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SCHOOL OF CHEMICAL AND BIO ENGINEERING

Production of Bioethanol from watermelon peel using dilute acid hydrolysis and fermentation

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By
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A thesis submitted to the School of Chemical and Bio Engineering presented in partial fulfillment of the requirement for the degree of Master of Science (Process engineering), Addis Ababa Institute of Technology, Addis Ababa University, Addis Ababa, Ethiopia

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Advisor: Dr.Eng. S. Anuradha Jabasingh Signature: Date:

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DECLARATION

I declare that this thesis entitled “Production of Bioethanol from watermelon peel using dilute acid hydrolysis and fermentation” has not been submitted in any form for another degree, diploma or an award at any university or other institution of the tertiary education. Whenever contributions of others are involved, every effort is made to indicate this clearly, with due reference to the literature and discussions. Information taken from published and unpublished work of others has been acknowledged in the text and a list of references is given. The work was under the guidance of Dr.Eng.S.Anuradha Jabasingh, School of Chemical and Bio Engineering.

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List of Acronym

ANOVA	Analysis of Variance
CCD	Central Composite Design
ECEA	Ethiopia Commodity Exchange Authority
EIO	Ethanol Industry Outlook
FTIR	Fourier Transform Infrared spectroscopy
GAP	Good Agricultural Practices
GHG	Green House gas
LCW	Lignocelluloses Wastes
MSW	Municipal Solid Waste
NUEP	United Nation Environmental Program
RFA	Renewable Fuels Association
Rpm	Rotation Per Minute
RSM	Response Surface Methodology
USA	United State of America

Abstract

The objective of this study was the production of bioethanol from watermelon peel in order to minimize the energy cost and substitute the non-renewable energy by using renewable resources. The conversion of watermelon peel to ethanol can be achieved mainly by four process steps. Pretreatment of watermelon peel to remove different contaminants, drying at 65°C for 48 h, followed by grinding to the particle size of 2 mm are the initial steps. Then, after soaking in dilute acid, the pretreated watermelon peel was converted into reducing sugar (glucose). This followed by the fermentation of the sugars to ethanol using *Saccharomycese cerevisiae* and distillation. Box Behnken Design (BBD) designed the experiment with three factors (mass of reducing sugar concentration, pH and Inoculum level). Seventeen runs were carried out and analyzed using Design Expert 11 software, to investigate the effects of fermentation parameters on yield of ethanol. The mass of reducing sugar was varied from 2.551g to 4.891 g, pH was varied from 4 to 6 and Inoculum was varied from 10 to 20 mL during fermentation. The maximum yield was observed at inoculum level of 15 mL; mass reducing sugar 3.721g and a pH of 5. Mass of reducing sugar and inoculum level has a statistically significant effect on the yield with p-values of 0.0082 and 0.0001, respectively. However, high inoculum level causes a decline in the ethanol yield. The statistical analysis also showed that the ethanol yield of (0.482mL/g) were obtained at optimized values of the variables, 3.698 g mass of reducing sugar, 5.02 pH, and inoculum level of 15.82 mL. This reveals a good agreement with the observed value of the ethanol yield (0.497 mL/g). Characterization of the bioethanol produced was performed using Fourier Transform Infrared Spectroscopy (FTIR) and High Performance Liquid Chromatography (HPLC). From result, it was observed that the ethanol produced from watermelon peel contains OH, CO, CH_2, and CH_3 functional groups, when compound with standard ethanol confirming the presence of ethanol in the product. The HPLC result showed that the product (Bioethanol) had an R_T value of 21.871 min similar to the R_T value of 21.86 min for the standard, confirming the presence of ethanol.

Keywords: Bioethanol, watermelon peel, hydrolysis, fermentation and *Saccharomycese cerevisiae*,FTIR,HPLC.

1. Introduction

1.1. Background

The world energy consumption has been increasing steadily with population growth and industrialization processes. Since 1900 Fossil fuels, e.g., Crude oil and natural gas are currently 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)). (Campbell et al., (1998)) also predicted that 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.

Bio renewable energy is one of the important energy alternatives to reduce world dependence on notorious fossil based fuels. 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 et al., 1994).

The production of bioethanol and its consumption as a fuel could substantially lower CO2 emissions compared with those from fossil fuels. The production of renewable biomass and its conversion to bioethanol could also generate jobs for local communities. Ethanol has been widely used as a gasoline additive worldwide. The production of ethanol fuel has been increasing over the last 10 years, and reached a level of 85.2 billion liters in the year 2012. The United States is the world's largest producer of bioethanol fuel, accounting for nearly 47% of global bioethanol production. Brazil is the world's largest exporter of bioethanol and second largest producer after the United States (Balat et al., 2009).Using ethanol blended fuel for automobiles can significantly reduce petroleum use and greenhouse gas emissions.

Bioethanol can be produced via several processes based on the properties of the feedstock, i.e. sugar, starch and cellulose platforms (Cheng et al., (2002).).Corn grain is currently the dominant feedstock for bioethanol production in the United States. However, using corn for fuel production is inevitably competing for limited cropland for food/feed production because corn is also an important food/feed source (Endo et al., (2008)) .Moreover, intensive corn production has raised environmental concerns. Corn production has high requirements for agricultural inputs, and its cultivation causes more total soil erosion than any other crop. Sugarcane molasses is also the main feedstock for ethanol production in India. Sugarcane grows in tropical regions and its planting area is very limited. The same dilemma of food/feed versus fuel also exists in ethanol production using other feedstock containing abundant carbohydrates, such as sweet potato and cassava. It is evident that lignocellulosic biomass is of great potential importance for ethanol production because the material is abundant in many regions of the world. However, conversion of lignocellulosic biomass to bioethanol is difficult and prohibitively expensive because of the tight structure of the biomass (Sarkar et al., (2012)).

The use of renewable biomass resources to produce liquid biofuels such as bioethanol offers attractive solutions to reducing greenhouse gas emissions, decreasing reliance on foreign oils, addressing energy security concerns, strengthening rural and agricultural economies, and increasing sustainability of the world transportations system. Apart from biofuels, many other valuable products for chemical and pharmaceutical industry can be produced from organic byproducts through microbial fermentation (Ye Sun, 2002).

Most current bioethanol production processes (1st generation) utilize more easily degradable biomass feedstock’s such as cereals (corn or grain) and sugarcane juice. However, the utilization of these agricultural crops exclusively for energy production is heavily conflicting with food and feed production. Great effort is enforced on advancing a cellulosic bioethanol concept (2nd generation) that utilizers lignocellulosic biomass (Zhaohui et al., 2011). Lignocelluloses wastes (LCW) refer to plant biomass wastes that are composed of cellulose, hemicellulose, and lignin. They may be grouped into different categories such as wood residues (including sawdust and paper mill discards) grasses, waste paper, agricultural residues (including straw, Stover, peelings, cobs, stalks, nutshells, nonfood seeds, bagasse, and domestic wastes. Currently, the second generation bio-products such as bioethanol, biodiesel, bio hydrogen and methane from lignocellulose biomass are increasingly been produced from wastes rather than from energy crops (Jatropha, switch grass and hybrid poplar) because the latter competes for land and water with food crops that are already in high demand (Abdel et al., 2014) .

Watermelon is grown in more than 96 countries worldwide. China is the world-leading producer of watermelon, with 70.3% of the total production in 2017. Other leading countries are Turkey (4.7%), Iran (2.3%), the United States (2.2 %) and Egypt (1.7%) (Ufoegbune et al., 2014) Ethiopia produces 15,090,750 tons of watermelon and the peels are discarded. Hence, this research investigated the application of this watermelon peel for bioethanol production (Duduyemi et al., (2013)).

1.2. Statement of problem

The economic growth of any country extremely depends on the availability of reliable sources of energy. Ethiopia is a country with different potentials on renewable energy sources such as hydropower, geothermal, solar and biomass. However, about 95 %, the energy demand of the country is mainly dependent on the use of traditional biomass fuels and only 5% is from the modern energy source of which petroleum product takes the largest share (Abadi et al., (2017)) .Ethiopia imports fuel on average 3,439,266.6 gallon per annum and this covers 77 % of the total export earnings. It is seen that the total amount of imported petroleum products is increased from year to year although the expenditure varies with the currency exchange rate (National et al., 2015). The transportation sector in general and the road transport segment in particular which accounts about 52% is one of the key sector which consumes the majority of the imported petroleum in 2010 (Abreham et al., 2015.) .In addition, now a days the country's population growth ,also increases steadily leading to more fuel consumption .

Ethiopia has more than 500 thousand hectares irrigable agricultural land for watermelon development and produces 15,090,750 tons of watermelon and the peels were discarded. Watermelon peel is an important byproduct for every 1165kg of watermelon fruit approximately 326.52 kg of watermelon peel is produced. While such peels may contain valuable materials such as 59.03 % cellulose, 30% hemicellulose,7.89 % lignin (Abdu et al., 2018). Hence, this research investigated the application of this watermelon peel for bioethanol production.

Bioethanol fermentation from edible, cellulosic feedstock's using enzymatic hydrolysis has carried out with success; very little research has done on fermenting bioethanol from non-edible, lignocellulosic material using dilute acid pretreatment with dilute acid hydrolysis on watermelon peel. Currently the cost of enzymes is also too high. Acid is low cost, non-volatilizes, easily available, and productive (Victoria, 2013). Therefore, this research Therefore, the main aim of this research work is production of cellulosic bioethanol from watermelon peel, which are economically competitive, environmentally acceptable, and capable of fulfilling an increasing energy demand. Minimize the volume of gasoline to be imported which in turn save hard currency of the nation and reduce the GHG emissions.

1.3. Objective

1.3.1 General Objective

The main objective of this study was the production of Bioethanol from watermelon peels using dilute acid hydrolysis and fermentation.

1.3.2 Specific objectives

The specific objectives of the thesis were the following;

- To characterize the of watermelon peel using proximate analysis (moisture content, volatile constitutes, and ash content), and chemical composition analysis (lignin, cellulose, hemicellulose and extractives).
- To investigate the effect of process variables (acid concentration, temperature and time) on the reducing sugar yield.
- To determine the amount of reduced sugar after the dilute acid hydrolysis.
- To investigate the effects of process variables (reducing sugar concentration, pH and inoculum) on the bioethanol yield,
- To characterize the quality of bioethanol produced from watermelon peel using Fourier Transform Infrared Spectroscopy (FTIR) and High Performance Liquid Chromatography (HPLC).

1.4. Significance of the study

Currently in Ethiopia, the demand for modern energy sources such as petroleum fuels is increasing with increase in population and economic growth. The country imports its entire petroleum fuel requirement by spending over 46 % of the foreign earning annually. To overcome the above problem the government of Ethiopia gives attention to environmental-friendly renewable energies such as ethanol since importing of petroleum ads a lot of cost to the country besides to higher environmental pollution through emitting of CO_2. Therefore, this study is significant on solving problems related to environmental concern, energy security, and economy. Bioethanol is a clear liquid alcohol that is made by the fermentation of different biological materials. Bioethanol developed from watermelon peel reduces the environmental pollution, greenhouse emission and has higher combustion efficiency because it is non-toxic, renewable, and biodegradable.

The production of bioethanol from locally available resource is reliable, renewable, and domestically distributed; this reduces dependency on imported petroleum and energy crisis. The other significance of this research is bioethanol production from watermelon peel 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, sugarcane and soybean oil. Therefore, that need to increase domestic ethanol production and to further develops the production from alternative feedstock rather than sugar cane molasses. The main importance of this study was to enhance the importance of watermelon peel for the production of bioethanol using dilute acid hydrolysis.

1.5. Scope of the study

This research generally covers bioethanol production from watermelon peel using dilute acid hydrolysis. It includes characterization of watermelon peel such as proximate analysis (moisture content, volatile constitutes, and ash content), and chemical composition, determining reducing sugar using UV/visible spectrophotometer, to investigate the effects of process variables such as sugar concentration, pH and inoculum in the fermentation process and hence to characterize the bioethanol using Fourier Transform Infrared Spectroscopy(FTIR) and High Performance Liquid Chromatography (HPLC).

2. Literature Review

2.1. Introduction to energy demand

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

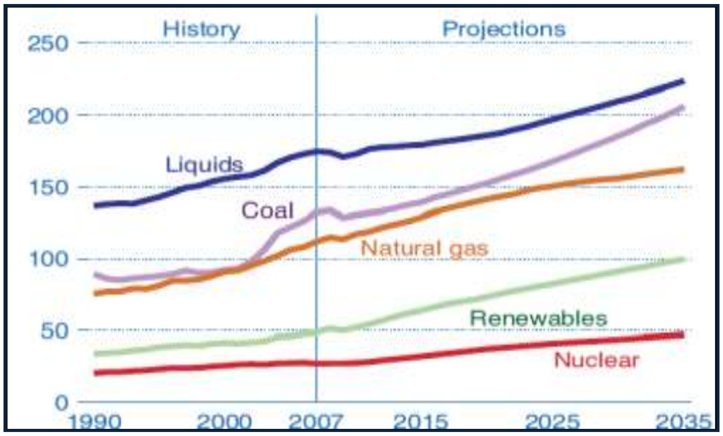

Figure 2-1: World market energy use by fuel type

Source: (Alabama and Auburn, 2012)

2.2. Types of biofuels

Bioethanol, biodiesel, biogas, and bio methanol are major biofuels among many others. Bioethanol as a general transport fuel has been developed for over 30 years since it was a natural extension of brewing technology. The combustion value of bioethanol has been measured by gasoline gallon equivalency (GGE) value, in which 1.5gallons of bioethanol generates the equivalent energy of one gallon of gasoline. Biodiesel is the product of the trans-esterification of plant oils (Nazia et al., 2017) .The particulate emissions from biodiesel are less harmful than conventional diesel. Biogas (methane) is generated from the anaerobic digestion of organic wastes, such as animal manure and sewage. This mature technology is applied on a small domestic scale in India and China (Mabee, 2007).It has also been systematically developed for industrial production in Germany and Denmark and more recently in the United Kingdom.

2.3. Bio-ethanol

A distilled colorless liquid fuel obtained from numerous potential feedstock varieties such as sugar beet, wheat, corn, cassava, fruits, bagasse, barley, molasses ,potatoes, sorghum, switch grass and cellulose biomass such as wood, paper, straw and other cellulose wastes such as grasses, others includes municipal solid wastes (Nazia et al., 2017) . These various waste streams for Ethanol production have their particular properties and generally differ. Ethanol as an alternative fuel, offers a sustainable economy by reducing the use of imported petroleum, emitting neutral CO_2 (g), boost economy providing value added market opportunities for the Agricultural sector. Ethanol has been made since ancient times by the fermentation of sugars. All beverage ethanol and more than half of industrial ethanol is still made by this process. Simple sugars are the raw material, Zymase and enzyme from yeast, changes the simple sugars into ethanol and carbon dioxide. The fermentation reaction, symbolized by the simple equation is actually very complex and impure cultures of yeast produce varying amounts of other substances, including glycerin and various organic acids.

2.3.1 Uses of Bioethanol

The main use of ethanol is as a motor fuel and fuel additive. Ethanol and other alcohols can be used to power motor vehicles instead of gasoline. In almost all cases, the ethanol is mixed with gasoline. Efficient method for conversion of biomass into fuel is by ethanol production because ethanol is an economical as well as environmentally friendly fuel. Ethanol has the advantages of being renewable, cleaner burning and produces no GHG (Stella et al., 2017).

A number of market segments are available in the ethanol industry serving a wide range of uses in the medical sectors, pharmaceuticals, beverages, industrial, household, and transport uses. The market potential for bioethanol is therefore not just limited to transport fuel or energy production but has potential to supply the existing chemicals industry and house hold uses. However, the most prevalent use of bioethanol in Ethiopia is as a transport fuel in spark ignition engine vehicles, the current amount of ethanol fuel blended with gasoline is 10%, and the government is working to increase the share. The government is also working to start export in two years of time and to substitute household cooking fuel in the future (Yacob, 2013). The major uses of ethanol are in alcoholic beverages. Alcoholic beverages vary considerably in their ethanol content and in the foodstuffs from which they are produced. Most alcoholic beverages can be broadly classified as fermented beverages, beverages made by the action of yeast on sugary foodstuffs, or beverages whose preparation involves concentrating the ethanol in fermented beverages by distillation (Tekle, 2008).

2.4. World market of ethanol

Today, bio-ethanol is the most dominant bio-fuel and its global production showed an upward trend over the last 25 years with a sharp increase from 2000. As of 2005, worldwide production capacity for bio-ethanol fuel was about 45 billion liters per year, with approximately 15% annual growth between 2000 and 2005. This value increased to 49 billion liters in 2006, when the Americans produced 75% of the total world ethanol output, followed by Asia/Pacific and Europe/Africa with respective values of 15 and 10% (Talebnia and Farid, 2008) .The industrial alcohol market showed a rather modest rate of growth similar to the increase in Gross Domestic Product in many countries. The market for beverage alcohol in most developed countries is stagnating, due to increased health awareness. In 2009, production of fuel ethanol reached an estimated 76 billion liters, an increase of 10 percent over 2008. The United States and Brazil accounted for 88 percent of global ethanol production in 2009. Most of the increased production occurred in the United States (Talebnia and Farid, 2008).After a significant downturn in the U.S. fuel ethanol market in 2008, U.S. production rose 16 percent to about 41 billion liters in 2009.

The highest sugar prices in years, combined with adverse weather conditions in a major producing region, resulted in a drop in Brazil's ethanol production from 27.1 billion liters in 2008 to 26.3 billion liters in 2009. All ethanol produced in Brazil is from sugar cane. All fueling stations in Brazil sell pure ethanol and gasoline, a 25 % ethanol and 75 % gasoline blend. Flex-fuel cars, which can use pure ethanol, gasoline, or any blend of the two, provide the flexibility to choose fuel based on price at the pump. However, Brazilian ethanol export declined by almost 31 % in 2009. International demands declined in great part because of the global economic crisis(Talebnia and Farid, 2008) .

Table 2-1: World Fuel Ethanol Production by Country or Region (millions of Gallon)

Country	2007	2008	2009	2010	2011	2012	2013	2014	2015
USA	6,521	9,309	10,938	13,298	13,948	13,300	13,300	14,300	14,806
Brazil	5,019	6,472	6,578	6,922	5,573	5,577	6,267	6,190	7,093
Europe	570	734	1,040	1,209	1,168	1,179	1,371	1,445	1,387
China	486	502	542	542	555	555	696	635	813
Canada	211	238	291	357	462	449	523	510	436
Rest of World	315	389	914	985	698	752	1,272	1,490	1,147
World	13,123	17,644	20,303	23,311	22,404	21,812	23,429	24,570	25,682

Source: Data Source: F.O. Licht, cited in Renewable Fuels Association, Ethanol Industry Outlook 2008-2015 reports. Available at www.ethanolrfa.org/pages/annual-industry-outlook (RFA, EIO)

2.5. The status of bioethanol in Ethiopia

The initiative for biofuels development in Ethiopia originally came from the private sector, though it did not take too long to get the government to buy. Governments often present mitigation of climate change as a key policy goal for biomass fuel developments, but in the case of Ethiopia, the government is explicit about its reasons for promotion of biofuels. The reasons, among others, are energy security using biofuels and to improve the balance of trade by import substitution and new export market development. Following population growth and economic development, the need for more modern fuels has increased significantly over the years. In general the demand for petroleum in the country indicates that there is a gradual increase from a year of 2000 to 2010 (Jaiswal et al., 2016) . A Biofuels Development and Utilization Strategy has been formulated by the Ministry of Mines and Energy in August 2007 (Yacob, 2013) . The objective of the strategy is to facilitate sufficient production of biofuels from indigenous resources to substitute imported petroleum and export excess products. The biofuels strategy document identified some energy crops such as sugarcane, Jatropha, castor, and palm trees as potential feedstock for biofuels production.

Table 2-2: Ethiopia Ethanol production in liters

Year		Ethanol produced (Litres)		
		Fincha sugar factory	Metehara sugar factory	Total
1991	1998/99	1,907,000		1907,000
1992	1999/00	720,000		720,000
1993	2000/01	1,790,571		1,790,571
1994	2001/02	209,444		209,444
1995	2002/03	894,624		894,624
1996	2003/04	911,431		911,413
1997	2004/05	1,636,047		1,636,047
1998	2005/06	6,847,816		6,847,816
1999	2006/07	6,066,860		6,066,860

2000	2007/08	5,330,337		5,330,337
2001	2008/09	5,878,516		5,878,516
2002	2009/10	7,116,585		7,116,585
2003	2010/11	7,127,895	6,373,775	13,501,670
2004	2011/12	6,794,000	7,658,000	14,452,000
2005	2012/13	7,620,500.00	7,063,000.00	14,683,500.00
2006	2013/14	11,678,000.00	7,767,000.00	19,445,000.00
2007	2014/15	10,999,000.00	8,806,000.00	19,805,000.00

Source: (Ethiopia Sugar Corporation)

2.6. Feedstock for bioethanol production

Biofuels originate from plant oils, sugar beets, organic waste and the processing of biomass. Biological feedstock that contain appreciable amounts of sugar or materials that can be converted into sugar, such as starch or cellulose can be fermented to produce bioethanol to be used in gasoline engines. Bioethanol feedstock can be conveniently classified into three types: (i) sucrose containing feedstock (e.g. sugar beet, sweet sorghum and sugar cane), (ii) starchy materials (e.g. wheat, corn, and barley), and (iii) lignocellulosic biomass (e.g. wood, straw, and grasses) (Badger, (2002)).

2.6.1 Sugars

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

(*Saccharomycese cerevisiae* also known as Bakers‟ yeast, since it is commonly used in the baking industry) is frequently used to ferment glucose to ethanol.

2.6.2 Starches

Starch is a biopolymer and defined as a homo polymer consisting only one monomer, D-glucose. To produce bioethanol from starch it is necessary to break down the chains of this carbohydrate for obtaining glucose syrup, which can be converted into bioethanol by yeasts. Starch consists of long chains of glucose molecules and can also be converted to fermentable sugar by a method called ‘‘the hydrolysis technique’’. Hydrolysis is a reaction of starch with water, which is normally used to break down the starch into fermentable sugar (Badger, (2002)) . There are two types of hydrolysis enzymatic hydrolysis and acid hydrolysis.

2.6.3 Lignocellulosic Biomass

Agricultural residues are a great source of lignocellulosic biomass, which is renewable, chiefly unexploited, and inexpensive. Such resources include leaves, stems, and stalks from sources like corncob, corn Stover, sugarcane bagasse, rice hulls, woody crops, and forest residues. Also, other multiple sources of lignocellulosic waste from industrial and agricultural processes include citrus peel waste, sawdust, paper pulp, industrial waste, municipal solid waste, and paper mill sludge (Kudirat et al., 2012).Cellulose materials represent the most abundant global source of biomass and have been largely unutilized. The global production of plant biomass, of which over 90% is lignocellulose, amounts to about 200×10^9 tons per year, where about 8 to 20×10^9 tons of the primary biomass remains potentially accessible .Cellulose is not used for food and the biofuels industries that use lignocellulosic materials do not compete for raw materials. Cellulosic biomass such as switch grass and agricultural wastes are cheaper to produce and requires fewer inputs in form of energy (Kudirat et al., 2012). Cultivation of such plants improves soil fertility and accompanied by less soil erosion. Moreover, the process also represents a means of effective and efficient waste management as a large proportion of agricultural and municipal wastes are lignocellulosic. Another benefit of cellulosic ethanol is the reduction in greenhouse gas emission. Compared to gasoline, ethanol burns cleaner with greater efficiency thereby releasing up to 85% less carbon dioxide (Zhaohui et al., 2011) .

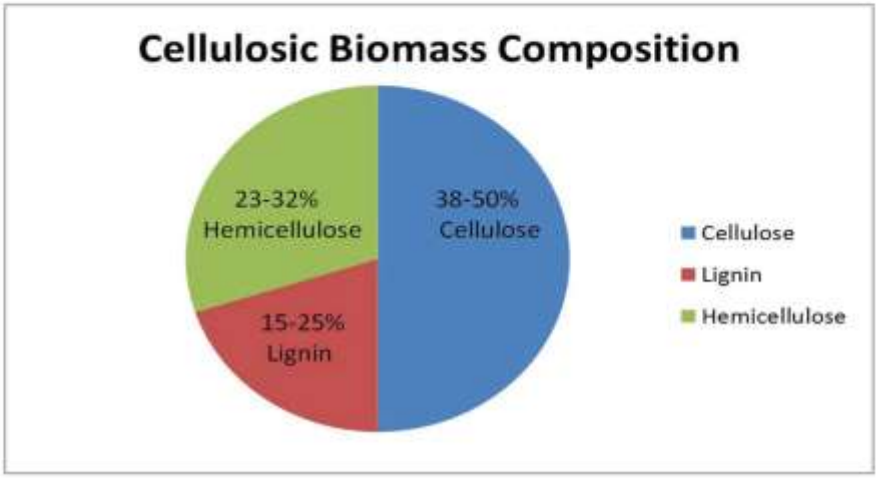

Figure 2-2: Composition of Cellulosic Biomass

Source :(Ufoegbune et al., 2014)

2.6.3.1 Cellulose

Cellulose is an un branched homo polysaccharide composed of β-D glucose units linked by (1, 4) glycosidic bonds. However, the basic building block of cellulose is a dimer of two glucose units known as cellobiose. Cellulose is the most abundant material on Earth, and it is the main constituent of plants. It is also present in bacteria, fungi, algae and even in animals (Talebnia and Farid, 2008).

2.6.3.2 Hemicellulose

Hemicellulose or polios' is a mixture of polymers comprising pentoses, hexoses hexuronic acids and deoxy-hexoses. Hemicelluloses differ from celluloses by composition of various sugar units and by much shorter and branched molecular chains. In contrast to cellulose that is crystalline, strong, and resistant to hydrolysis, hemicellulose has a random, amorphous structure with little strength. Therefore, it is easily hydrolyzed by dilute acid or base, as well as hemicellulose enzymes (Talebnia and Farid, 2008).

2.6.3.3 Lignin

Lignin is a complex, hydrophobic, cross-linked, three-dimensional aromatic polymer of phenyl propane building blocks. The mechanical strength properties of plants are mainly due to incorporation of lignin into their cell walls, whereby huge plants such as trees can remain upright. Lignin is one of the most complicated natural polymers with respect to its structure and heterogeneity, which make it extremely resistant to chemical and biological degradation (Talebnia and Farid, 2008) .

2.7. Watermelon

Watermelon (*Citrullus lanatus*) is a vine-like flowering plant originally from southern Africa. It is a worldwide economically important member in family Cucurbitaceous. It has been cultivated for a long time in Africa, the Middle East, and Egypt. One fruit that can investigate for its ethanol production via fermentation is watermelon. *Cirullus lanatus* (cucurbitaceous) commonly known as watermelon is cultivated in all parts of the world and is locally found in Guyana(Ufoegbune et al., 2014) . Watermelon contains 7-10 % (w/v) ready to ferment sugars. The pulp of the watermelon contains three types of fermentable sugars (7-10%): sucrose, fructose, and glucose while the peel contains cellulose, which can be can be converted to glucose by enzymatic or acid hydrolysis of the peel. The watermelon fruit has a smooth thick rind (exocarp) and fleshy center (mesocarp and endocarp) including red pulp and watery juice. Watermelon fruit contains 60% flesh, of which 90% is a juice that contains 7 to 10% w/v sugars. Thus, over 50% of the watermelon fruit is readily fermentable liquid (Muhammad et al., 2014).

Figure 2-3: Watermelon

2.8. Watermelon peel

Watermelon peel contains cellulose; cellulose which can be can be converted to glucose by enzymatic or acid hydrolysis of the peel but , In dilute acid hydrolysis, the hemicellulose fraction is depolymerized at lower temperature than the cellulosic fraction,& cost effective than enzymes (Swapna et al., 2017).based on this acid hydrolysis is best

Figure 2-4: Watermelon Peel

2.9. Geographic Distribution

2.9.1 Overview of watermelon production in the world

Watermelon (*Citrullus lanatus*) belongs to the family Cucurbitaceous (Swapna et al., 2017). Its center of origin has traced to both the Kalahari and Sahara deserts in Africa, and these areas have regarded as points of diversification to other parts of the world. In Nigeria, though there are no official figures recorded for its production, the crop has a wide distribution as a garden crop, while as a commercial vegetable production; its cultivation is confined to the drier savanna regions of Nigeria (Wayne et al., 2009) . Watermelon is grown in more than 96 countries worldwide. China is the world-leading producer of watermelon, with 70.3 % of the total production in 2015. Other leading countries are Turkey (4.7%), Iran (2.3%), United States (2.2%), and Egypt (1.7 %). Watermelon is a sweet, crisp, and juicy fruit grown worldwide particularly in tropical countries. In India 1.83 million metric tons of watermelon was produced in 2017-2018. Watermelon (Citrullus lanatus) is a vine-like flowering plant originally from southern Africa (Muhammad et al., 2014). It is a worldwide economically important member in family Cucurbitaceous. It has been cultivated for a long time in Africa, the Middle East, and Egypt. In 2016, global production of watermelons was 117 million tones, with China alone accounting for 68% of the total. Secondary producers with more than 1% of world production included Turkey, Iran, Brazil, Uzbekistan, Algeria, the United States, Russia, Egypt, Mexico, and Kazakhstan.

In Africa, watermelon is grown not only in dry, low altitude tropical areas like Cape Verde, Mali, Mauritania, Chad, Senegal and Nigeria, but also in equatorial countries like Gabon and Democratic Republic of Congo (Swapna et al., 2017). In Nigeria, watermelon production has increased significantly in the last one decade with the major production areas being located in the Sahel, Sudan, and Guinea agro-ecological zones. In recent times, its cultivation has extended down to the forest belts of southwestern Nigeria. However, the northern fringes of the Sudan and Sahel savanna ecological zones and the shores of the Lake Chad remain the major production areas (Swapna et al., 2017) .

Table 2-3: Major watermelon producers, 2017-2018

Country	Millions of tones
China	79.2
Turkey	3.9
Iran	3.8
Brazil	2.0
World	111.0

Source: (EPA, 2017), and EBB (2018)

2.9.2 Overview of watermelon production in Ethiopia

The growth of Ethiopia watermelon dates back to the mid-20th century when East Shoa (Shewa) farmers utilized the waters of Lake Koka for growing the nutritious fruit. This fruit-farming area lies in the central part of the agricultural Oromia zone that also produces tropical fruits like strawberries. To date, the region serves as the mere production area thanks to a man from Italy who brought the seeds in that the place, around 1950 (Ufoegbune et al., 2014) . The Ethiopia climate provides the best grounds for planting the crop as it can survive in warm temperatures of at least 21°C. The fruit needs a lengthy season that runs for a minimum eighty days, or at most 3 months. The Ethiopia watermelon is usually ready for harvesting in 3 to 4 months. Because we understand that watermelons are sweetest now of picking, we ensure that our sources only harvest them when they are just about to ripen. They contain no extra starch upon harvest and thus the need to pick them when they are just mature (Ufoegbune et al., 2014). The best signs to look for are usually a yellowish ring at the lower parts of the gourd-like striped fruit. If the bottom section is still green even when pressed, it is then immature and not ready for harvesting. It is also characteristic of a mature watermelon to be dull in the skin compared to the brilliant green tinge when it is still developing (Hiben, 2013). Ethiopia watermelon also undergoes processing to make various products. The most popular include juice, pickle, and spread. It is made of 92% water, which restores the fluid balance in the body. Its range of vitamins include B6, A as well as C (Ufoegbune et al., 2014).

Table 2-4: Major watermelon Producing Administrative Zones of Ethiopia

Zone	Average production (in tone)
Lake Koka	7,020,250
West Welega	5,030,400
Raya	3,040.100
Total	15,090,750

Source:(Duduyemi et al., (2013))

Fuel properties of bioethanol

Bioethanol (ethyl alcohol, CH_3–CH_2–OH) is a liquid biofuel which can be produced from several different biomass feed stocks 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 (Swapna et al., 2017) . 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 (Urbanchuk, 2018) .

2.10. Bioethanol production process

2.10.1 Pretreatments

An effective pretreatment is characterized by several criteria, it should avoid the need for reducing the size of biomass particles, should preserve the hemicellulose fractions, should reduce formation of inhibitors that hinder growth of fermentative microorganisms, and minimize energy demands and limit cost (Zhaohui et al., 2011).Various pretreatments are being investigated for their effectiveness in subsequent enzymatic scarification. The material treated by effective single or combined pretreatments is more accessible and susceptible to enzymatic scarification and more fermentable sugars are obtained for subsequent fermentation (Nathan et al., 2005). However, the application of pretreatment is likely to vary from material to material.

2.10.1.1 Physical pretreatment

Physical pretreatments aim to degrade the cellulose crystal to improve biomass digestibility by changing the physical character of materials using pyrolysis and mechanical commination including dry, wet and vibratory ball mills. Mechanical combination is a combination process involving chipping, grinding and milling which reduces the material size (Nathan et al., 2005). Dry and wet mill processes are economically effective when applied in starch-to-ethanol production. Vibratory ball milling has been used to effectively generate smaller particles than other mechanical commination methods.

2.10.1.2 Chemical pretreatment

Chemical pretreatments assist in specifically removing unwanted compounds, such as lignin or hemicellulose compounds (Ye Sun, 2002), and their application is therefore changed based on the nature of the biomass. Ozonolysis can effectively remove 60 % lignin (e.g. In wheat straw) and also avoid the generation of toxic products at room temperature conditions (Ye Sun, 2002). Dilute acid hydrolysis (using H_2SO_4 , HCl) has been successfully developed to achieve high sugar yield and avoid the toxic, corrosive issues trigger by concentrated acid hydrolysis (Ye Sun, 2002).

2.10.1.3 Physical chemical pretreatment

Physical-chemical pretreatment is an efficient process the advantages of both individual physical and chemical pretreatments, in which fine pretreated materials for downstream process are obtained and the cost and energy demands are also dramatically decreased. Steam explosion a recognized thermal hydrolytic method is one typical physical-chemical pretreatment and is extensively applied to enhance ethanol production for lignocellulosic biomass. The process explodes biomass by sudden decompression following high pressure and temperature conditions. Recent studies tend to use lower temperatures combined with longer retention time. Steam exploded cellulose is more accessible to cellulose (Zhaohui et al., 2011). The advantages of steam explosion are the high yield of glucose and xylose attributed to the considerable lignin transformation and hemicellulose degradation. However, disadvantages are seen as the high energy demand, a consequence of high pressure and temperature requirements, and the formation of fermentation inhibitors. Addition of H_2SO_4 or CO_2 into steam explosion could improve the degradation of hemicellulose and enzymatic hydrolysis and decrease the formation of inhibitory compounds (Ye Sun, 2002).Ammonia fiber explosion (AFEX) is another classic physical-chemical pretreatment. It involves exposing lignocellulosic biomass to steam explosion with additional liquid ammonia. AFEX does not effectively decompose lignin and hemicellulose but generates only trace levels of inhibitors (Ye Sun, 2002).

2.10.1.4 Biological pretreatment

Microorganisms have been studied and used for decomposition of plant cell wall material. Fungi are employed in the decomposition of lignocellulosic materials due to the many saccharifing enzymes they produce. Diverse fungi involving brown, white, and soft rot fungi have been used to target different compounds (Nathan et al., 2005) .For instance, brown rot fungi mainly degrade cellulose, while white and soft rots can degrade both cellulose and lignin (Hiben, 2013). One novel approach for biological degradation of lignocellulosic biomass has been found by the wood-boring marine crustacean Limnoria quadripunctata can digest crystalline cellulose directly to fermentable glucose showed that these crustaceans potentially possess all of the enzymes for lignocellulose digestion due to the absence of the gut microbes. Although biological pretreatment requires low energy at moderate conditions, the extent of decomposition is less.

2.10.2 Hydrolysis

After pretreatment, there are two types of processes to hydrolyze the feedstock into monomeric sugar constituents required for fermentation into ethanol. The hydrolysis methods most commonly used are acid (dilute and concentrated) and enzymatic. To improve the enzymatic hydrolytic efficiency, the lignin-hemicellulose network has been loosened for the better amenability of cellulose to residual carbohydrate fraction for sugar recovery. Dilute acid treatment is employed for the degradation of hemicellulose leaving lignin and cellulose network in the substrate. Preferably, act upon lignin leaving cellulose and hemicellulose network in the residual portion. However during both treatment processes a considerable amount of carbohydrates are also degraded, hence the carbohydrate recovery is not satisfactory for ethanol production (Jaiswal et al., 2016).

2.10.2.1 Acid hydrolysis

There are two types of acid hydrolysis process commonly used, dilute and concentrated acid hydrolysis. The dilute acid process is conducted under high temperature and pressure and has reaction time in the range of seconds or min. The concentrated acid process uses relatively mild temperatures, but at high concentration of H_2SO_4 and a minimum pressure involved, which only creates by pumping the materials from vessel to vessel. Reaction times are typically much longer than for dilute acid.

2.10.2.1.1 Dilute Acid Hydrolysis

In dilute acid hydrolysis, the hemicellulose fraction is depolymerized at lower temperature than the cellulosic fraction. Dilute H_2SO_4 is mixed with biomass to hydrolyze hemicellulose to xylose and other sugars. Thus hemi-cellulosic fraction of plant cell wall is depolymerized and was lead to the enhancement of cellulose digestibility in the residual solids (Suhas et al., 2013) . Dilute acid hydrolysis has some limitations, if higher temperatures (or longer residence time) are applied, the hemi-cellulosic derived monosaccharide's will degrade and give rise to fermentation inhibitors like furan compounds, weak carboxylic acids and phenolic compounds. These fermentation inhibitors are known to affect the ethanol production performance of fermenting microorganisms (Nathan et al., 2005) .

2.10.2.2 Enzymatic Hydrolysis

The acid, alkaline or fungal pretreated lignocellulosic can be saccharified enzymatically to get fermentable sugars(Suhas et al., 2013) .Bacteria and fungi are the good sources of celluloses, hemicelluloses that could be used for the hydrolysis of pretreated lignocellulosic. The enzymatic cocktails are usually mixtures of several hydrolytic enzymes comprising of celluloses, xylanases, hemicelluloses, and mannanases. In the last decade, new celluloses and hemicelluloses from bacterial and fungal sources have continued been isolated and regular efforts have been made for the improved production of enzymatic titers .However, the celluloses were produced at a concentration too low to be useful.

2.10.3 Fermentation process

Fermentation is a metabolic process of microorganisms to obtain energy by breaking down organic compounds. While microorganisms derive their energy, some byproducts are lactic acid, butane, carbon dioxide, ethanol, cellulose. In ethanol fermentation, derivation of energy from sugars by yeast or bacteria, produce carbon dioxide and ethanol. Because yeasts produce their energy without the need for oxygen, ethanol fermentation is a facultative anaerobic process (JAGESSAR1, 2017) .A variety of microorganisms, generally either bacteria, yeast, or fungi, ferment carbohydrates to ethanol under oxygen free conditions.

$$3C_5H_{10}O_5 \rightarrow 5C_2H_5OH + 5CO_2 \tag{2.1}$$

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 \tag{2.2}$$

The efficiency of fermenting process depends on several factors, choice of microorganism, raw material, pretreatment method, hydrolysis method and environmental factors such as pH, temperature, substrate and ethanol concentration. Common conditions for fermentation with *Saccharomyces cerevisiae* are normally pH 5.0 and a temperature of maximum 37°C (Badger, 2002) .The performance of the process is affected by different inhibitors generated from the upstream process steps. The hydrolyzers contain, together with fermentable sugars, inhibitors, which restrict the fermenting microorganisms and thus, decrease the ethanol yield. Recirculation of the process water increases these compounds further (MARUTI, 2010).

The optimal pH range for *Saccharomyces cerevisiae* varies between 4.0 and 6.0 depending upon the fermentation medium. The pH affects the efficiency of ethanol fermentation by influencing the activity of plasma proteins. If the fermentation are deactivated by pH < 4.0 the yeast will not be able to grow and produce ethanol efficiently observed an increase in ethanol production as well as fermentation efficiency with an increase in pH from 4.0 to 5.0 and found the optimum pH for *Saccharomyces cerevisiae* species around pH 5 (Rocha et al., 2017).

The optimum temperature for the fermentation with *Saccharomyces cerevisiae* is 37°C. The temperature affects the efficiency of ethanol fermentation by influencing the activity of yeasts. If the fermentation are deactivated by temperature < 37°C the yeast will not be able to grow and produce ethanol efficiently observed an increase in ethanol production as well as fermentation efficiency found the optimum temperature for *Saccharomyces cerevisiae* species around 37°C (Rocha et al., 2017).

Distillation

Distillation is one of the steps of the purifications. Distillation is the method to separate two liquid utilizing their different boiling points. However, to achieve high purification, several distillations are required. This is because all materials have intermolecular interactions with each other, and two materials will co-distill during distillation. This means that proportion between two materials, in this case, ethanol and water can be changed, and still, there are two materials in layers, the liquid, and the vapor layers. Simple distillation requires components which have a large difference in boiling points in order to achieve high purities of the separated components (Services, 2011). However, simple distillation could not purify ethanol with 100 % effectiveness because vapor is enriched in ethanol but moisture always remains. This situation is called azeotrope. Azeotrope means the components of mixture at certain proportion have similar boiling point, which leads the expected component was not separated by distillation. If the ethanol distillation proceeds over time, the purified ethanol was collect more water, which negatively affects final ethanol concentration (MARUTI, 2010). Fractional distillation is a technique developed from simple distillation that enables a number of components possessing similar boiling points to be separated (JAGESSAR1, 2017). The presence of azeotrope in ethanol distillation limits the purity of final distilled ethanol less than 95.6 %. However, the use of fractional distillation can obtain higher pure ethanol than 95.6 %.

The principle of fractional distillation is according to a temperature gradient (coolest in the top and hottest at the bottom) generated by the distance from the heat source. When the mixed vapor (ethanol and water) ascends through the temperature gradient, ethanol remains in the vapor and water condenses back to refluxing liquor. After several cycles of vaporization and condensation, ethanol is purified and relatively water free. Industrial distillation is the process of repeated vaporization and condensation in a huge refluxing distillation column.

3. Material and Methods

3.1. Material and Equipment's

3.1.1 Equipment

The materials that were used: Plastic bags, grinder, test tubes, different size of conical flasks and beakers, crucible furnace, desiccator, gloves, filter paper, Oven (Gallenkamp), Digital Balances (Adam,pw124), pH meter (pH meter3310, Jenway), Thermostats, Vessels Rack, Autoclave, Density meter(Dma4100m), Spectrophotometer (Spector uv-vis double beam pc 8 scanning auto cell uvd -3200), Shaker, Fermentation and distillation set ups, Vacuum Filter (model-bn 3 Staatliche, berlin), Sieves (mesh size of 2.0 mm,Sortmks-3332, pfeuffr, Germany), Fourier Transform Infrared spectroscopy (FTIR) and High Performance Liquid Chromatography (HPLC).

3.1.2 Chemicals

Sodium Hydroxide (NaOH, min. assay 98% BDH Chemicals Ltd pool England cellulose), sulphuric acid (H_2SO_4, (98%, England)), Dextrose sugar, Yeast extract, Urea, and $MgSO_4.7H_2O$.

3.1.3 Yeast sources

Yeast (*Saccharomyces cerevisiae*) obtained from Ethiopian biodiversity institute, Addis Ababa Ethiopia.

3.2. Proximate analysis and determination of the chemical composition

3.2.1 Sample collection

Watermelon rind was collected from the local juice and fruit shops. The sample were packed in plastic bags and transported to Addis Ababa Institute of Technology (AAiT) ,School of Chemical and Bio engineering laboratory.

3.2.2 Proximate analysis

All analytical determinations were performed according to standards coupled with triplicate replication.

3.2.2.1 Determination of moisture content

Samples were weighed in a clean preheated moisture crucible of known weight by using sensitive balance. The sample and a crucible were kept in an oven 105°C for a two hour. The crucible was covered and transferred to desiccators, and weighed after the room temperature was attained. The crucible was heated in the oven for another two hours and was re-weighed. This was repeated until constant weight was obtained. The loss of weight was calculated as percent of weight and expressed as moisture content.

$$\text{Moisture content } \% = \frac{W_1 - W_2}{W_1} \times 100 \tag{3.1}$$

Where; W_1 = Original weight of the sample before drying (g)

W_2 = Weight of the sample after drying (g)

3.2.2.2 Determination of ash

A crucible was weighed empty, and then a sample was loaded. The sample and the crucible were placed in a muffle furnace for 5 hours at 550°C. The crucible was removed from furnace and placed in desiccators to cool, then was re-weighed.

$$\text{Ash content } (\%) = \frac{\mathrm{W}_1 - \mathrm{W}_2}{\mathrm{W}_1} \times 100 \tag{3.2}$$

Where: W_1= Original weight of the sample (g)

W_2 = Weight of sample after cooling (g)

3.2.2.3 Determination of Volatile content

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

$$\text{Volatile content } (\%) = \frac{W_1 - W_2}{W_1} \times 100 \tag{3.3}$$

Where: W_1= Original weight of the sample (g)

W_2 = Weight of sample after cooling (g)

3.2.3 Chemical composition of watermelon peels (lignin, cellulose, hemicellulose, and extractives).

3.2.3.1 Determination of extractives

Oven dried sample was placed on to thimble and placed in a soxhlet extraction tube. An exhaustive ethanol extraction was completed in 4 hours using the Soxhlet method (Sarkar et al., (2012)).

$$\text{Extractive}\ (\%) = \frac{W_1 - W_2}{W_1} \times 100 \qquad (3.4)$$

Where: W_1 = oven dry sample (g)

W_2 = extracted residue (g)

Figure 3-1: Soxhlet extraction unit set up

3.2.3.2 Determination of cellulose content

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

$$\text{Cellulose }(\%) = \frac{W_1 - W_2}{W_1} \times 100 \qquad (3.5)$$

Where: W_1 = oven dry sample (g)

W_2 = extracted residue (g)

3.2.3.3 Determination of lignin content

Extractive free sample was placed in flask and 72% sulfuric acid was added. The flask was kept in water bath at 30°C, during the dispersion of the material for 1hr. Deionized water was added and sample was placed in autoclave for 121°C, to 1h. Next, the insoluble material (lignin) was filtered by the vacuum filtration. The lignin was washed until it became acid free (with hot water) then it was dried and weighed.

$$\text{Lignin }(\%) = \frac{W_1 - W_2}{W_1} \times 100 \qquad (3.6)$$

Where: W_1 = oven dry sample (g)

W_2 = extracted residue (g)

3.2.3.4 Determination of hemicellulose content

The hemicellulose content, WH, was calculated by difference, assuming that extractives, cellulose, lignin, ash, and cellulose are the only components of the entire biomass(Thomas Karsch, 1983).

$$100 = W_C + W_H + W_E + W_L$$

$$WH = 100 - W_C + W_E + W_L \qquad (3.7)$$

Where: $W_C, W_H, W_E\ W_L$

are cellulose content, hemicellulose content, extractive, and lignin content respectively in (g).

3.3. Experimental procedure

3.3.1 Raw material preparation

Watermelon rind was collected from the local juice and fruit shops. The sample was packed in plastic bags and transported to Addis Ababa Institute of Technology (AAiT), School of Chemical and Bio engineering laboratory. The watermelon rind was washed twice with distilled water in order to remove unwanted matter. The rinds were chopped in to small pieces and dried in a hot air oven at 65°C for 48h. The dried sample was ground using milling machine to the size of 2 mm and sieve analysis was performed ,because the standard size range for lignocellulose material varies from 1-3mm (Sarkar et al., (2012)). Grinding of watermelon peel into fine powder gives increased surface area, which enhances the mass transfer.

Figure 3-2 : (a) watermelon rind sample b) Ground sample

3.3.2 Acid pretreatment

The goal of any pretreatment method is to alter or remove the structural and compositional impediments to hydrolysis in order to improve the rate of dilute acid hydrolysis and increase the yield of fermentable sugars from cellulose or hemicellulose(Nathan et al., 2005). Watermelon peel powder was feed as batches and every batch contains 70 g of screened watermelon peel powder with a ratio of 10:1(v/w) distilled water to the sample. Dilute sulfuric acid 1.5% concentration was used and watermelon peel powder was pretreated in an autoclave and heated at temperature of 120℃, for 30 min. After that, it was cooled and filtered. The filtrate was preserved in another conical flask and stored for fermentation. The residue were washed twice by distilled water to remove sulfuric acid from it and kept for hydrolysis purpose.

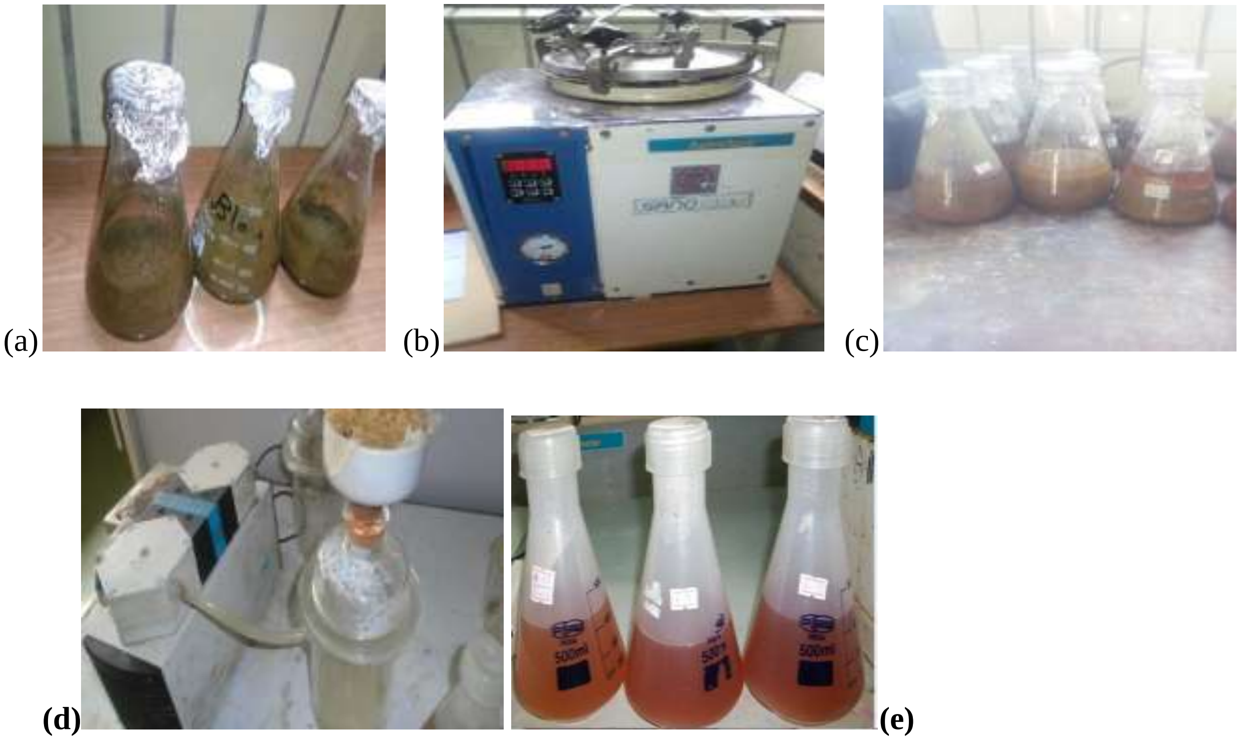

Figure 3-3: (a) Raw materials ready for pretreatment (b) Autoclave (c) Sample after pretreatment (d)Vacuum filtration, (e) Filtered sample

3.3.3 Dilute Acid Hydrolysis

Dilute acid hydrolysis is an easy and productive process. Research work on the dilute acid hydrolysis of different lignocellulosic materials have defined optimal process conditions: temperature 80-200°C, sulfuric acid concentration 0.25–8 w%, and reaction time 10-2000 min (Yulia, 2011).With 40 g of dried sample from the pretreatment

Table 3-1 : Experimental factors and levels for dilute acid hydrolysis

Factor name	Level		
Temperature (°C)	120	130	140
Acid-concentration (%)	3	4	5
Time (min)	30	60	90

Procedures for Acid Hydrolysis

- Different concentration of sulfuric acid was prepared and added to the non-soluble component from the pretreatment steps.
- 40g watermelon peel was then hydrolyzed in the reactor at a temperature of 120, 130 and 140°C, acid concentration 3, 4 and 5% and time of 30, 60, and 90 min.
- After hydrolysis, the solid part was separated from the liquid by vacuum filtration unit to remove the non-fermentable component.

Figure 3-4: Samples after hydrolysis

3.3.4 Filtration

The monomeric sugar was separated in a pressurized filter or bag filter. Then, the sugar solutions (hydrolyzed) were neutralized and introduce into fermentation. The solid part obtained from this filtration process was dried and weighed.

3.3.5 Determination of residual glucose after the hydrolysis

The concentration of total reducing sugar content of hydrolyzed, obtained from dilute acid hydrolysis was determined using a uv-vis spectrophotometer (pc 8 scanning auto cell uvd -3200), by measuring absorbance vs. sugar concentration.

3.3.5.1 Phenol sulphuric acid method for total carbohydrate

The phenol sulphuric acid method to estimate reducing sugar determination is described below. In the hot acidic medium. Glucose is dehydrated to hydroxymethyl furfural. This forms a green colored product with phenol and has absorption maximum at 490 nm (Dubois et al., 1956).

Standard Preparation:

A 0.009g/L standard stock solution of glucose was prepared by dissolving 0.9g of glucose in 100mL distilled water. Working standards were prepared by pipetting 2.5, 5.0, 7.5, and 1 g aliquots of the standard stock solution into separate 100 mL volumetric flasks and diluting to volume with 50mL distilled water. Five tubes were prepared for the standard preparation; one tube for blank, the four tubes for glucose standard. 2mL of sample containing glucose standard was pipetted 2mL of 5% phenol were added to all tubes and mixed. Then 10mL of 96% concentrated sulphuric acid was added, simultaneously the tubes were shaken to effect fast and complete mixing. For color development, the tubes are allowed to stand for 10 min and then placed in water bath (25℃ - 30℃) to cool and display color. Blank solutions were prepared in the same way as above, except that the 2 mL of the standard solution was replaced by distilled water. The amount of reducing sugar present in the sample solution was calculated using the standard graph.

$$\mathrm{y} = mx + b \tag{3.8}$$

Where: y = absorbance
x = concentration
m =slope and
b = intercept

$$\text{Conc. of unknown sample} = \frac{(\text{absorbance of unknown sample}) - (\text{y} - \text{intercept})}{\text{Slope}} \tag{3.9}$$

Where

$$\text{Mass of reducing sugar (g)} = \text{Sugar concenteration} \times \text{volume of sample} \quad (3.10)$$

$$\text{Bioethanol yield} \left(\frac{\text{mL}}{\text{g}}\right) = (\text{volume of ethanol produced} \times \%\ \text{ethanol})/\text{gram glucose useed} \quad (3.11)$$

Where: sugar concentration was found from the glucose concentration vs Absorbance standard curve

:%ethanol was found from pycnometer

pH Adjustmen

Before the addition of any microorganism to the above prepared samples, pH of these samples has to be adjusted to avoid the decease of micro-organism in hyper acidic or basic state. A pH of around 4, 5&6 was maintained. First, the pH meter was calibrated by using buffer solution. Pretreated and hydrolyzed solutions were mixed, shaken, and primarily checked for pH using a digital pH meter. Since, the mixed sample was more acidic to maintain the pH of around 4-6 sodium hydroxide solution was added.

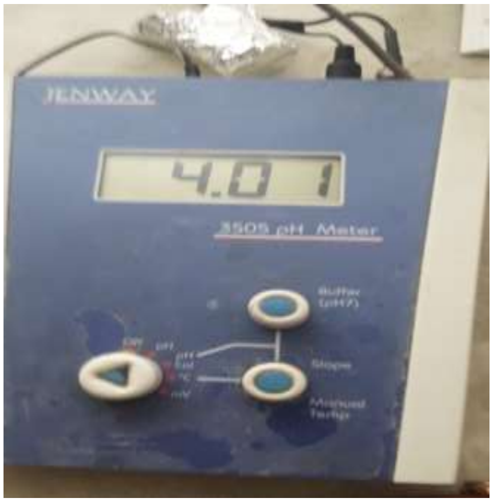

Figure 3-5: Adjustment of pH after the hydrolysis (pH meter3310, Jenway)

3.3.6 Fermentation

The fermentation process was carried out in a shaker incubator, at 30°C, with stirring at 180 rpm, for a 72h because this fermentation conditions are good for the production of bioethanol using *Saccharomyces cerevisiae* (Sumphanwanich et al., (2008).).The prepared hydrolyzates were adjusted to pH of 4-6, optimum for *Saccharomyces cerevisiae* using 2M sodium hydroxide solution. In order to prepare the media the favorable condition for yeast growth must be established to supply the required amount of nutrients.

Fermentation medium

The aim of this step is to culture the microorganism that is used for the fermentation. Yeast (*Saccharomyces cerevisiae)* employed for the conversion of glucose to ethanol. Before conducting fermentation, the media for the preparation of yeast was prepared. 200 mL of production medium was prepared according to the requirements of *Saccharomyces cerevisiae*, containing 4 g dextrose, 2g malt yeast extract, 4g peptone, 1g Urea; 1g $MgSO_4.7\ H_2O$ and 200 mL make up distilled water and adjusts the pH to 5. This was autoclaved at 121°C, for 15 min. After that, 1g of yeast *Saccharomyces cerevisiae* was added to the above 200 mL media in a 500 mL conical flask and then measure the absorbance using Spectrophotometer before placed in to the incubater. Then the conical flasks were properly covered with an aluminum foil. Finally, the conical flask was placed in a shaker incubator for 24 h, at a temperature of 30°C and 180rpm and measure absorbance using Spectrophotometer the density of the Medium was increased (IGELIGE, 2015).

Figure 3-6: Cultured media

3.3.7 Sterilization

The aim of this step was to eliminate the contaminant microorganisms, which could share the reducing sugar prepared for ethanol production by *Saccharomyces cerevisiae*. All the equipment's that were used for fermentation purposes were sterilized (autoclaved). The sterilization was carried out at a temperature of 121°C for 15 min & at 1bar.

Figure 3-7: Autoclave for sterilization

Fermentation

The prepared sterilized sample and media were mixed in the 500mL flasks with the ratio of 10 % (1% media with 10% sample) (Sumphanwanich et al., (2008).) . Then, it was placed on shaker incubator at a temperature of 30°C and at 180rpm for 72 h. In addition, after 72 h of fermentation, the samples were take-out and distilled.

3.3.8 Design of the Experiment for fermentation

The laboratory experiment was based on response surface method design where the different factors was analyzed for the different combinations of their test the effect of different sugar concentration, inoculum levels and pH levels using response surface method were determined. Randomization of the experimental runs as well as appropriate analysis technique was ensured through software of design expert 11 (Box-Behnken Design). The response variable is bioethanol yield and data analysis of the effect of the above parameters was done by analysis of variance

(ANOVA). The responses function (Y) was partitioned into linear, quadratic, and interactive components. Experimental data were fitted to the second-order regression equation:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \ldots \quad (3.12)$$

Where β_0 is the intercept; β_1, β_2, and β_3 are linear coefficients; β_{11}, β_{22}, and β_{33} are squared coefficients; β_{12}, β_{13}, and β_{23} are interaction coefficients

Table 3-2: Experimental factors and levels for fermentation

Factor name	Level		
Mass of reducing sugar (g)	Minimum	Middle	Maximum
Inoculum(mL)	10	15	20
pH	4	5	6

3.3.9 Distillation

Whatever method of preparation is used, the ethanol is initially obtained in a mixture with water. The ethanol is extracted from this solution by fractional distillation

Distillation was the final step in the production of bioethanol from watermelon peel. Distillation is the method used to separate two liquid based on the difference of their boiling points The fermented product was distilled using fractional distillation at a temperature of 85°C for 2-3h. Although the boiling point of ethanol, 78.3°C, is significantly lower than the boiling point of water, 100°C, Temperature of sample to be distilled was measured by immersion of thermometer. 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 the stillage, hade been used as byproduct.

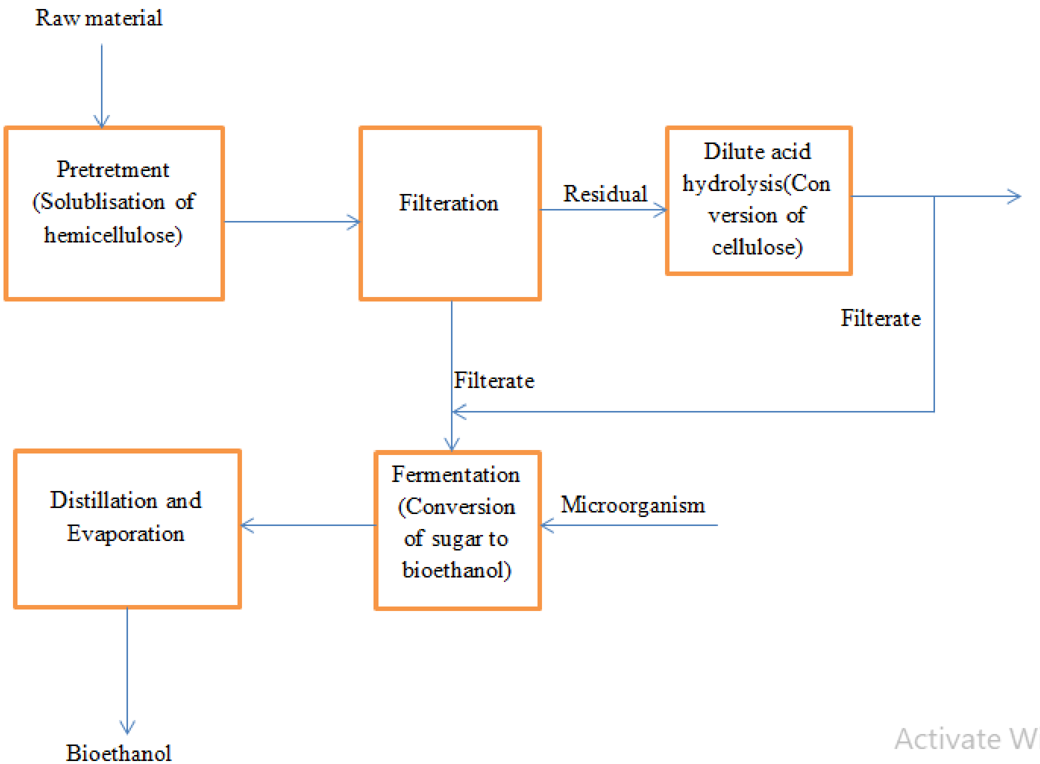

Figure 3-8: Schematic Design of Bioethanol Production

3.4. Characterization of the product

3.4.1 FTIR spectroscopy analysis of bioethanol

Infrared spectrometer passes infrared radiation through a sample of an unknown compound and uses a detector to plot percent transmission of the radiation through the molecule versus the wavenumber of the radiation. A downward peak on the plot represents absorption at a specific wavenumber. In sum, IR spectroscopy is useful in determining chemical structure because energy that corresponds to specific values allows us to identify various functional groups within a molecule. An IR spectrum usually extends from radiation around 4000 cm^{-1} to 600 cm^{-1} and can be split into the functional group region and the fingerprint region. The fingerprint region is different for each molecule just like a fingerprint is different for each person. Two different molecules may have similar functional group regions because they have similar functional groups, but they always have a different fingerprint region.

3.4.2 High performance liquid chromatography analysis of bioethanol

High-performance liquid chromatography (HPLC; formerly referred to as high-pressure liquid chromatography) is a technique used to separate, identify, and quantify each component in a mixture. The schematic of a HPLC instrument typically includes a degasser, sampler, pumps, and a detector. The sampler brings the sample mixture into the mobile phase stream which carries it into the column. The pumps deliver the desired flow and composition of the mobile phase through the column. The detector generates a signal proportional to the amount of sample component emerging from the column, hence allowing for quantitative analysis of the sample components flow rates for the different components and leading to the separation of the components as they flow out of the column. The components of the sample mixture are separated from each other due to their different degrees of interaction with the adsorbent particles. operational pressures are significantly higher (50–350 bar) &typical HPLC runs are 20 to 30 min, while ordinary liquid chromatography typically relies on the force of gravity to pass the mobile phase through the column. The column temperature & RI detected temperature is 75℃ & 30℃ respectively.(Karger et al., (1997)).

4. Results and Discussion

4.1. Characterization of watermelon peel

4.1.1 Proximate analysis

Table 4-1 : Proximate analysis of watermelon rinds

Physical composition	Weight percentage (% wt. dry basis)	Standard weight percentages (%)(Abdu et al., 2018)
Moisture	0.833	1.1
Volatile	52.7	59.03
Ash	1.428	2
Fixed carbon	9	7.87

The results from this study are in a comparable range with the values reported by the aforementioned researcher, but there is a significant difference between weight percentage of the sample &standard weight, the reason for this difference can be the efficiency of the material (equipment) used for the process. Moisture content is a measure of the amount of water in the watermelon peel. 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(Abdel et al., 2014). The analysis of total moisture was used to determine other properties such as volatile matter, ash content, and fixed carbon. The sample of watermelon peel with higher moisture content needs more heat for moisture vaporization. Ash is a measure of inorganic impurities in the watermelon peel. In this study, low ash content of watermelon peel constituents could decrease the sludge formation during the bioethanol production. Finally, fixed carbon (FC), the carbon found in the material, which is left after volatile materials are driven off, is used for the determination of carbon in the watermelon peel. Having high amount of carbon is important for the production reducing of sugar, because if the raw material have high amount of carbon it produced high amount of sugar.

4.1.2 Chemical composition analysis

Table 4-2: The results of chemical composition of Watermelon peel sample

Chemical composition	Weight percentage (w/w %)	Standard weight percentage (%)(Abdu et al., 2018)
Extractive	0.75	1
Cellulose	55	59.03
Hemicellulose	23.5	30
Lignin	5.26	7.89

The results from this study are in a comparable range with the values a reported by the aforementioned researcher, but there is a significant difference between weight percentage of the sample &standard weight, the reason for this difference can be the efficiency of the material (equipment) used for the process. Therefore, the determination of cellulose and hemicellulose can applied to quantify the theoretical production of bioethanol. However, *Saccharomyces cerevisiae* only converts glucose. In this study, watermelon peel, contained high contents of the total cellulose of approximately 55% cellulose. The low level of lignin present in the sample of watermelon peel used in this study appears more advantageous than the other types. The lower, the lignin content the easier the hydrolysis condition, and hence a decrease in the formation of toxic chemicals (aromatic, polyaromatic, phenolic and aldehydic) (Abdel et al., 2014).

4.2. Measurement of reducing sugar

In this study, the total reduced sugar content after the dilute acid hydrolysis process was investigated. The powdered watermelon peel was hydrolyzed at different acid concentration, hydrolysis time, and temperature on the amount of sugar produced was investigated. The standard glucose concentration and its absorbance (glucose calibration curve) shown below.

Table 4-3 : Glucose concentration and its absorbance

Glucose concentration, g/L	Absorbance
2	0.163
4	0.378
6	0.576
8	0.786
10	0.987

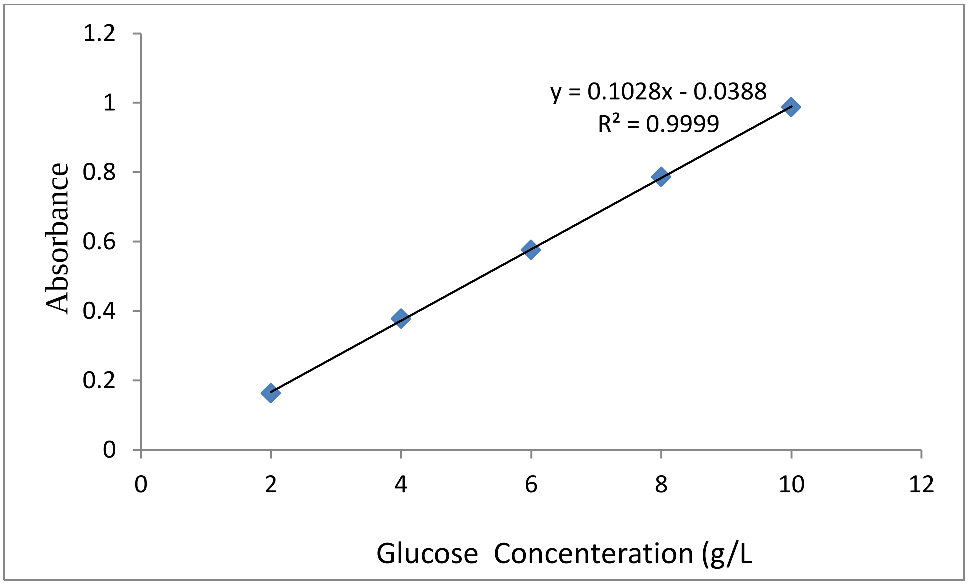

Figure 4-1: Calibration curve of glucose standard for determination of glucose content

The concentrations of unknown sugar samples were determined from a standard curve of glucose ($y = 0.1028x - 0.0388$; $R^2 = 0.9999$).

Table 4-4: Glucose yield at various temperature, acid concentration, and reaction time

Std	A: Acid conc. (%)	B:Temperature (°C)	C:Time (min)	Mass of reducing sugar (g)
1	3	120	60	2.551
2	5	120	60	2.553
3	3	140	60	2.923
4	5	140	60	2.895
5	3	130	30	3.878
6	5	130	30	3.900
7	3	130	90	3.950
8	5	130	90	3.609
9	4	120	30	3.798
10	4	140	30	2.978
11	4	120	90	3.721
12	4	140	90	2.955
13	4	130	60	4.750
14	4	130	60	4.810
15	4	130	60	4.866
16	4	130	60	4.889
17	4	130	60	4.891

Regarding the acid hydrolysis of watermelon peel, table 4.4 the maximum yield of glucose concentration was obtained at 4% of acid concentration, at a temperature of 130°C and hydrolysis time of 60 min (mass of reducing sugar 4.891g). The minimum mass of reducing sugar was observed at 4% of acid concentration, at a temperature of 140°C and hydrolysis time of 90 min (mass of reducing sugar 2.55 g). The medial mass of sugar was observed for 4% of acid concentration, at a temperature of 120°C and hydrolysis time of 90 min (mass of 3.721g). The mass of sugar were observed to decrease at high acid concentration, increasing time and temperature. This may be due to; at higher acid concentration, hydrolysis produces sugar degradation products such as pentose sugar monomers may dehydrate to the inhibitor furfural, hexose sugars (e.g. glucose) may degrade to the toxic hydroxymethyl-furfural (HMF) which leads to decreased glucose yield & carrying reaction. Maximum reducing sugar produced from jackfruit and Pineapple rinds was 4.64 g and 4.38g respectively(Suhas et al., 2013). In comparison to the other studs, watermelon peel has high sugar content.

Table 4-5: Design summary for dilute acid hydrolyses

Study type	Response surface
Initial point	Box-Behnken Design
Center point	5
Design Model	Quadratic polynomial
Run	17
Blocks	No

Table 4-6: ANOVA for Quadratic model for dilute acid hydrolysis

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

F- Value is a test for comparing the model variance with residual (error) variance. If the variances are close to each other, the ratio will be close to one and it is less likely that any factors have a significant effect on the response. It is calculated by model mean square divided by residual mean square. Here the model F- Value of 13.51 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, C, B^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. The "Lack of Fit F-value" of 1.57 implies the Lack of Fit is not significant relative to the pure error. There is a 32.21 % chance that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit is good. The "Pred R-Squared" of 0.9121 is in reasonable agreement with the "Adj R-Squared" of 0.9427. "Adeq Precision" measures the signal to disturbance ratio due to random error. A ratio greater than 4 is desirable. Here ratio of 7.526 indicates an adequate signal. Therefore, this model can be used to navigate the design space.

4.3. Effect of fermentation on the bioethanol yield

In this study, the total reducing sugar content after the hydrolysis process was investigated. The sample from hydrolysis was fermented at different mass of reducing sugar (Maximum, Minimum & middle), inoculum levels (10-20 mL), and pH levels (4-6), and there results are shown in Table 4.7.

Table 4-7: Ethanol yield at various inoculum levels, sugar concentration and pH levels.

	Factor 1	Factor 2	Factor 3	Response
Run	A:mass of reducing sugar (g)	B:innoculem level (mL)	C:pH level	Ethanol Yield (mL/g)
1	2.551	15	6	0.365
2	3.721	15	5	0.470
3	4.891	20	5	0.391
4	2.551	10	5	0.309
5	3.721	20	4	0.400
6	3.721	20	6	0.393
7	3.721	15	5	0.484
8	4.891	15	4	0.394
9	3.721	15	5	0.497

10	4.891	10	5	0.338
11	3.721	10	4	0.348
12	3.721	15	5	0.473
13	4.891	15	6	0.357
14	3.721	15	5	0.475
15	2.551	15	4	0.347
16	2.551	20	5	0.372
17	3.721	10	6	0.357

Regarding fermentation of watermelon peel, From Table 4.7 the maximum bioethanol 0.497 mL/g was obtained at an experiment number 9 at 15 mL of Inoculum level, 3.721g of reducing sugar, and at 5 pH level. While the minimum yield, 0.309 mL/g was obtained at experiment number 4, at 10 mL of Inoculum level, 2.551g of reducing sugar, and at 5 pH level. The decrease and increase of the yield was depending on the level of factors because there are specific conditions for the production of bioethanol using *saccharomyces crevasse*. . Maximum ethanol produced from jackfruit and Pineapple rinds was 0.67 mL/g and 0.59mL/g respectively (Suhas et al., 2013). In comparison to the other studs, ethanol produced from watermelon is less this is may be due to the efficiency of the material & the efficiency of the yeast. There resulting data, From Table 4.7, were analyzed using Design expert® 11 software to determine the effect of reducing Sugar, Inoculum level and pH level. The dependent variable used as a response parameter was the ethanol yield. All experiments were carried out in a randomized order to minimize the effect of unexpected variability in the observed response due to extraneous factors.

Table 4-8: Design summary

Study type	Response surface
Initial point	Box-Behnken Design
Center point	5
Design Model	Quadratic polynomial
Run	17
Blocks	No

To determine whether the quadratic model is significant, it was crucial to perform analysis of variance (ANOVA) (Table 4.9). The probability values (p-values) were used to perform as a device to check the significance of each coefficient, which also showed the interaction strength of each parameter. The smaller the p- values the bigger, the significance of the corresponding variables.

Table 4-9: Analysis of variance (ANOVA)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0557	9	0.0062	86.78	< 0.0001	significant
A-Mass of reducing sugar	0.0009	1	0.0009	13.28	0.0082	
B-Inoculum level	0.0063	1	0.0063	87.82	< 0.0001	
C-pH level	0.0002	1	0.0002	2.28	0.1749	
AB	0.0000	1	0.0000	0.4105	0.5421	
AC	0.0008	1	0.0008	10.67	0.0137	
BC	0.0003	1	0.0003	4.74	0.0658	
A^2	0.0208	1	0.0208	292.43	< 0.0001	
B^2	0.0135	1	0.0135	189.98	< 0.0001	
C^2	0.0080	1	0.0080	112.87	< 0.0001	
Residual	0.0005	7	0.0001			
Lack of Fit	0.0000	3	7.200E-06	0.0603	0.9781	not significant
Pure Error	0.0005	4	0.0001			
Cor Total	0.0562	16				

F- Value is a test for comparing the model variance with residual (error) variance. If the variances are close to each other, the ratio will be close to one and it is less likely that any factors have a significant effect on the response. It is calculated by model mean square divided by residual mean square. Here , The model F-value of 86.78 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B, AC, A^2, B^2, C^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. The Lack of Fit F-value of 0.06 implies the Lack of Fit is not significant relative to the pure error. There is a 97.81% chance that a Lack of Fit F-value this large could occur due to noise. Non-significant lack of fit is good. Coefficient of variation, the standard deviation expressed as a percentage of the mean; predicted residual sum of squares, which is the a measure of how the model fits each point in the design; the R- squared, measure of the amount around the mean explained by the model; Adj R- squared, a measure of the amount of variation in new data explained by the model, and Adequate precision, this is a signal to disturbance ratio due to random error, are used to decide whether the model can be used or not.

The Predicted R^2 of 0.9797 is in reasonable agreement with the Adjusted R^2 of 0.9806; i.e. the difference is less than 0.2 as one might expect. The difference between Adj R-Squared and Pred R-Squared is 0.0009 (i.e. they are reasonably close to each other). This indicated a close fit of the model to the actual response data. “Adeq Precision” measures the signal to noise ratio. A ratio greater than 4 is desirable. Here the ratio of 26.0416 indicates an adequate signal. This model can be used to navigate the design space.

4.3.1 Development of regression model equation for mass of reducing sugar & ethanol yield

The application of RSM gives an empirical relationship between the response function and the independent variables. The mathematical relationships between the response, ethanol yield and the independent variables mass of reducing sugar (A), inoculum (B) and pH (C) in terms of coded and actual factors can be determined by Design Expert software 11.

$$\begin{aligned}\text{Mass of reducing sugar}\\ &= +4.83 - 0.0449 \times \text{A} - 0.1097 \times \text{B} - 0.0400 \times \text{C} - 0.0186 \times \text{AB} - 0.0906 \times \text{AC} \\ &+ 0.0136\text{BC} - 0.8126 \times A^2 - 1.28B^2 - 0.1861C^2 \qquad (4.1)\end{aligned}$$

Where; A = Acid concentration (%)

B = Temperature (°C)

C = Time (min)

$$\text{Ethanol Yield} = +0.4802 + 0.0109 \times \mathrm{A} + 0.0280 \times \mathrm{B} - 0.0045 \times \mathrm{C} - 0.0027 \times \mathrm{AB} - 0.0138 \times \mathrm{AC} - 0.0092 \times \mathrm{BC} - 0.0704 \times \mathrm{A2} - 0.0567 \times \mathrm{B} - 0.0437 \times C^2 \quad (4.2)$$

Where; A = Reducing sugar

B = Inoculum level

C = pH

Final Equation in Terms of Actual Factors

$$\text{Ethanol yield} = -2.3137 + 0.4576 \times \text{mass of reducing sugar} + 0.08456 \times \text{innoculem level} + 0.5040 \times \text{pH level} - 0.0004 \times \text{mass of redusing sugar} \times \text{innoculem level} - 0.0117 \times \text{mass of redusing sugar} \times \text{pH level} - 0.0018 \times \text{innoculem level} \times \text{pH level} - 0.0005 \times \text{mass of redusing sugar}^2 - 0.00226 \times \text{innoculem level}^2 - 0.0437\ \text{pH level}^2 \quad (4.3)$$

The result, showed that yield of ethanol produced from watermelon peel was dependent on the linear terms, on the quadratic terms and on the interactions of variables.

4.4. Model Adequacy Check for mass of reducing sugar & ethanol yield

The model was tested for adequacy by analysis of variance. The regression model was found to be highly significant with the correlation coefficients of determination of R -Squared, adjusted R-Squared and predicted R-Squared having a value of 0.9911, 0.9806, and 0.9797 respectively. The quality of the model developed could be evaluated from their coefficients of correlation and the value of R-squared for the developed correlation is 0.9911. It implies that 99.11% of the total variation in the percentage of conversion is attributed to the experimental variables studied. The graph of the predicted values obtained using the developed correlation versus actual values is shown in Figure below. The results in Figure demonstrate that the regression model equation provided a very accurate description of the experimental data, in which all the points are very close to the line of perfect fit. This result indicates that it was successful in capturing the correlation between the three variables to the yield of ethanol.

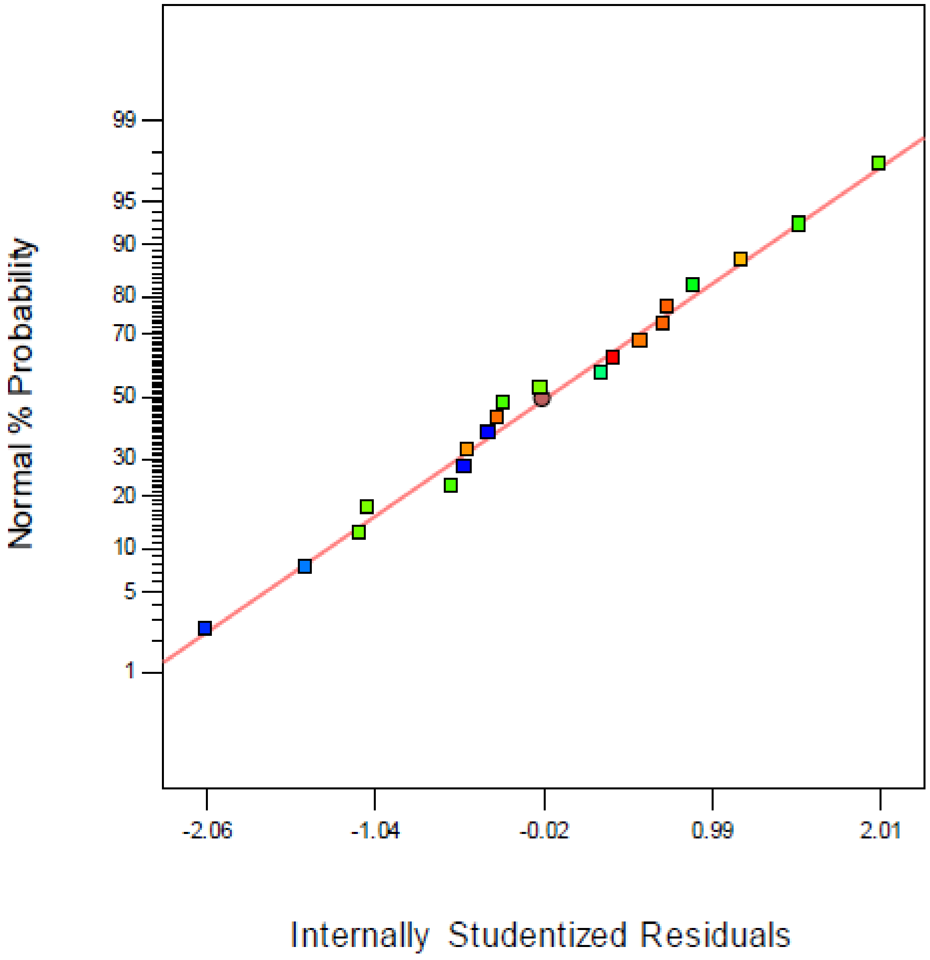

Figure 4-2: Normal plots of residuals for mass of reducing sugar

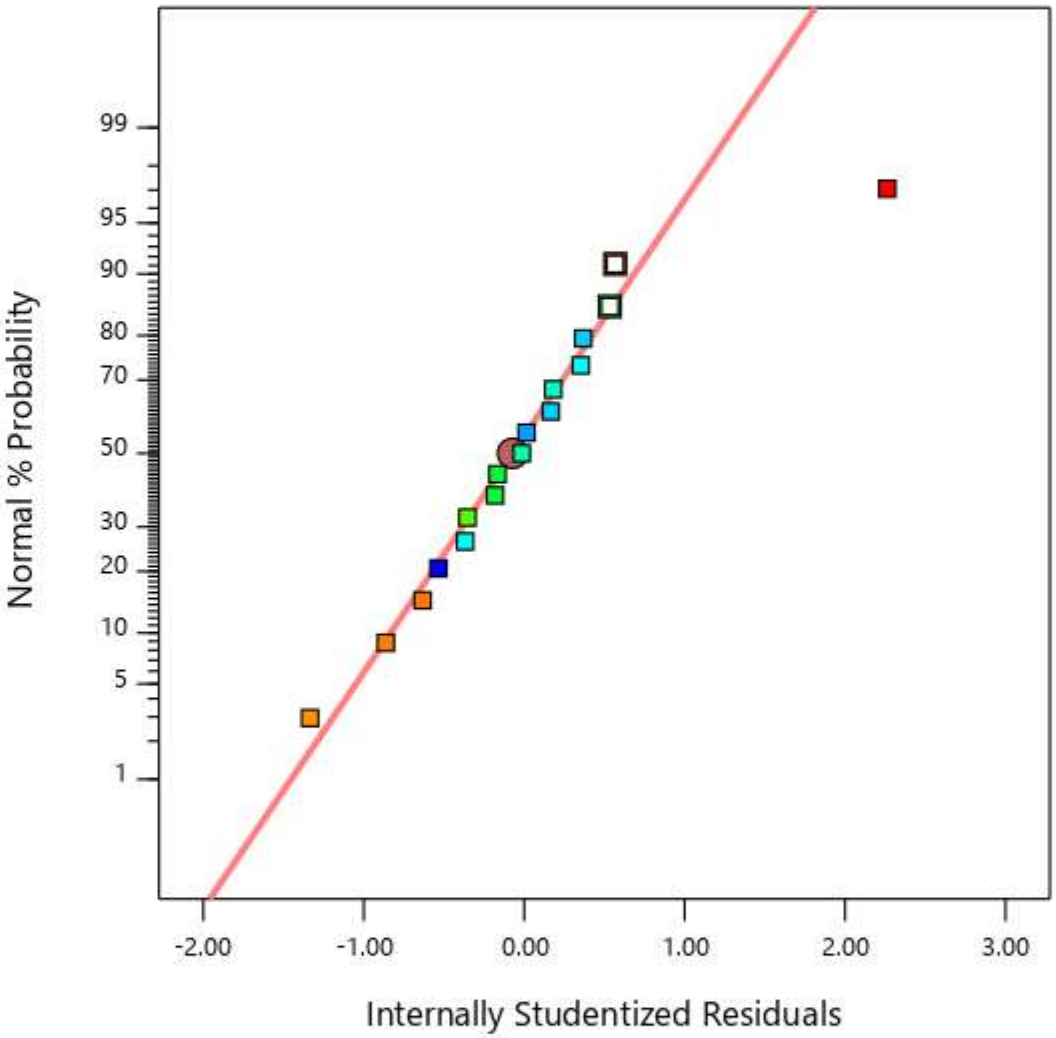

Figure 4-3 : Normal plots of residuals for ethanol yield

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

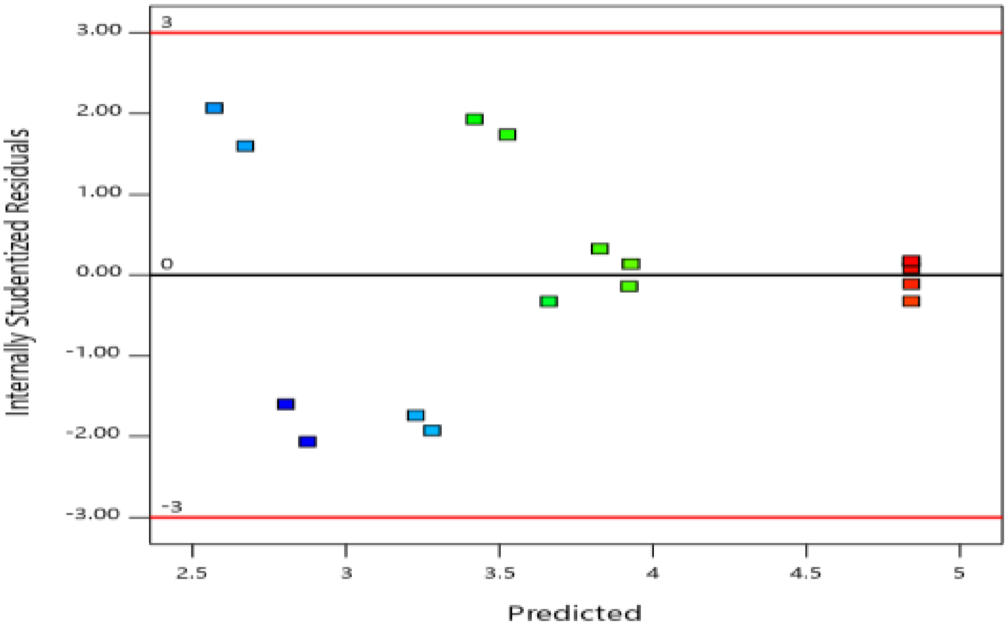

Figure 4-4 : Plot of residuals versus model predicted values for mass of reducing sugar

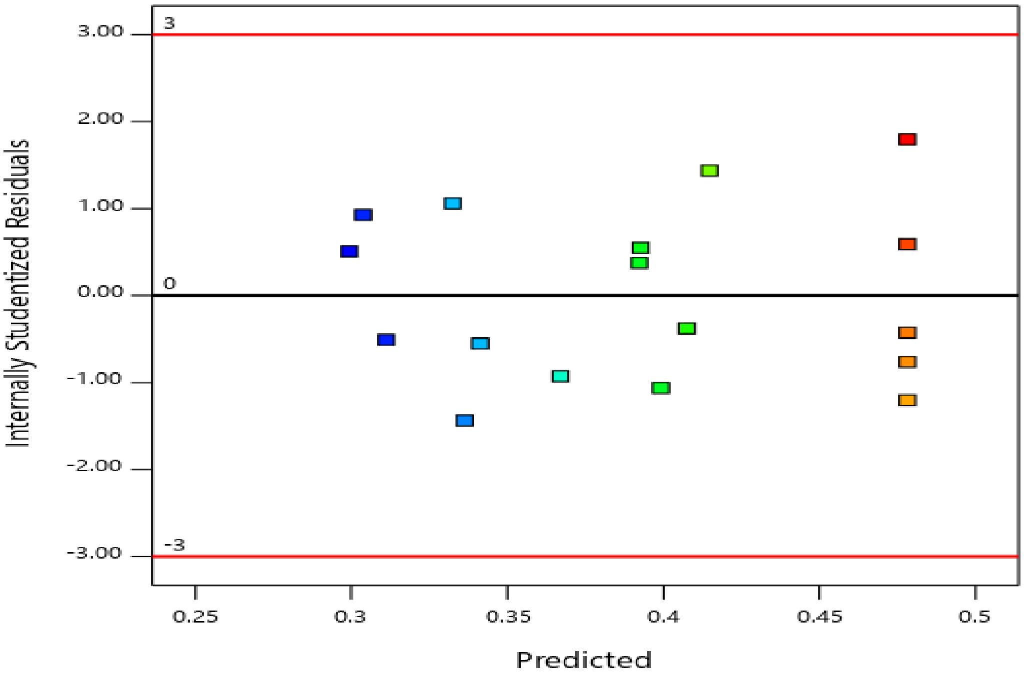

Figure 4-5: Plot of residuals versus model predicted values for bioethanol yield

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

4.4.1 Individual effect of experimental variables on the mass of reducing sugar

4.4.1.1 Effect of acid concentration on the mass of reducing sugar

The resulting plot of acid concentration versus the mass of reducing sugar, when temperature and hydrolysis time were actual factors, was depicted in Figure 4.6. As shown from the plot increasing acid concentration from 3% to 4%, mass of reducing sugar increased from 2.55g to 4.89g. Beyond 4%, acid concentration the mass of reducing sugar decreased to 2.55g is due to degradation of pentose, hexoses, and the lignin present. Therefore, optimum acid concentration was found to be 4% and the mass off reducing sugar at this acid concentration was 4.89g.

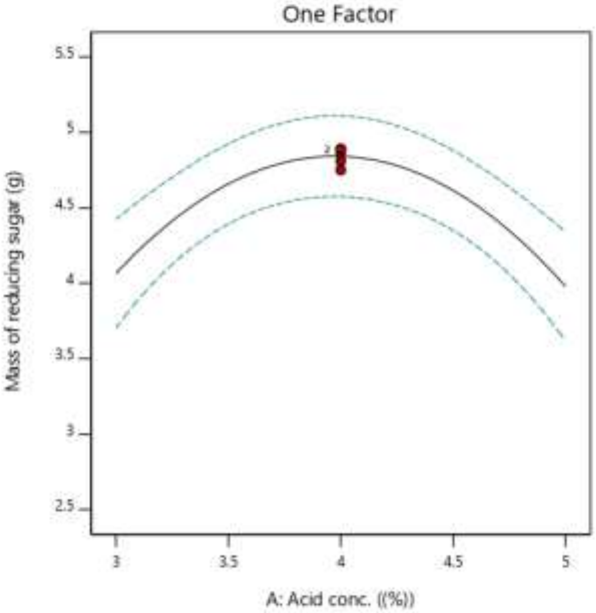

Figure 4-6: Effect of acid concentration on the mass of reducing sugar

4.4.1.2 Effect of temperature on mass of reducing sugar

The resulting plot of temperature versus the mass of reducing sugar, when Acid concentration and hydrolysis time were actual factors was depicted in Figure 4.7. From the plot, as the temperature increases from 120℃ to 130℃, mass of reducing sugar increased to 4.89 g by. Beyond 130°C, mass of reducing sugar decreased to 2.55g, is due to further conversion sugar degradation products such as pentose sugar monomers may dehydrate to the inhibitor furfural, hexose sugars (e.g. glucose) may degrade to the toxic hydroxymethyl-furfural (HMF) which leads to decreased glucose yield. Therefore, the optimum temperature was found to be 130℃ and the mass of reducing sugar at this temperature was 4.89g.

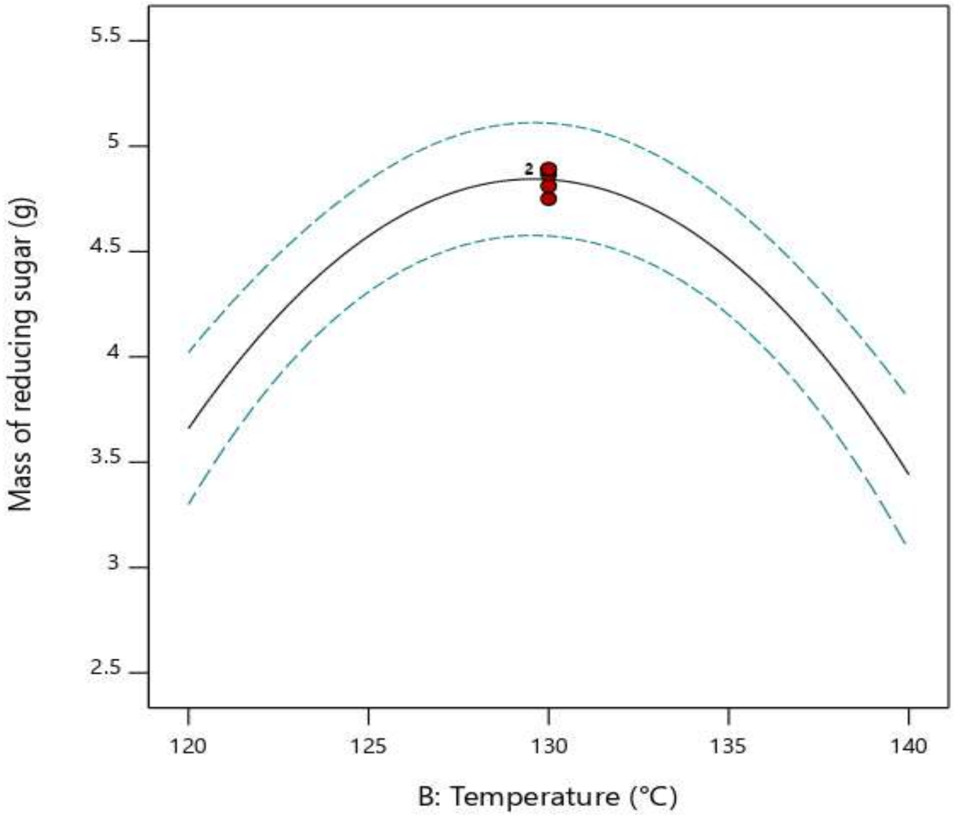

Figure 4-7: Effect of temperature on the mass of reducing sugar

4.4.1.3 Effect of time on mass of reducing sugar

The resulting plot of time versus the ethanol yield, when Acid concentration and hydrolysis temperature were actual factors, was depicted in Figure 4.8 below. As shown from the plot increasing time from 30 to 90 min, mass of reducing sugar decreased the reason was due to formation sugar degradation products such as pentose sugar monomers may dehydrate to the inhibitor furfural, hexose sugars (e.g. glucose) may degrade to the toxic hydroxymethyl-furfural (HMF) which leads to decreased glucose yield.

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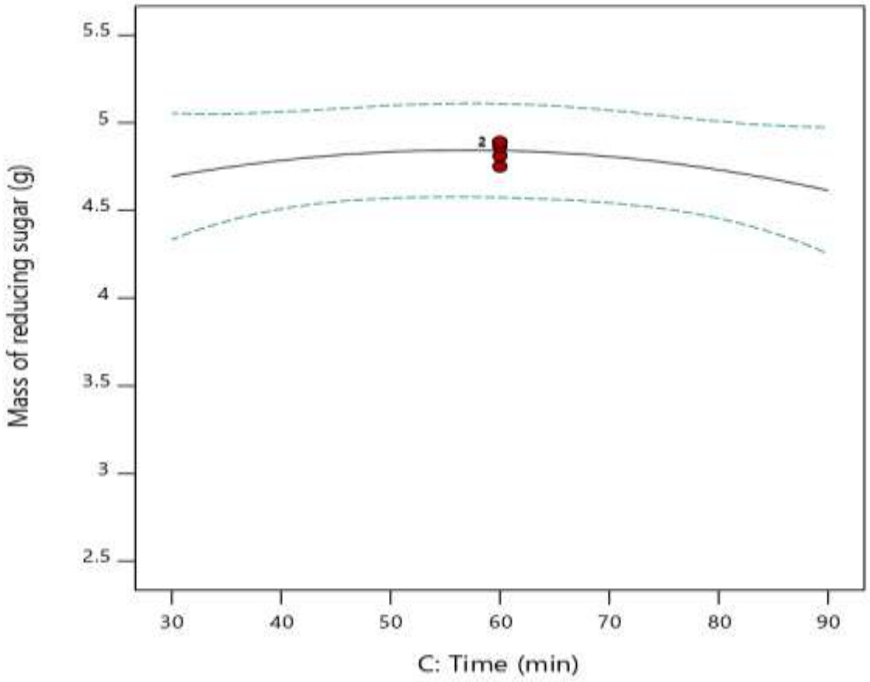

Figure 4-8: Effect of time on the mass of reducing sugar

4.4.2 Effects of Experimental variables on dilute acid hydrolysis

The hydrolysis steps has a complex connection with independent variables. The best way of showing, the effects of this parameter for the mass of reducing sugar are to generate response surface plots of the equation. The three-dimensional i.e. interactions, contours and response surfaces effect as a function of the interactions of any two of the variables by holding the other value of the variable at center.

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

4.4.2.1 The effect of acid concentration and temperature on the mass of reducing *sugar*

The figure 4.9 shows the effect of acid concentration and temperature on the mass of reducing sugar, when time was at the center point. As observed from the acid concentration and temperature had no interaction and effect on the mass of reducing sugar. When acid concentration was at high (5%) and, temperature was low (120°C), the amount of mass of reducing sugar was low (2.553 g), and when acid concentration is at low level (3%) and at low temperature (120°C) the mass of reducing sugar is low (2.551 g), this might be due to the acid concentration is insufficient for hydrolysis of glucose. The second case is at high acid concentration (5%) and temperature (140°C), the mass of reducing sugar of is low (2.855 g) compared to the middle maximum point. This might be due to the reason that at high temperature glucose was converted to sugar degradation products such as pentose sugar monomers may dehydrate to the inhibitor furfural, hexose sugars (e.g. glucose) may degrade to the toxic hydroxymethyl-furfural (HMF) which leads to decreased glucose yield.

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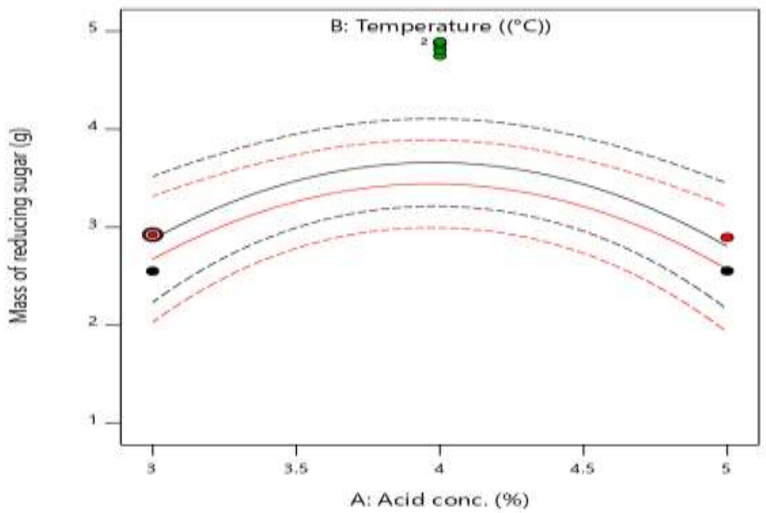


Figure 4-9: The effects of acid concentration and temperature on the yield of ethanol, when the temperature was at the center point.

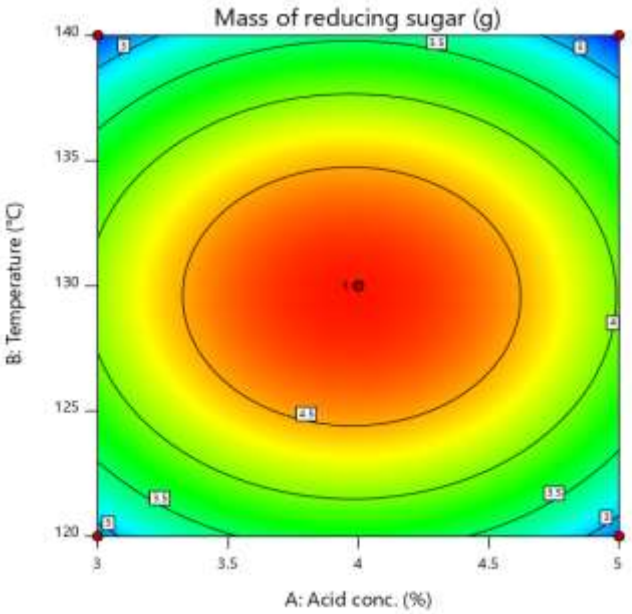


Figure 4-10: Contour plot of the effects of acid concentration and temperature on the mass of reducing sugar

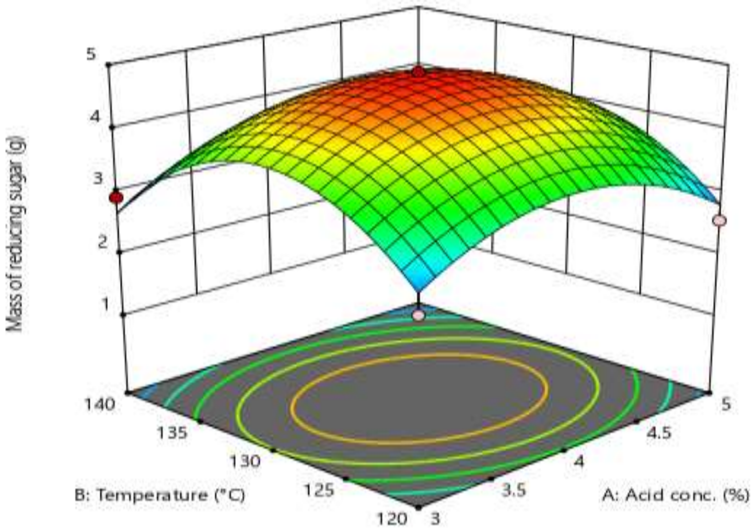


Figure 4-11: Surface plot of the effects of temperature and acid concentration on the mass of reducing sugar

4.4.2.2 The Effects of acid concentration and Time

The graph shown in Figure 4.12 Shows the effect of acid concentration and fixed time on mass of reducing sugar yield but the temperature kept 130°C. The graph shows effect acid concentration on mass of reducing sugar at fixed time when the temperature held at center point. When the acid concentration increases for both high level and level time the mass of reducing sugar increases and reaching the maximum and it starts to decrease.

When acid concentration was at high (5%) and, time was low (30 min), the amount of mass of reducing sugar was low (3.90 g), this might be due to the acid concentration is insufficient for hydrolysis of glucose. The second case is at high acid concentration (5%) and time (90 min), the mass of reducing sugar of is low (3.06g). This might be due to the reason that at high time glucose was converted to These inhibitors have toxic effects on the fermenting organisms, thus reducing the ethanol yield and productivity.

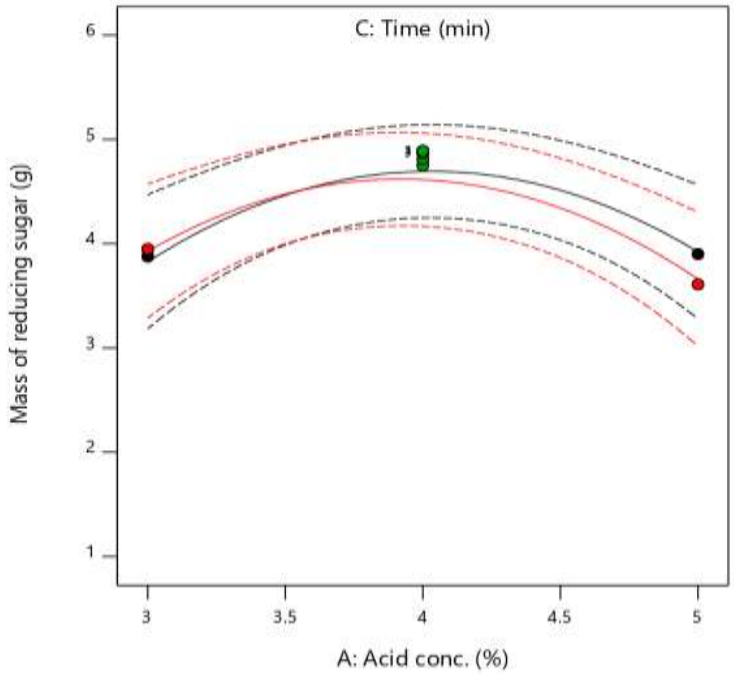


Figure 4-12: Effect of acid concentration and time (fixed) on the mass of reducing sugar center of temperature

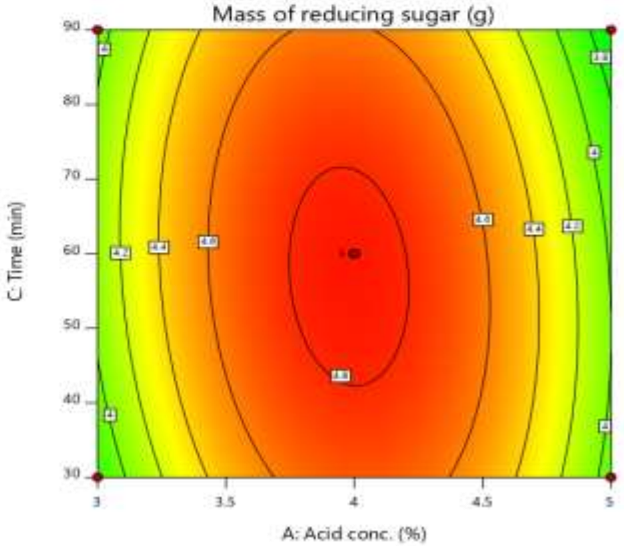


Figure 4-13: Contour plot of the effect of acid concentration and time on the mass of reducing sugar at constant temperature

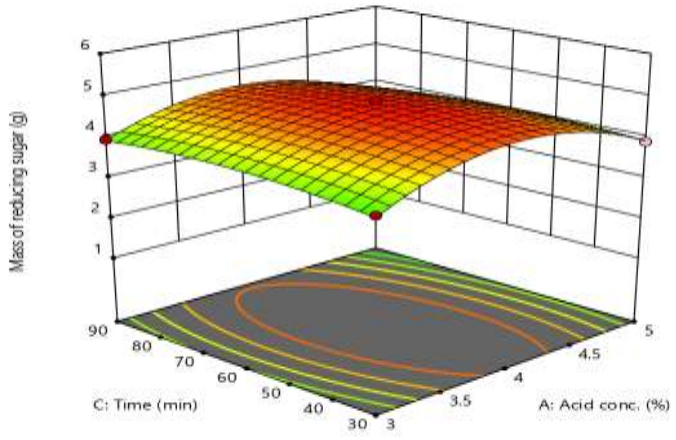


Figure 4-14: Response surface plot of the effect of acid concentration and time on the yield of ethanol at constant temperature

4.4.2.3 The Effects of temperature and time on the mass of reducing sugar

The figure 4.15 shows the effect of temperature and time on the ethanol yield when acid concentration was at the center point. As it observed from figure 4.15, the temperature and time have no interaction on the mass of reducing sugar and it has maximum effect on the mass of reducing sugar at higher temperature until the time reaches 60 min. At high temperature (140°C), the mass of reducing sugar is low compared to low temperature. There are two cases, the first case is, when temperature is at high level (140°C), and at low time (30 min) the mass of reducing sugar is low (2.97g), and when temperature is low (120°C) and at low time (30min), the mass of reducing sugar is low (3.72 g). The 2nd case is at high temperature (140°C) and high time (90 min) the mass of reducing sugar is low (2.95g) compared to the middle maximum point. This might be due to the reason that, at high temperature glucose was converted to sugar degradation products such as pentose sugar monomers may dehydrate to the inhibitor furfural, hexose sugars (e.g. glucose) may degrade to the toxic hydroxymethyl-furfural (HMF) which leads to decreased glucose yield.

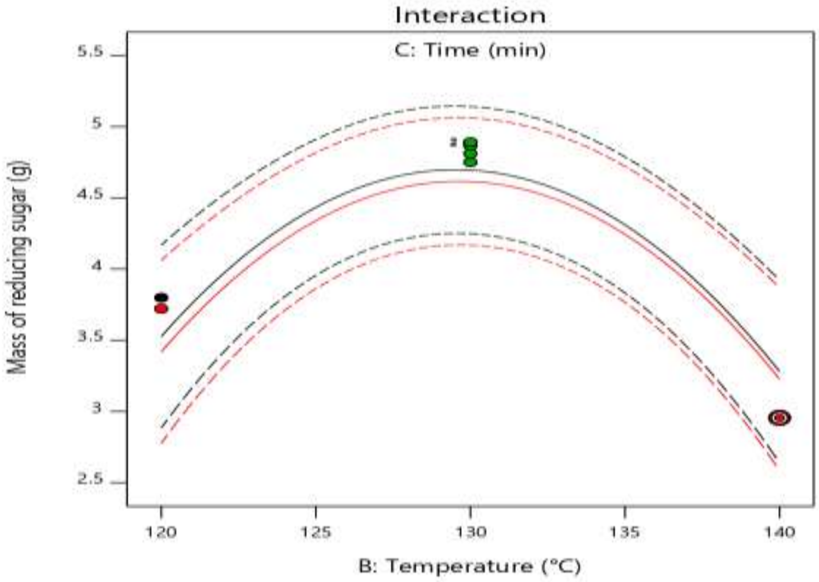


Figure 4-15: Effect of acid concentration and time (fixed) on the mass of reducing sugar at center of temperature

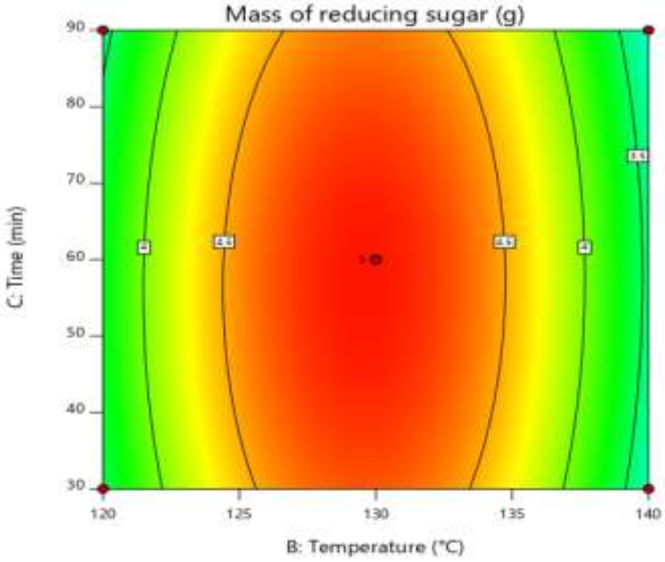


Figure 4-16: Contour plot of the effects of acid concentration and time on the mass of reducing sugar

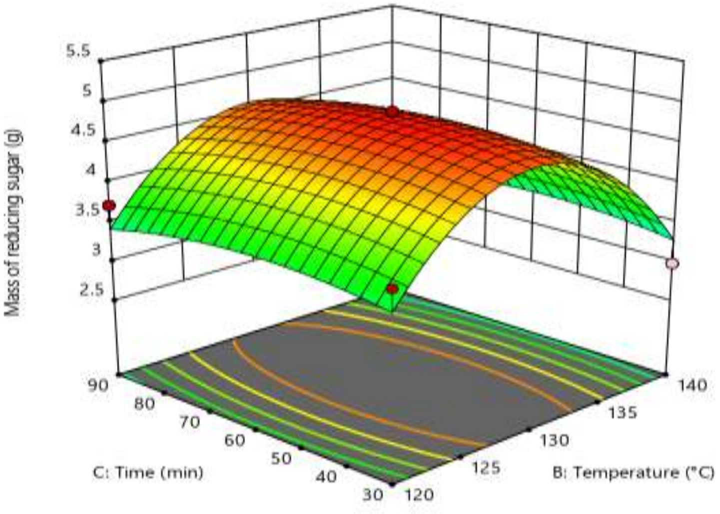


Figure 4-17: Surface plot of the effects of time and acid concentration on the mass of reducing sugar

4.4.3 Individual effect of experimental variables on the yield of ethanol

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

4.4.3.1 Effect of mass of reducing sugar on the ethanol yield

The resulting plot of mass of reducing sugar versus the ethanol yield, when inoculum level and pH level were actual factors, were depicted in Figure 4.18. From the plot, as the mass of reducing sugar increases from 2.551g to 3.721g, ethanol yield increased from 0.309mL/g to 0.497mL/g by weight. Beyond 3.721g, the yield decreased to 0.338mL/g, is due to further conversion in to other products. Therefore, the optimum mass of reducing sugar was found to be 3.721 g and the yield at this mass of reducing sugar was 0.497mL/g.

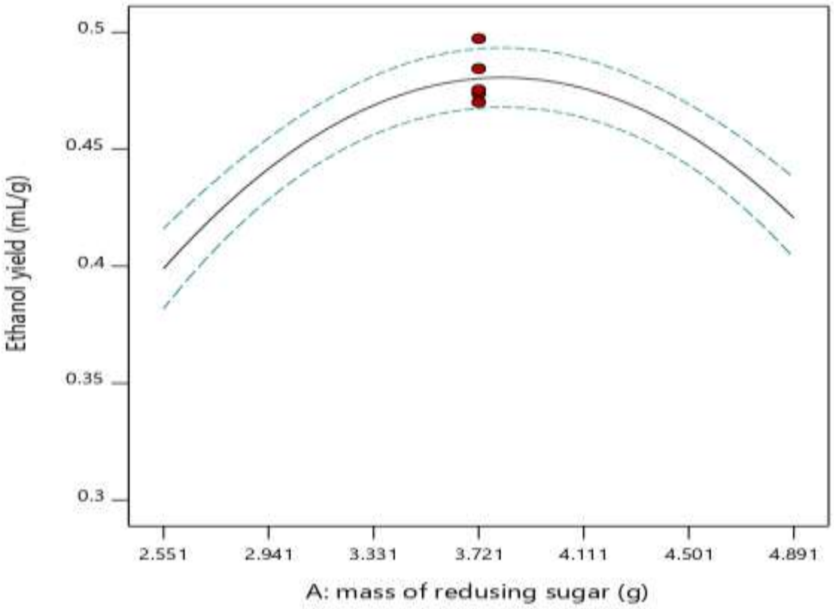


Figure 4-18: Effect of mass of reducing sugar on the ethanol yield

4.4.3.2 Effect of inoculum level on ethanol yield

Figure 4.19 shows the effect of inoculum level on the yield of ethanol at constant mass of reducing sugar and pH level in the center point. As shown in figure 4.19, the yield of ethanol was affected slightly by inoculum level, as the inoculum level increase from 10mL to 15mL the yield slightly increase from 0.309 mL/g to 0.497 mL/g, beyond 15mL the yield of ethanol slightly decrease to 0.447 mL/g.

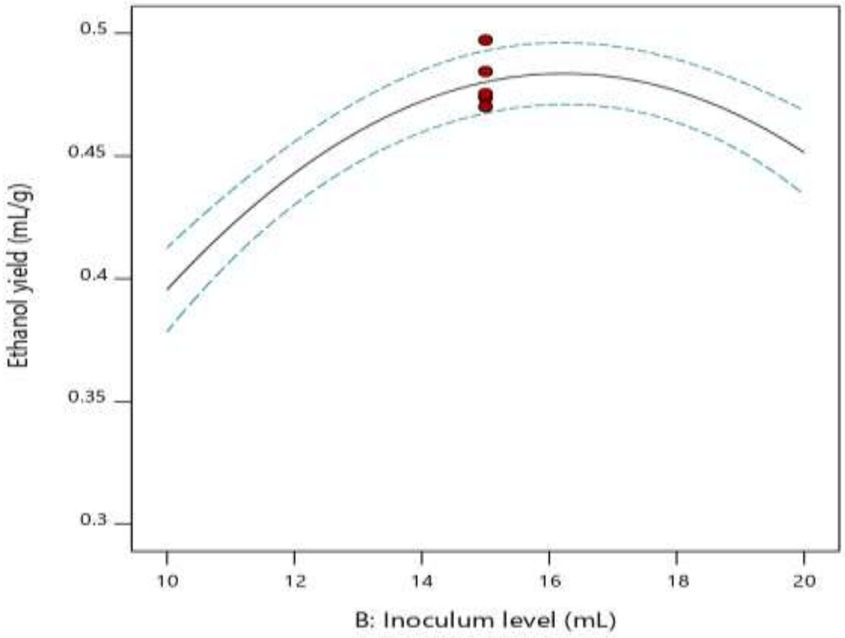


Figure 4-19: Effect of inoculum level on the ethanol yield

4.4.3.3 Effect of pH level on the ethanol yield

The resulting plot of pH level versus the ethanol yield, when inoculum level and mass of reducing sugar were actual factors, was depicted in Figure 4.20. As shown from the plot increasing pH level from 4 to 5, ethanol yield were increased from 0.309 mL/g to 0.497 mL/g Therefore, the optimum pH level was found to be 5 and the yield at this pH level was 0.497 mL/g. If the fermentation are deactivated by pH < 4.0 the yeast was not be able to grow and produce ethanol efficiently observed an increase in ethanol production as well as fermentation efficiency with an increase in pH from 4.0 to 5.0 and found the optimum pH for *Saccharomyces cerevisiae* species around pH 5 (Rocha et al., 2017).

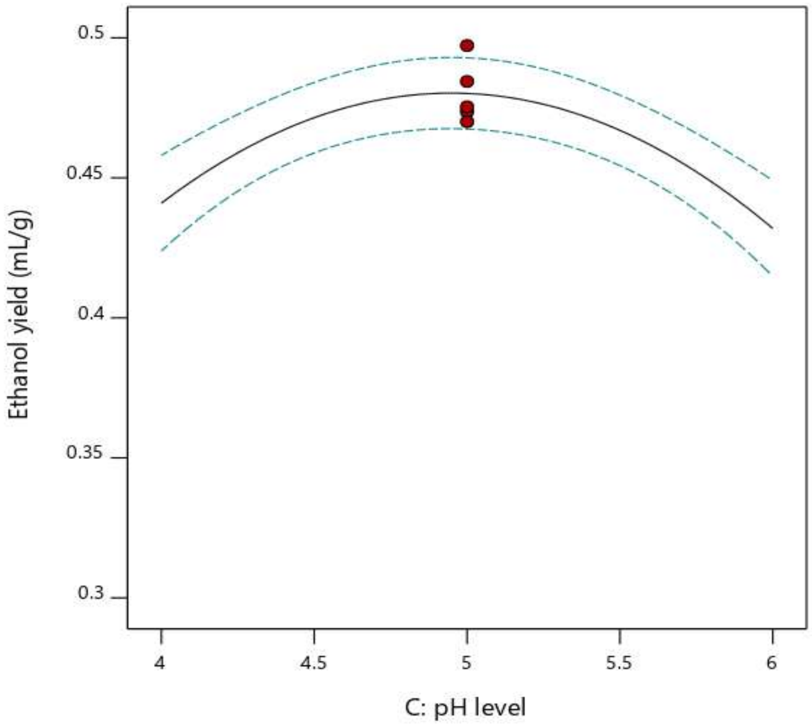


Figure 4-20: Effect of pH level on the ethanol yield

4.4.4 Effects of experimental variables on fermentation

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

Interaction effects are effects that independent variable impose on one another. All controllable factors are obvious variables, which affect the output of the response variable. In this research, there are three controllable factors in the fermentation step namely: mass of reducing sugar, pH level, and inoculum level.

4.4.4.1 The effect of mass of reducing sugar and inoculum level on ethanol yield

The figure 4.21 shows the effect of mass of reducing sugar and inoculum level on the ethanol yield, when pH was at the center point. As it observed from figure 4.21, the mass of reducing sugar and inoculum level had not interact and effect on the ethanol yield and it has maximum effect on the yield of ethanol at higher mass of reducing sugar until the inoculum level reach 15mL. At higher level of mass of reducing sugar, the amount of ethanol yield was high compared to lower sugar concentration. There are two cases, the first case is when mass of reducing sugar was high (4.891g) and, inoculum level was low (10 mL), the amount of ethanol yield was low (0.338 mL/g) and, when mass of reducing sugar was low (2.551g) and at low inoculum level (10mL) ,the amount of ethanol yield was low (0.309 mL/g). This might be due to this insufficient inoculum for fermentation of glucose to ethanol yield is low. The 2nd case is at high inoculum level (20mL) and mass of reducing sugar high (4.891g), when the amount of ethanol yield is low (0.391mL/g) compared to the middle maximum point. This might be due to the reason that the shortage of glucose or the glucose was not enough for the yeasts based on this the yeasts could be died (Iliassou et al., 2019) .

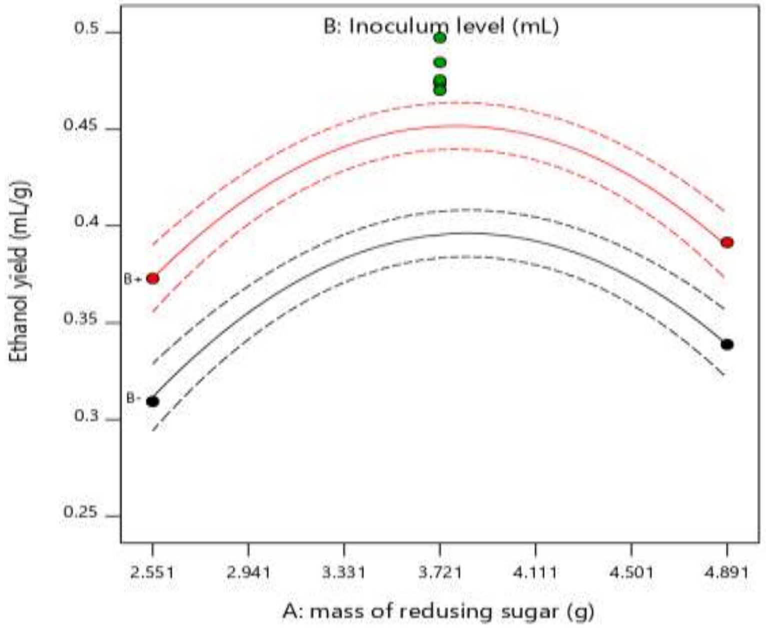


Figure 4-21: The effects of mass of reducing sugar and inoculum level on the yield of ethanol, when the pH level was at the center point

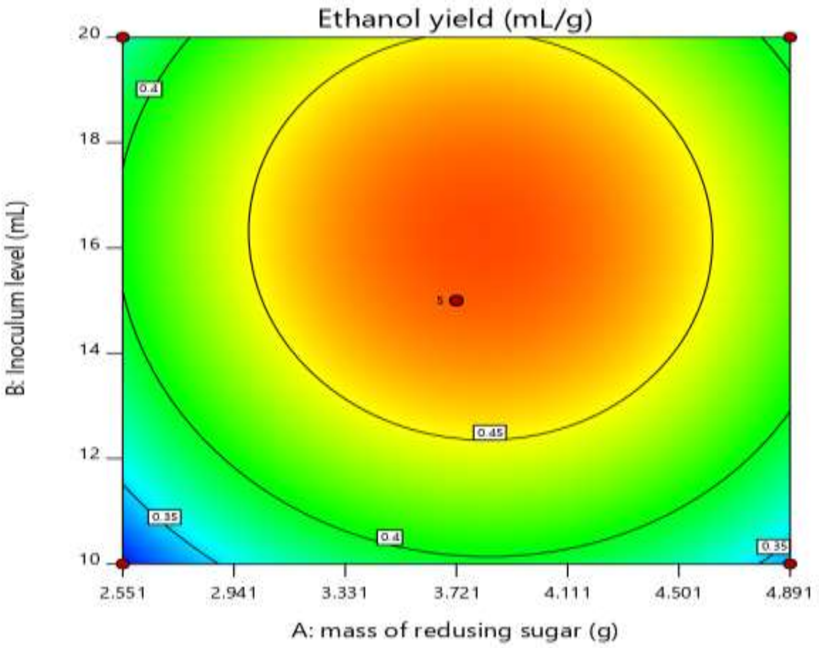


Figure 4-22: Contour plot of the effect of mass of reducing sugar and inoculum level at constant pH in the center

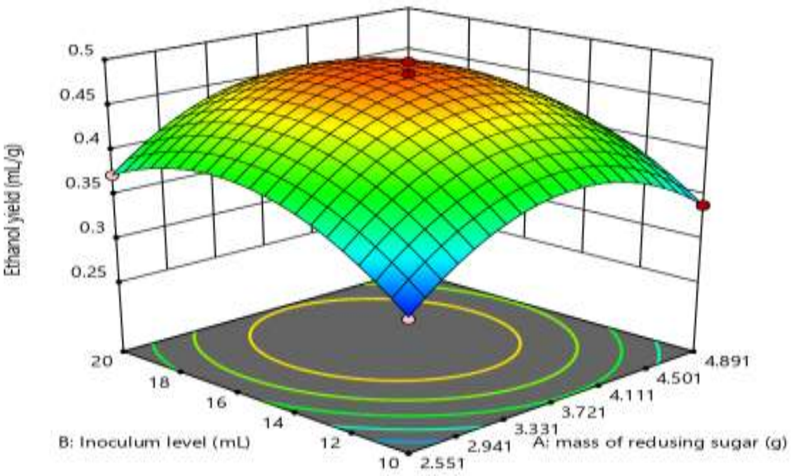


Figure 4-23: Response surface plot of the effect of mass of reducing sugar and inoculum level at constant pH in the center

Figure 4.22 and 4.23 represents contour and the response surface plots developed as a function of mass of reducing sugar and inoculum level respectively, while pH was kept constant at 5. The yield of ethanol increased slightly with the mass of reducing sugar from 2.551g to 3.721g and inoculum level from 10mL to 15mL. However, upon increasing the mass of reducing sugar beyond 3.721g, and inoculum level beyond 15mL there was a gradual decline in the yield, due to the degradation of sugars occurred.

As shown from the Figure 4.23 contour plot, the maximum yield of ethanol (0.497 mL/g) in the region of 3.721g of reducing sugar and 15mL inoculum. There is a color change on the graph and the response variable increases from green 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.

4.4.4.2 Effects of inoculum level and pH on yield of ethanol

The graph shown in figure 4.24 shows the effect of inoculum level and pH on ethanol yield when the mass of reducing sugar was kept 3.721g. The graph shows the effect of inoculum level on ethanol yield at fixed pH, when the mass of reducing sugar is held at center point. As it was observed from figure 4.24 the inoculum level and pH, has maximum effect on the yield of ethanol at higher pH until the inoculum level reached 15mL .But, beyond 15mL inoculum level, at fixed pH the yield of ethanol slightly decreased. There are two cases, the first case is when inoculum level is at higher level (20mL) and at high pH (6), the amount of ethanol yield is high (0.39mL/g) ,and the 2nd case is when inoculum level is at low level (10mL) and at low pH level (4) the amount of ethanol yield is low (0.34mL/g). This might be due to the insufficient inoculum for the fermentation of glucose, and hence yielding a low amount of ethanol yield .This could be due to the reason that the amount of glucose is low, at low level of the inoculum level and pH. At high level of inoculum level and pH, the amount of ethanol yield is low compared to the middle point**.**

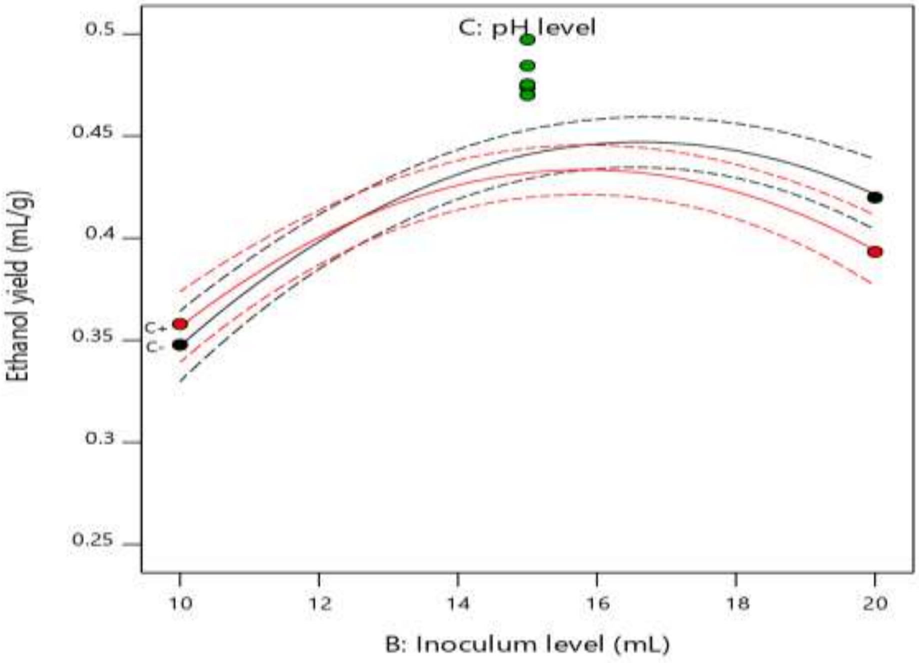


Figure 4-24: Effect of inoculum level and pH at center of mass of reducing sugar

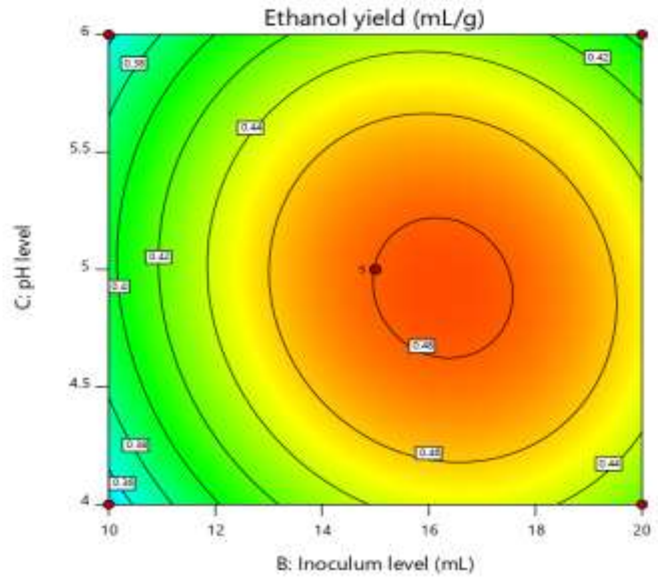


Figure 4-25: Contour plot of the effect of inoculum level and pH on the yield of ethanol at constant mass of reducing sugar ion.

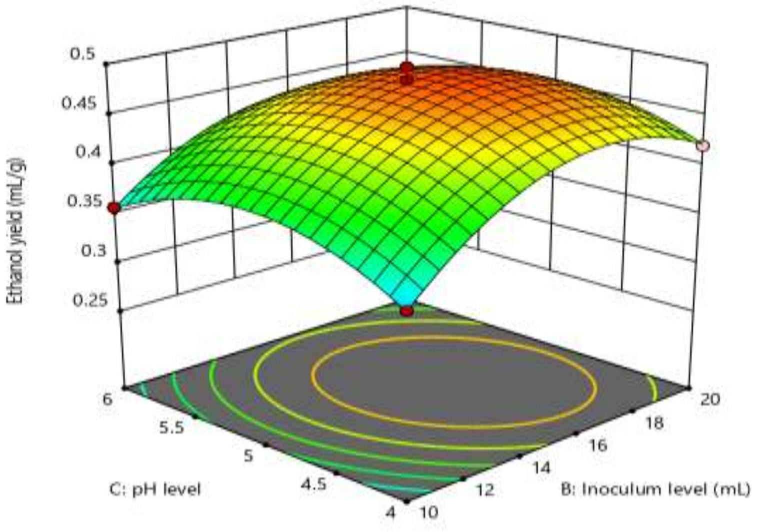
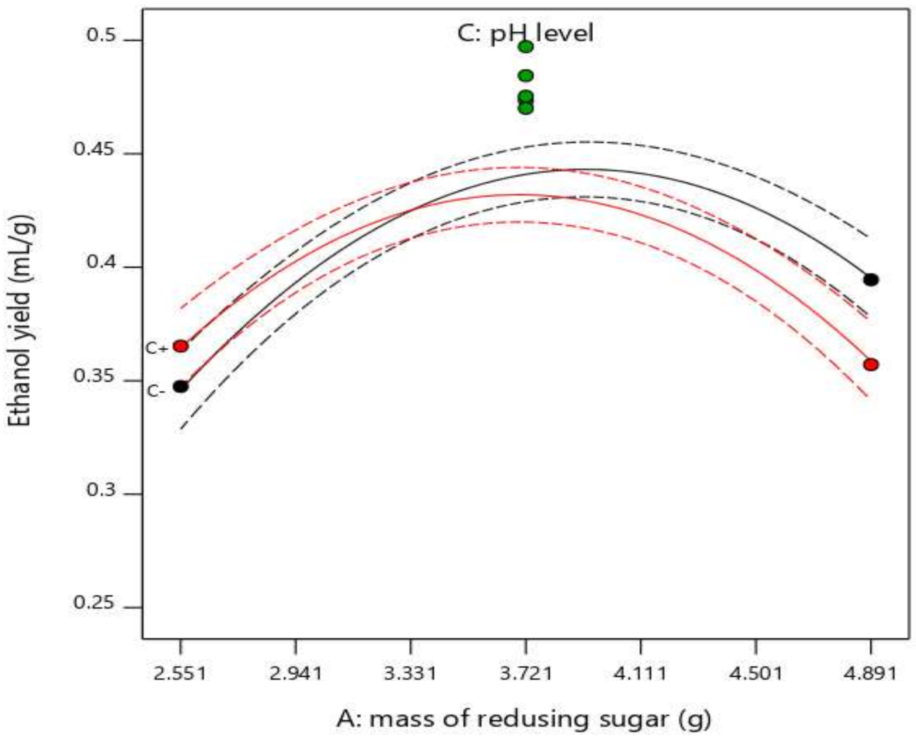


Figure 4-26: Response surface plot of the effect of inoculum level and pH on the yield of ethanol at constant mass or reducing sugar

Figure 4.25 and 4.26 shows the contour and response surface plots developed as a function of inoculum level and pH respectively, while the mass of reducing sugar was kept constant at 3.721g. Upon increasing the inoculum level from 10mL to 15mL, with an increase of pH level from 4 to 5, the yield of ethanol increased in a smaller extent. Beyond 5 pH the yield of ethanol was gradually decreased. The highest yield (0.497 mL/g), was obtained at 5 pH and 15mL inoculum level. The decrement of ethanol yield with increasing of inoculum is due to the decomposition of sugar and the formation of some inhibitors.

4.4.4.3 Effects of mass of reducing sugar and pH on yield of ethanol

To study the effects of mass of reducing sugar and pH on the yield of ethanol, inoculum level were selected at the center point, are shown in figure 4.27. The maximum yield of ethanol (0.49 mL/g), was observed at middle mass of reducing sugar (3.721g) and middle pH (5).



level

Figure 4-27: Effect of mass of reducing sugar and pH at center of inoculum

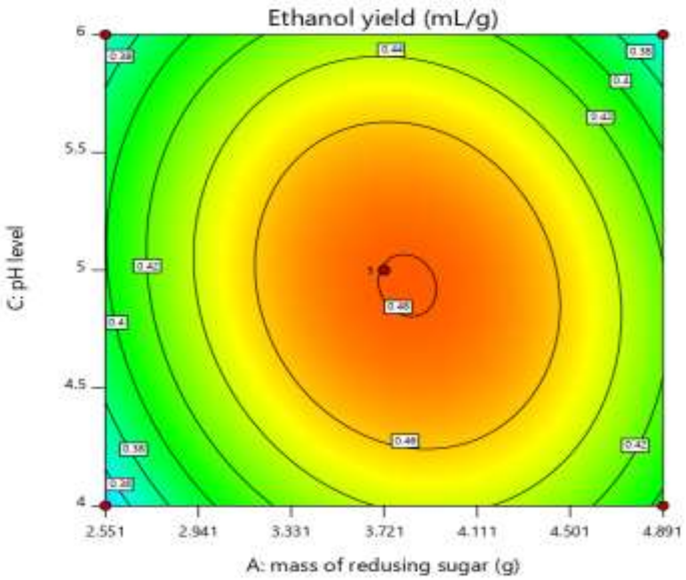


Figure 4-28: Contour plot of the effect of mass of reducing sugar and pH on the yield of ethanol at constant inoculum level.

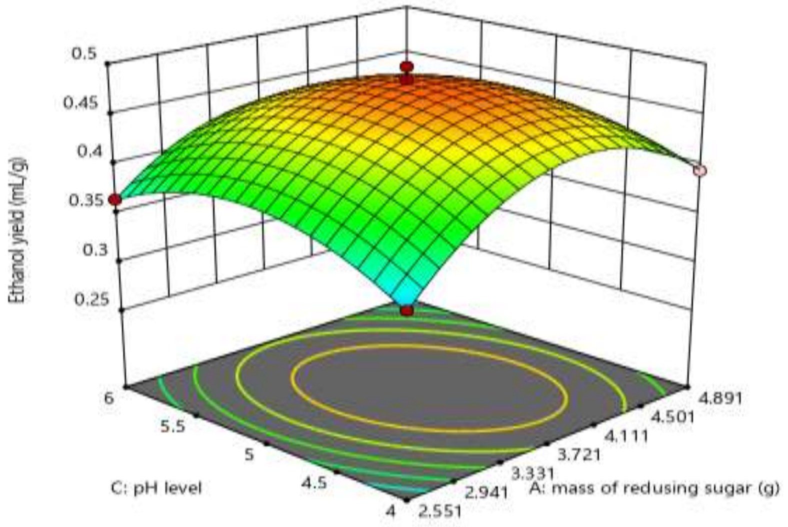


Figure 4-29: Response surface plot of the effect of mass of reducing sugar and pH on the yield of ethanol at constant inoculum level

4.5. Optimization of operating process variables in Fermentation process using RSM

One of the primary objectives of the present study was to find the optimum process parameters for maximizing the quantity of ethanol yield. The process variables such mass of reducing sugar, inoculum level, and pH have been optimized using Box Behnken Design (BBD) and their output values are executed using Design-expert software 11. In the optimizing process, the mass of reducing sugar, Inoculum level and pH are a set of process parameters that should be "in range" while the ethanol yield, need to be "maximized". Table 4.9 shows the summary of factors responses and goals and the corresponding set of specific objectives that was optimized .The table 4.11 exhibits the desired combinations of process parameters that would provide the highest responses by using Numerical optimization. Numerical optimization was used to optimize any combination of one or more goals. The goals may be apply either factors or responses. The model capable of predicting the maximum ethanol yield, the optimum values of the process variables were the operating process variables are putting on the range.

Table 4-10: Constraints applied for optimization

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
A: mass of reducing sugar	is in range	2.551	4.891	1	1	3
B:innoculem level	is in range	10	20	1	1	3
C:ph level	is in range	4	6	1	1	3
Ethanol Yield	Maximize	0.30927	0.49725	1	1	3

By using the numerical optimization criteria in Table 4.11, the Design expert 11 solution was obtained. The possible solution for this model with the given factors that change the amount of the produced ethanol yield is shown in Table 4.11. The optimum possible solutions in fermentation of watermelon peel for yield of maximum of bioethanol are presented in table 4.11.

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Table 4-11: Optimum possible solutions

Number	Mass of reducing sugar (g)	inoculum level (mL)	pH level	Ethanol Yield (mL/g)	Desirability	
1	3.721	15.000	5.000	0.480	1.000	
2	3.721	20.000	4.000	0.406	1.000	
3	3.721	10.000	4.000	0.348	1.000	
4	3.661	19.842	5.867	0.410	1.000	
5	3.601	13.456	5.747	0.439	1.000	
6	3.537	13.564	5.692	0.443	1.000	
7	3.502	10.198	5.497	0.387	1.000	
8	3.738	13.641	4.042	0.428	1.000	
9	3.645	11.121	4.838	0.422	1.000	
10	3.698	15.820	5.022	0.482	1.000	Selected
11	3.754	17.850	4.684	0.473	1.000	
12	3.729	19.510	5.939	0.409	1.000	
13	3.656	10.530	5.676	0.390	1.000	
14	3.662	16.252	4.880	0.481	1.000	
15	3.795	18.942	5.799	0.429	1.000	
16	3.762	16.225	5.728	0.456	1.000	

The desirability lies between 0 and 1 and it represents the closeness of a response to its ideal value. If a response falls within the unacceptable intervals, the desirability is 0, and if a response falls within the ideal intervals or the response reaches its ideal value, the desirability is 1. Based on the above analysis best local maximum for ethanol yield 48.2% was found at mass of reducing sugar 3.698g, inoculum level 15.820mL and pH 5.022 and the value of desirability obtained was 100%.

4.6. Model validation

According to the Box Behnken Design (BBD) result using Design-Expert® 11 software, an experiment with mass of reducing sugar, inoculum level, and pH were conducted in order to study the outcome or effect of the design. The experiment was carried out at the optimized conditions. Numerical optimization was carried out to maximize the yield of ethanol, using the response optimizer in Design expert®11. The optimal values test factors were sugar concentration 3.698 g, inoculum level 15.820 mL and pH 5.022 (obtained from Table 4.11). Ethanol yield of 4.82mL/g obtained, this was in good agreement with the predicted one. Therefore the model is considered to be accurate and reliable for predicting the yield of ethanol.

4.7. Fourier Transform Infrared spectroscopy (FTIR) for Bioethanol Characterization

Alcohols have characteristic IR absorptions associated with the O-H, C-O and the C-H stretching vibrations. When run as a liquid film the region 3500-3200 cm^{-1} with a very intense and broad band indicated the O-H stretch of alcohols, while the region 1260-1050 cm^{-1} confirms the C–O stretch. The bands at around 2880 and 2930 cm^{-1} were assigned as the symmetric stretching modes of the $–CH_2$ and $–CH_3$ groups, respectively (Bodîrlău et al., 2007).This assures that the product obtained from watermelon peel is exactly ethanol due to the confirmation of these regions as show in figure 4.30.

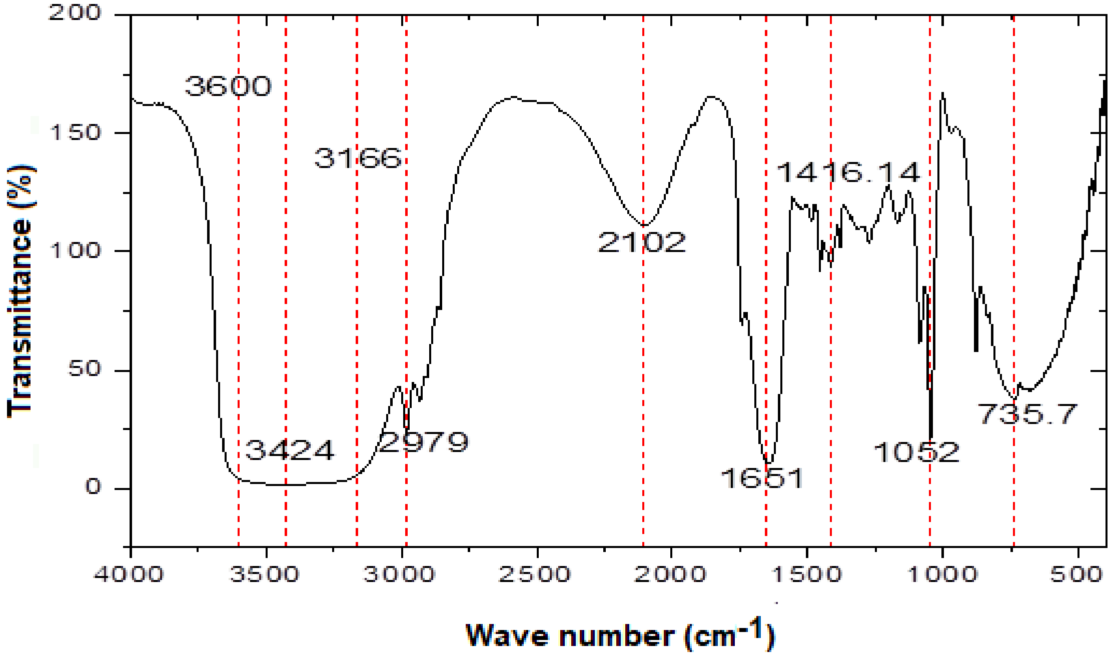

Figure 4-30: Fourier transforms Infrared spectra of the produced bioethanol from Watermelon peel

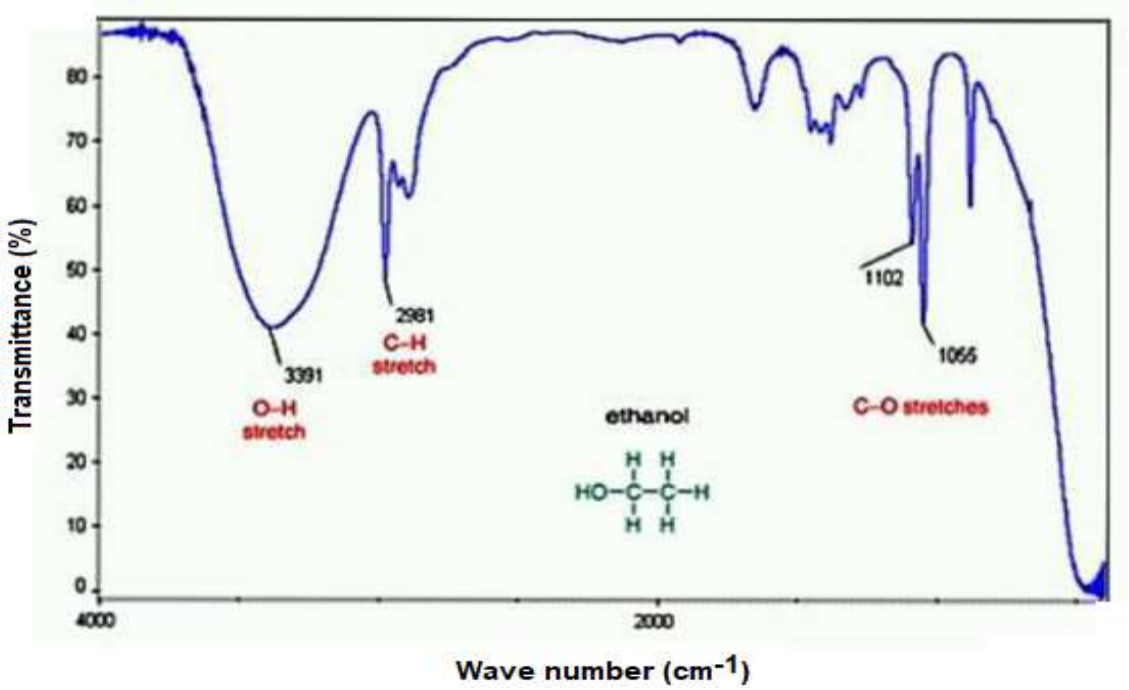

Figure 4-31: FTIR of standard ethanol. Source:(Bodîrlău et al., 2007)

4.8. Analysis of Bioethanol by High-Performance Liquid Chromatography (HPLC)

HPLC analysis can easily provide information the critical components. When run as a liquid film the region at around 21-23 min retention time the standard ethanol was observed as shown in figure 4.32 & Table 4.12. HPLC result showed that the product (Bioethanol) had an R_T value of 21.871 min similar to the R_T value of 21.86 min for the standard, This assures that the product obtained from watermelon peel is exactly ethanol due to the confirmation of these regions as show in figure 4.33 & table 4.13.

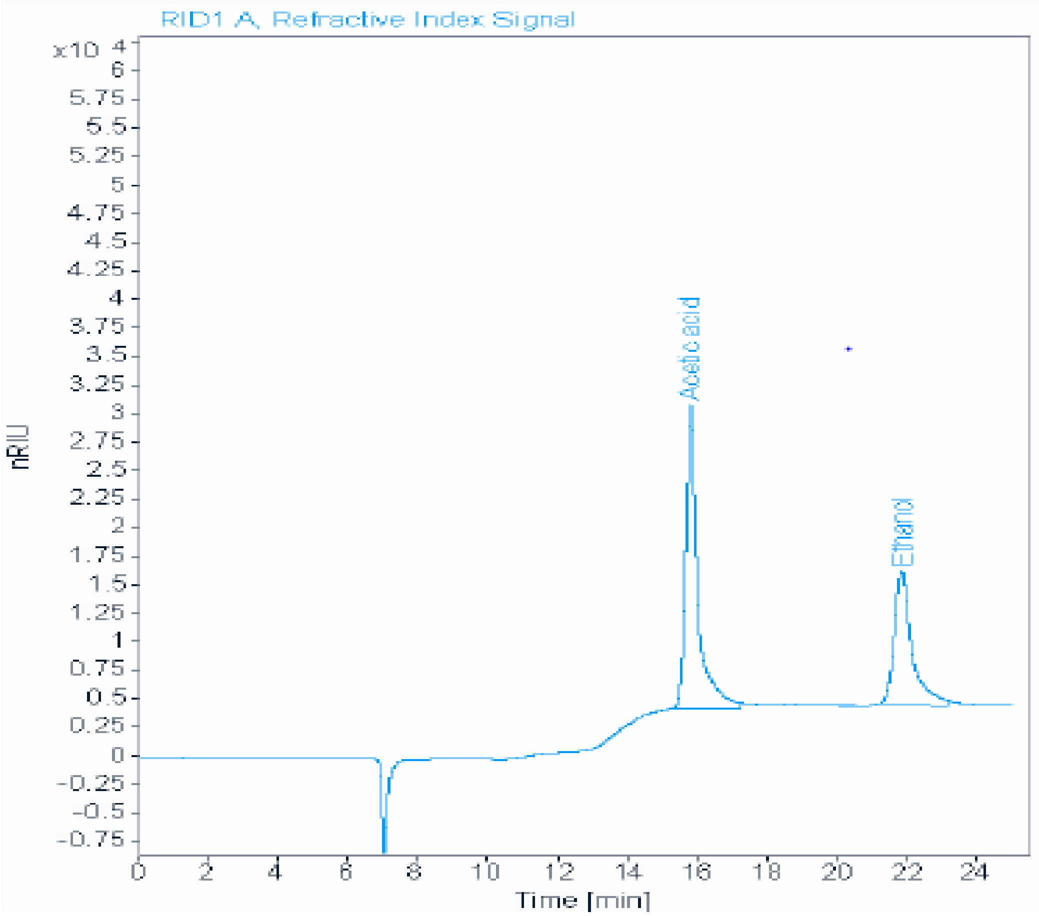

Figure 4-32: HPLC graph for the standard ethanol

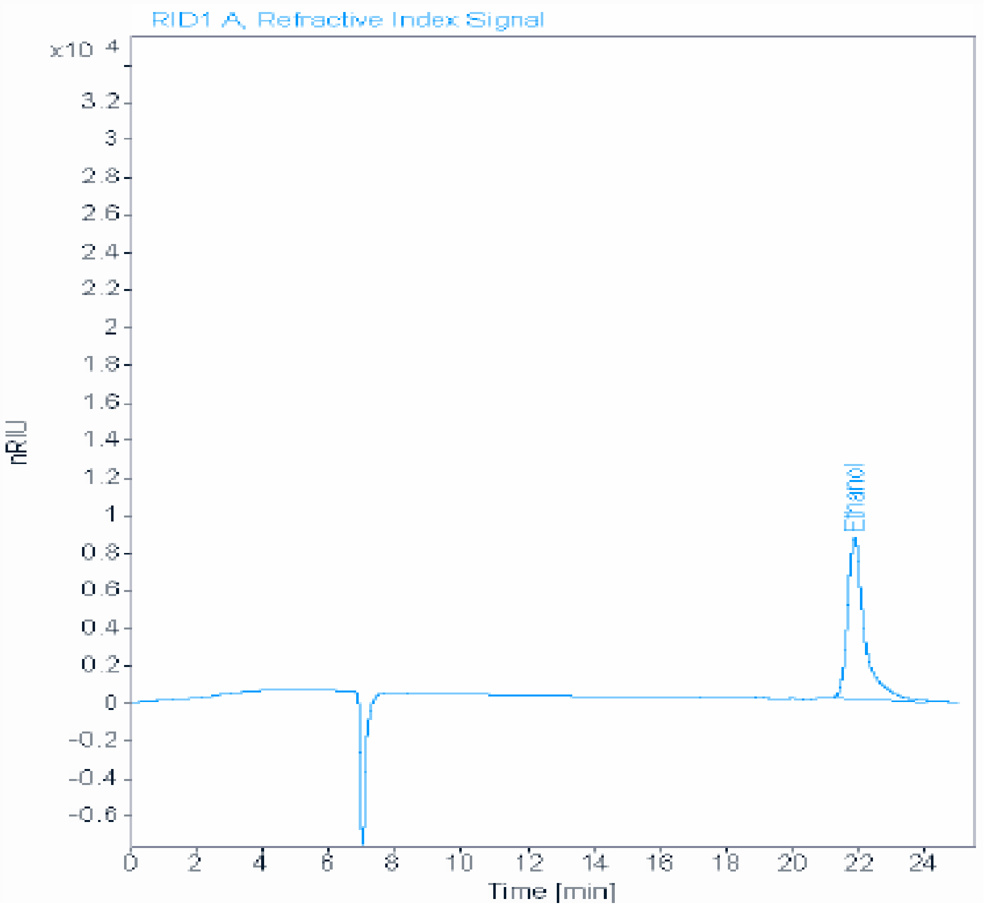

Figure 4-33 HPLC of the produced bioethanol from watermelon peel

Table 4-12: HPLC readings for the Standard ethanol

Line#	Location	Inj#	RT[min]	Unit	Area	Height	Sample Name
1	1	1	21.86	%	101146.29	3032.49	0.2%EtOH and AA2
2	2	1	21.86	%	211543.94	6008.35	0.4%EtOH and AA3
4	4	1	21.85	%	410047.00	11695.36	0.8%EtOH and AA5
5	5	1	21.86	%	509629.72	14277.89	1.0%EtOH and AA6

Table 4-13: HPLC reading for the bioethanol produced from Watermelon peel

Location	Inj#	RT [min]	Amount	Unit	Area	Height	Sample Name
6	1	21.871	6.326	%	310791.8438	8640.41992	J-0581-19

5. Conclusions and Recommendations

5.1. Conclusions

The main difficulty in ethanol production from conventional sources is that raw material availability is limited; on the other hand 75% food-material inflation (worldwide) is attributed to using conventional feedstock for ethanol production. As biofuels are very essential for the environment and the economy when they are produced from lignocellulosic biomass, selection of the cheap and appropriate raw material is big task. This study examines the possibility of Watermelon peel for ethanol production. The conversion of watermelon peel to ethanol was carried out with dilute acid pretreatment, dilute acid hydrolysis, fermentation and distillation process steps.

The experimental design was conducted by Box Behnken Design (BBD) to study the effects of three variables, mass of reducing sugar (2.551g, 3.721g and 4.891g),inoculum level (10, 15, and 20 mL), and pH (4, 5, and 6). The optimum operating condition was found to be at a mass of reducing sugar of 3.721g, inoculum level 15 mL, and a pH of 5. At these optimum operating conditions, the maximum yield of ethanol was found to be 0.497 mL/g. Quadratic model was employed to correlate the operating variables with the response. From the analysis of variance, mass of reducing sugar and inoculum level were found to have the most significant effect on productivity of bioethanol by using F-test ($p<0.05$).

The maximum, observed, value of ethanol productivity recorded was 0.497 mL/g and this is in a good agreement with the predicted value of 0.482 mL/g . Based on this study, it is evident that the chosen method of optimization was efficient, and reliable. From this, it can conclude that the selected model was adequate to fit the data of response variable. Chemical characterization of the bio-ethanol produced was performed using FTIR and HPLC. From result, it was observed that the ethanol produced from watermelon peel contains OH, CO, CH_2, and CH_3 functional groups, when compound with standard ethanol confirming the presence of ethanol in the product. the HPLC result showed that the product(Bioethanol) had an R_T value of 21.871 min similar to the R_T value of 21.86 min for the standard, confirming the presence of ethanol.

5.2. Recommendations

Based on the current investigation the following recommendations are forwarded:

- Researches needs to be carried out to increase the yield of bioethanol from the feedstock (watermelon peel) by employing other microorganisms, are capable of converting reducing sugar into bioethanol.
- In addition, to the hydrolysis and the pretreatment process, and the distillation step variables needs to be optimized, to obtain maximum yield of bioethanol from the watermelon peel.
- An economic feasibility analysis of the overall conversion process is necessary for the purpose of commercialization.

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Appendices

Appendix A: Properties of Ethanol

Physical properties	Description
Molecular formula	CH_3CH_2OH
Molar mass	40.06844(232) g/mol
Appearance	Colorless clear liquid
Density	0.789 g/cm^3
Melting point	- 114.3 ^oC
Boiling point	78.4^oC
Solubility in water	Fully miscible
Ack2idity (pKa)	15.9
Viscosity	1.200 mpa.s(cp) at 20^oC
Dipole moment	5.64 fc.fm (1.69 D) (gas)
EU classification	Flammable (F)
Flash point	286.15K(13^oC)

Appendix B: Density versus Percent Alcohol of Aqueous Ethanol Solutions at 20°C

%Ethanol(V/V)	Dencity
3.67	0.9531
4.734	0.9299
5.011	0.9690
2.422	0.9017
3.857	0.9503
3.859	0.9593
3.231	0.9620
3.014	0.9352
4.734	0.9424

4.338	0.9227
5.369	0.9487
3.956	0.9437
4.3	0.9299
3.178	0.9599
3.18	0.9599
3.665	0.9532
6.32	0.9503

Appendix C: Fit summary

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Mean vs Total	2.70	1	2.70			
Linear vs Mean	0.0061	3	0.0020	0.5364	0.6655	
2FI vs Linear	0.0009	3	0.0003	0.0584	0.9804	
Quadratic vs 2FI	**0.0480**	**3**	**0.0160**	**169.96**	**< 0.0001**	**Suggested**
Cubic vs Quadratic	0.0002	3	0.0001	0.5071	0.6982	Aliased
Residual	0.0005	4	0.0001			
Total	2.76	17	0.1622			

Appendix D: Laboratory work pictures

(a) (b) (c)

Fig.D-1: a)watermelon peel sample b) ground watermelon peel and c) Grinding Machine

(d) (e) (f)

Fig.D-2: d) sieve analysis, e) Sample ready for pretreatment and f) autoclave reactor

(g) (h) (i)

Fig.D-3: g) Hydrolysate product, h) Vacuum filtration, i) Filtered sample

(j) (k)

Fig.D-4: j) Sterilization machine and k) Media after sterilization,

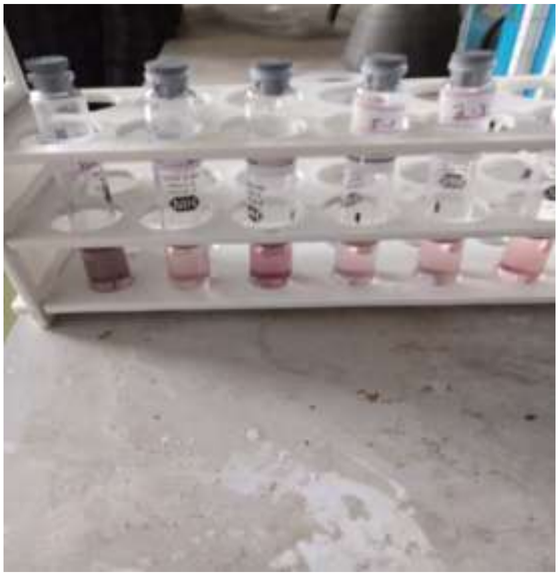
(l) (m)

Fig.D-5: l) pH adjacent and m) Filtered sample after neutralization

(n) (o)

Fig .D-6: n) Shaker incubator, and o) Distillation setup

(p)



(q)

Fig.D-7: p) Stock standard of glucose and q) Glucose standard after the addition of phenol Sulphuric acid.

(r)

(s)

Fig.D-8: r) Hydrolysate Sample, and r) spectrophotometer,

(t) (U)

Fig D-9: t)water bath and u) pycnometer