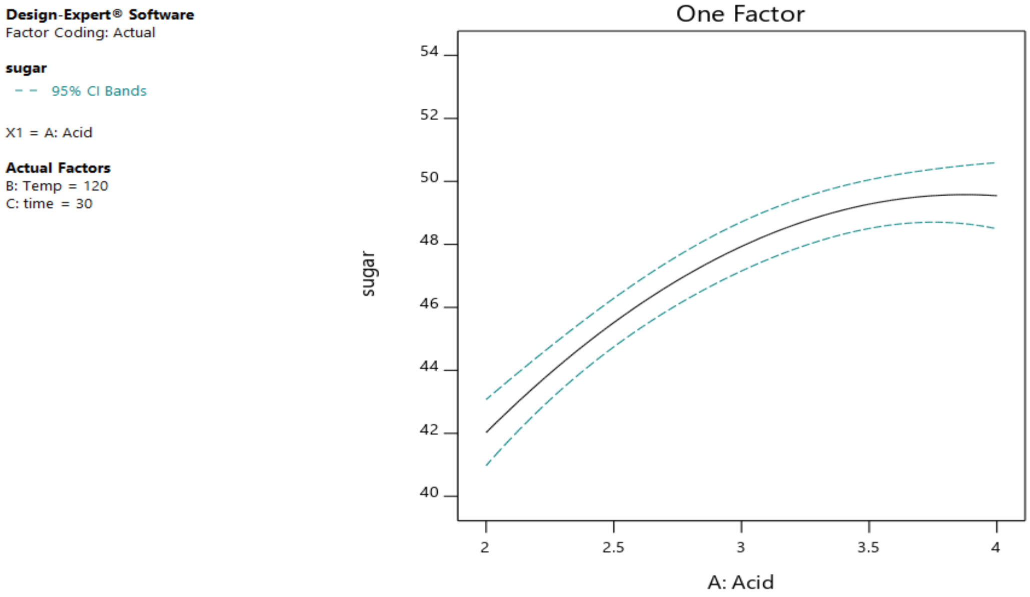


Figure C.2. Effect of acid concentration on hydrolysis process

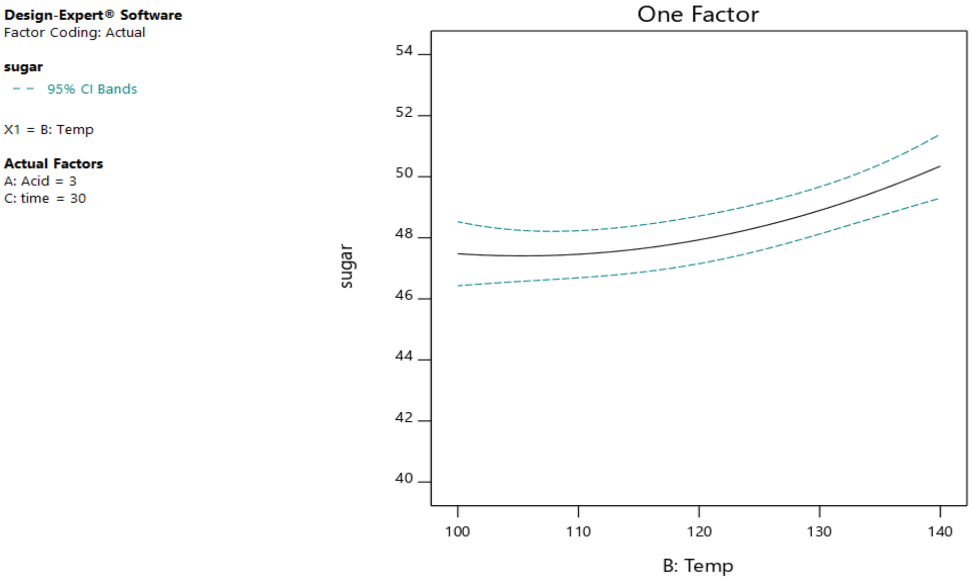


Figure C.3. Effect of temperature on hydrolysis process

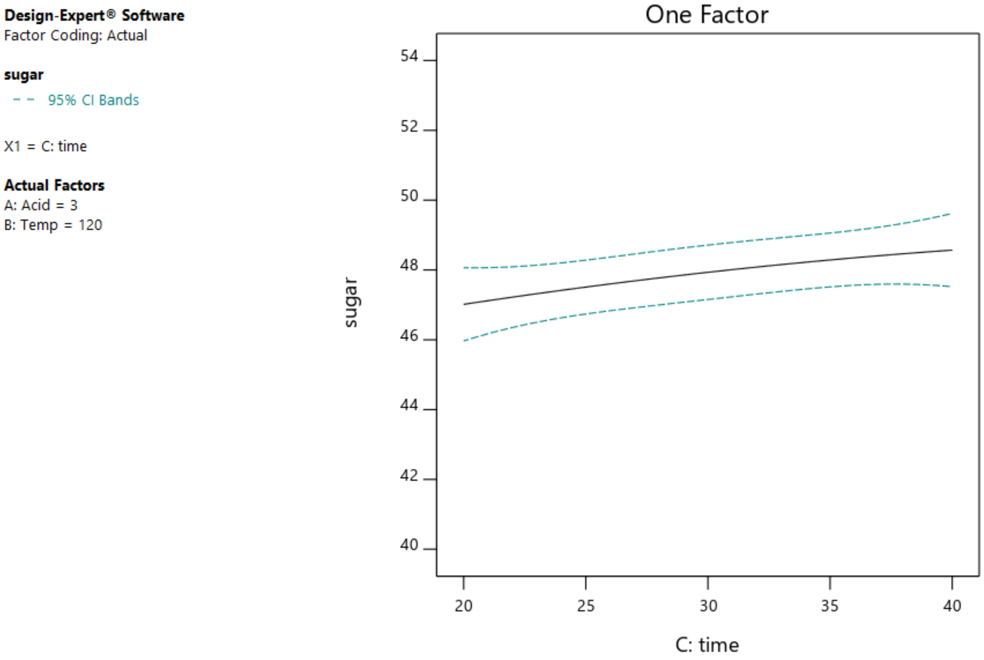


Figure C.3. Effect of time on hydrolysis process

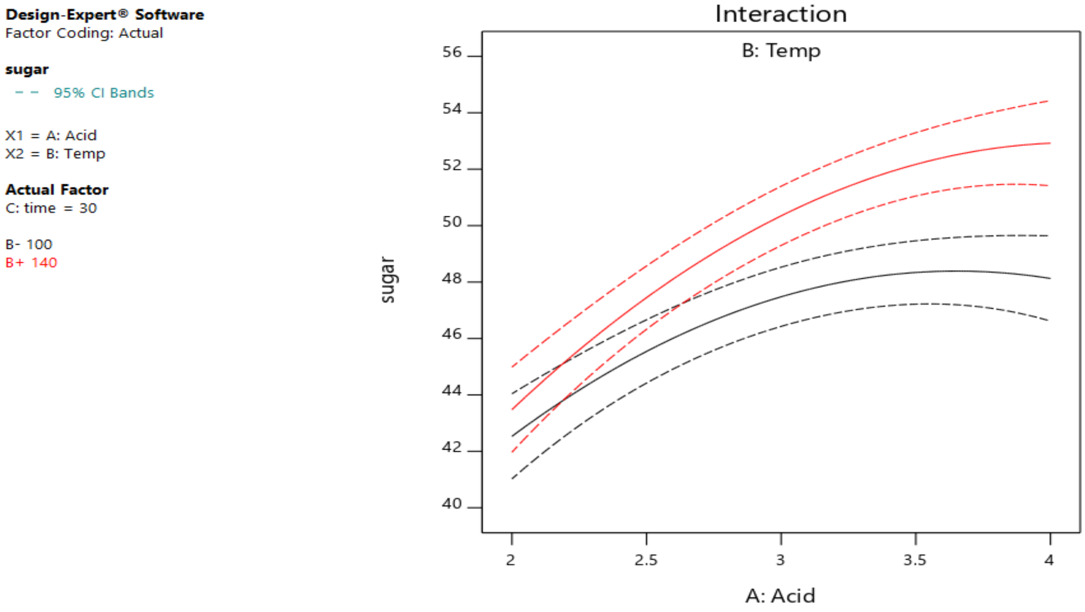


Figure C.4. Interaction effect of acid concentration and temperature on hydrolysis process

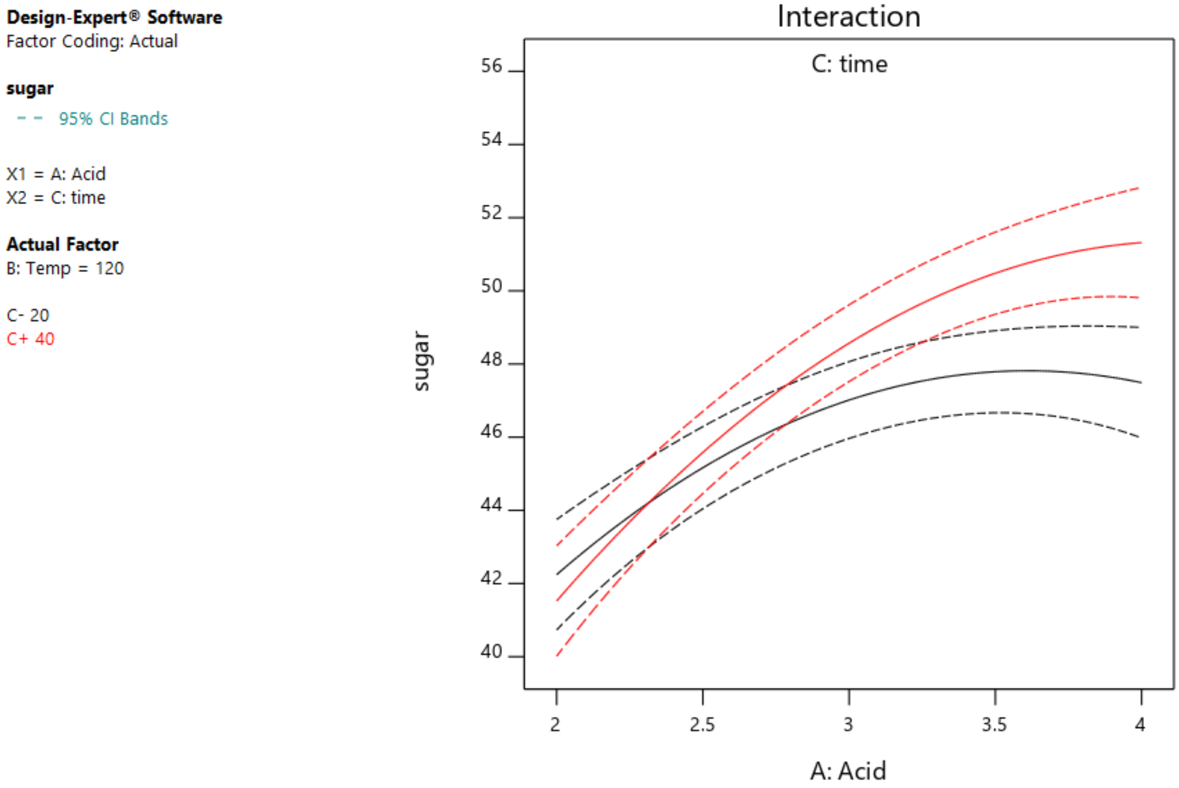


Figure C.4. Interaction effect of acid concentration and time on hydrolysis process

Appendix D: Ethanol volume percent and density

Ethanol %v/v and densities measured using density meter is in the following table

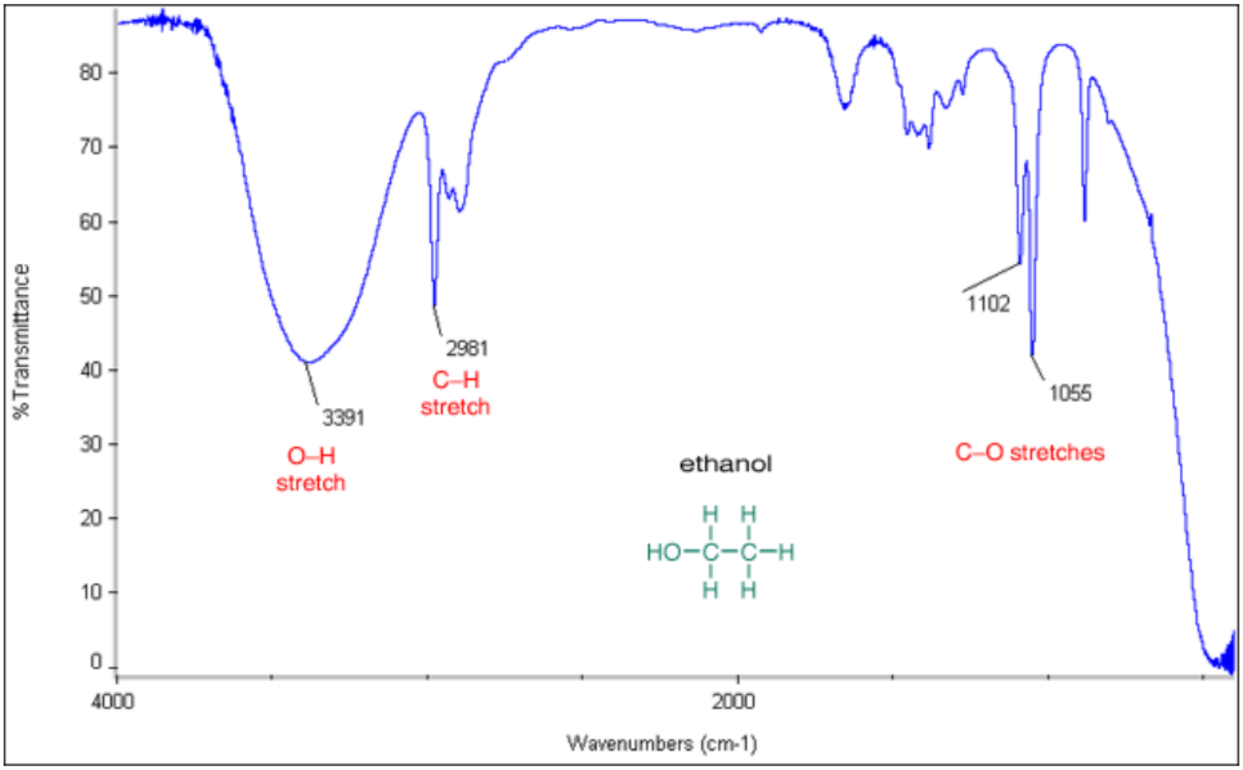
#	Ethanol % v/v	Density (g/cm^3)
1	2.313	0.99446
2	3.42	0.99193
3	3.821	0.99134
4	5.21	0.98791
5	3.106	0.99261
6	4.312	0.99001
7	3.518	0.99170
8	5.144	0.98803
9	4.324	0.9900
10	5.035	0.98910
11	5.058	0.98831
12	4.129	0.99095
13	5.051	0.98842
14	6.926	0.98645
15	4.66	0.98992
16	5	0.98938
17	4.152	0.99051

Appendix E: Possible solutions of fermentation optimization

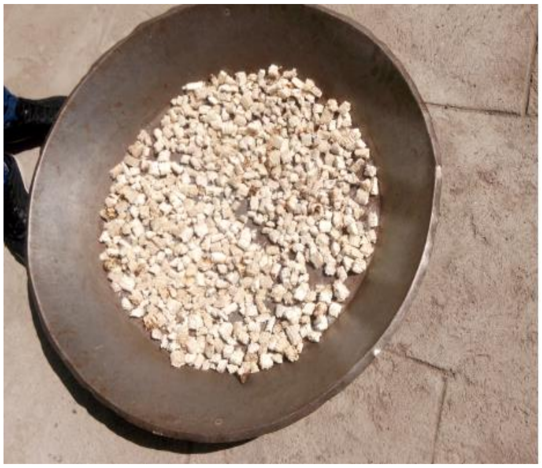
Number	Sugar	Inoculum	Temperature	Ethanol yield	Desirability
1	4.579	11.575	27.006	0.478	1.000
2	5.356	10.000	25.000	0.419	1.000
3	4.746	5.000	25.000	0.406	1.000
4	4.165	15.000	30.000	0.354	1.000
5	4.746	10.000	30.000	0.517	1.000
6	5.356	5.000	30.000	0.370	1.000
7	4.746	5.000	35.000	0.363	1.000
8	5.356	15.000	30.000	0.475	1.000
9	5.356	10.000	35.000	0.391	1.000
10	4.165	10.000	35.000	0.330	1.000
11	4.165	5.000	30.000	0.335	1.000
12	4.165	10.000	25.000	0.324	1.000
13	4.746	15.000	25.000	0.434	1.000
14	5.181	9.517	29.731	0.495	1.000
15	4.433	5.295	29.150	0.414	1.000
16	4.495	12.403	27.879	0.470	1.000
17	5.209	6.393	25.301	0.408	1.000
18	5.041	9.935	27.316	0.503	1.000
19	4.723	11.906	26.103	0.483	1.000
20	5.125	9.612	28.073	0.499	1.000
21	4.850	13.979	28.477	0.515	1.000
22	4.205	12.589	31.725	0.392	1.000
23	5.056	12.797	30.209	0.525	1.000
24	4.478	6.436	33.749	0.399	1.000
25	4.318	5.156	25.563	0.350	1.000
26	4.715	13.005	31.796	0.511	1.000
27	5.238	5.409	31.453	0.397	1.000
28	4.493	14.287	26.800	0.437	1.000
29	5.053	11.938	31.796	0.516	1.000
30	4.713	8.911	25.230	0.459	1.000
31	4.532	14.081	32.433	0.471	1.000
32	4.398	7.927	34.931	0.380	1.000
33	4.749	12.819	31.779	0.515	1.000
34	4.404	10.404	29.170	0.461	1.000
35	5.098	6.379	26.759	0.446	1.000
36	4.589	14.267	29.162	0.486	1.000
37	5.289	7.346	27.686	0.438	1.000
38	4.261	8.183	25.816	0.372	1.000
39	4.505	8.697	34.450	0.423	1.000
40	4.458	14.030	25.509	0.404	1.000
41	4.982	7.045	25.836	0.455	1.000
42	4.214	6.527	29.552	0.377	1.000
43	4.871	7.347	34.424	0.431	1.000
44	4.351	9.309	29.312	0.444	1.000
45	4.351	12.567	28.682	0.438	1.000
46	4.503	6.025	29.835	0.440	1.000
47	4.759	6.682	26.332	0.458	1.000
48	5.070	5.056	29.642	0.432	1.000

49	4.319	13.699	29.614	0.425	1.000
50	4.493	7.091	31.034	0.450	1.000
51	4.393	14.514	26.029	0.391	1.000
52	4.567	5.405	33.992	0.385	1.000
53	4.180	10.296	28.265	0.384	1.000
54	4.232	13.434	32.989	0.385	1.000
55	4.730	11.324	33.388	0.493	1.000
56	4.286	8.465	32.757	0.400	1.000
57	4.270	12.475	26.178	0.377	1.000
58	4.777	11.086	32.131	0.512	1.000
59	4.635	9.660	29.880	0.504	1.000
60	4.512	5.019	25.416	0.388	1.000
61	4.774	7.259	31.935	0.474	1.000
62	5.298	9.983	33.064	0.448	1.000
63	5.242	6.834	34.134	0.382	1.000
64	4.675	14.711	25.986	0.452	1.000
65	4.981	13.029	32.009	0.519	1.000
66	4.610	5.524	30.745	0.442	1.000
67	4.273	12.568	27.777	0.404	1.000
68	5.265	7.572	31.201	0.443	1.000
69	4.708	13.621	32.124	0.505	1.000
70	4.746	10.714	29.344	0.520	1.000
71	5.174	8.723	30.099	0.486	1.000
72	4.673	11.599	25.272	0.460	1.000
73	5.023	12.527	32.129	0.516	1.000
74	4.539	13.987	29.651	0.481	1.000
75	5.028	7.823	33.156	0.457	1.000
76	4.399	5.049	33.804	0.354	1.000
77	4.444	10.843	31.407	0.469	1.000
78	5.097	13.298	28.195	0.513	1.000
79	4.498	6.358	26.874	0.430	1.000
80	4.465	5.417	28.987	0.423	1.000
81	4.533	11.045	25.079	0.433	1.000
82	4.397	13.005	27.606	0.437	1.000
83	4.514	7.810	28.788	0.467	1.000
84	4.939	13.922	33.233	0.503	1.000
86	5.113	10.967	32.165	0.502	1.000
87	4.822	5.885	25.833	0.440	1.000
88	4.940	14.003	26.238	0.487	1.000
89	4.654	13.585	26.254	0.466	1.000
90	4.168	10.876	31.490	0.384	1.000
91	4.696	8.513	31.568	0.492	1.000
92	5.266	7.610	34.262	0.391	1.000
93	5.149	11.509	34.127	0.468	1.000
94	5.245	12.371	34.612	0.447	1.000
95	4.658	5.611	25.325	0.416	1.000
96	5.014	5.974	33.478	0.414	1.000
97	4.551	9.221	33.510	0.455	1.000
98	5.244	7.084	26.144	0.427	1.000
99	4.961	7.578	31.336	0.484	1.000
100	4.971	12.563	34.002	0.491	1.000

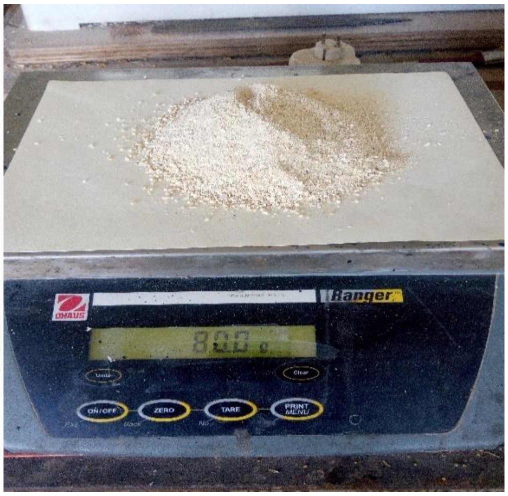
Appendix F: FTIR analysis of standard ethanol



Appendix G: Photos during experimental works



Pic G.1. Corn cob



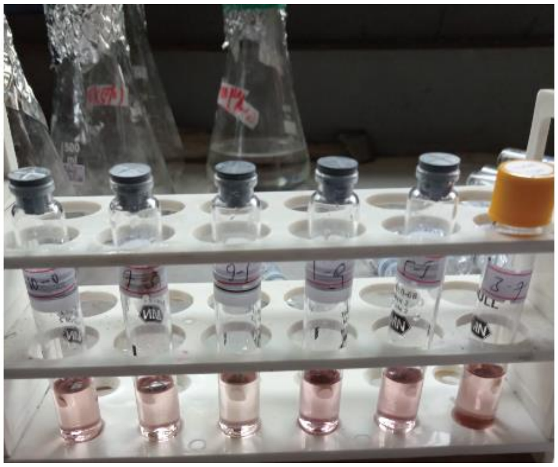
Pic G.2. Corn cob powder on digital balance

Pic G.3. Pretreated corn cob

Pic G.4. Dried residue of pretreated corn cob

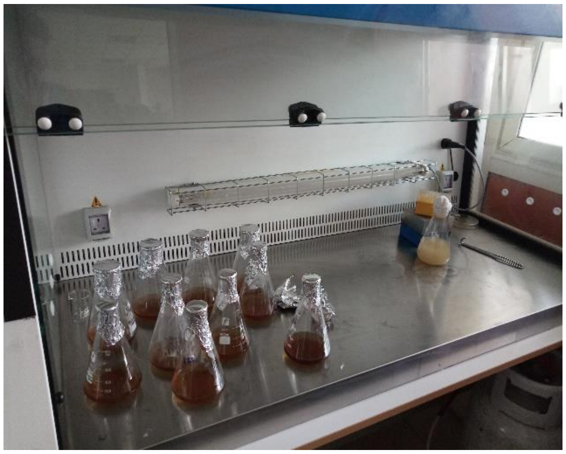
Pic G.5. Hydrolysate of corn cob

Pic G.6. Preparation of glucose dilution



Pic G. 7. Preparation of phenol-acid

Pic G.8. Reading absorbance of sugar on spectrophotometry



Pic G.9. Mixing of hydrolysate and inoculum

Pic G.10. Measuring ethanol density and percentage

Pic G.11. End product (ethanol) on plastic flask

Pic G.12. Infrared spectroscopy