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SCHOOL OF CHEMICAL ENGINEERING

PROCESS ENGINEERING STREAM

Hydrolysis Process Optimization and Characterization of Bioethanol from Food Waste

(Case Study on JIT Students' Cafeteria Food Waste)

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

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This is to certify that the thesis is prepared by **Temesgen Abeto,** entitled: **"Hydrolysis Process Optimization and Characterization of Bioethanol from Food Waste** (Case Study on JIT Students' Cafeteria Food Waste)**"** and submitted in partial fulfillment of the requirement for the degree of Masters of Science (Chemical Engineering) complies with the regulation of the university and meets the accepted standards with respect to originally and quality.

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DECLARATION

I, Temesgen Abeto, hereby declare that the work on which this thesis is based on and entitled: **Hydrolysis Process Optimization and Characterization of Bioethanol from Food Waste** (Case Study on JIT Students' Cafeteria Food Waste) is my original work not submitted for the degree in this or any other university, and all resources of material used for this thesis had been duly acknowledged. The work was under the guidance of Dr. Kumsa Delessa, Assistant professor in Addis Ababa Institute of technology (AAIT), Addis Ababa University and Mr. Samuel Gesesse Lecture in school of chemical Engineering in (JIT), Jimma University.

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ABSTRACT

Food waste from Jimma institute of Technology students' cafeteria was used as a raw material for bioethanol production. The major components of Food waste from JIT students' cafeteria collected on Tuesday were found to be injera, bread, rice, and potato. Obviously, these components are carbohydrate-rich biomass which can be converted to ethanol. The conversion of this biomass into ethanol involved upstream processing which includes hydrolysis and the downstream process which includes fermentation and distillation stages. This study mainly focused on investigating optimal conditions for the hydrolysis stage. The effects of three important parameters, (namely temperature, acid concentration and hydrolyzing time) on the yield of bioethanol during hydrolysis of the food waste were investigated. The optimum condition for the hydrolysis process was determined based on the maximum bioethanol yield obtained during processing at various interactions of operating parameters. From the results obtained the yield of ethanol has a positive relationship with temperature and hydrolyzing time. The yield of ethanol had a negative relationship with acid concentration. The relationship between ethanol yield and three parameters (namely temperature, acid concentration and hydrolyzing time) was obtained from experimental results which were interrelated with a model equation. The maximum ethanol produced was 30.6mL from 60g of food waste sample this was obtained at a temperature of 120^{o}C, hydrolyzing time of 1hour and acid concentration of 1%. The minimum ethanol was produced at a temperature of 120^{o}C, acid concentration of 5.00% and hydrolyzing time of 60 minutes was 16.41 mL from 60g of food waste sample. The ethanol produced after distillation had a density of 0.802g/cm3, viscosity of 1.2 cP, having an alcohol content of 70%, the pH value of 6.67, Flammability 15 ^{o}C and boiling point 79^{o}C. Ethanol produced in this experiment was used as solvents for different chemicals, to wash laboratory equipment's to prevent contamination and it's used for blended with fuel if it further purified.

Keywords; Bioethanol, Food Waste, Optimization, Hydrolysis, Fermentation

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List of Symbols and Abbreviations

A^2	doubling the effects of temperature
AB	interaction effects of temperature and acid concen.
AC	interaction effects of temperature and time
AD	Anaerobic Digestion
ANOVA	Analysis of Variance
A	Temperature
B^2	doubling the effects of acid concentration
B	Acid concentration
BC	interaction effects of acid concentration and time
C^2	doubling the effects of time
C	Time
FAO	Food and Agricultural Organization (United Nation)
FS	fermentable sugar
FTIR	Fourier-transform infrared spectroscopy
GHG	Green House Gas
JIT	Jimma Institute of Technology
RSC	reducing sugar concentration
SC	saccharomyces cerevisiae
KBr	Potassium Bromide

1. INTRODUCTION

1.1. Background

Fossil fuels have been utilized for centuries as primary energy sources. However, their improper utilization subjected the world to various problems such as global warming. Nowadays, issues related to climate change are forcing the world to focus on other sources of energy that are environmentally friendly and sustainable. In the 20th century, the world economy has been dominated by technologies that depend on fossil energy, such as petroleum, coal, or natural gas to produce fuels, chemicals, materials, and power. The continued use of fossil fuels for meeting the energy demand of generations is threatening the world by increasing the concentration of CO_2 in the atmosphere which is believed as the main cause of global warming. The combustion of fossil fuel is responsible for 73 percent of the CO_2 emission (Asif *et al.*, 2007).

One way to reduce problems associated with global warming is the use of bioethanol as a substituent for petroleum-based oil (Asif *et al.*, 2007). Bioethanol is one of the promising future energy alternatives contributing to the reduction of negative environmental impacts generated by the use of fossil fuels(Caspeta *et al.*, 2013). It can be produced from a variety of raw materials containing cellulose or starch. However, generating ethanol from energy-rich raw materials like corn and sugar cane can be processed. Since this feedstock has a high amount of fermentable sugars. But using these raw materials can lead to jeopardizing food security in any country and is a debated issue at the moment(Freris and Infield 2008). Besides, the utilization of virgin resources enhances the total cost of ethanol production to a large extent. Therefore, biomass wastes such as corn fiber, waste wood, waste cardboard, paper sludge, molasses, bread residues and bagasse can be used as an alternative source for producing bioethanol (Le Man, Behera et al. 2010). Waste biomass source is very attractive since it is the cheap raw material for ethanol production (Balat, 2009).

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. An Efficient method for conversion of biomass into fuel is ethanol an economical as well as environmentally friendly fuel. Ethanol has the advantages of

being renewable, cleaner-burning and produces less amount of greenhouse gas (Balat, 2009).

Food waste is a kind of organic solid waste discharged from households, restaurants, and food processing factories. Bioethanol can be produced by fermentation from several renewable sources, such as from food waste, sugarcane, and corn. The ethanol obtained from biomass-based waste materials or renewable sources is called bioethanol. It can be used as a fuel, chemical feedstock, and solvent in various industries. It has certain advantages as petroleum substitutes and can be produced from a number of renewable resources. Ethanol is biodegradable and thus, keeps a check on pollution and it is far less toxic than fossil fuels(Demirbas, 2008). Bioethanol, which is one of the energy sources, is known to be a potential alternative to petroleum-derived fuels and has the potential to meet the increasing demand for energy for industrial processes, heating and transportation (Cherubini, 2010).

1.2 Statement of the Problem

Fulfilling energy demand of generations in accordance with environmental policy and in a sustainable way is a very critical issue that requires the attention of everybody who has a concern for the future of our world. Nowadays, it is common to hear weather-related disasters happening here and there in the entire world which many scientists correlate them to global warming. Global warming in turn linked to excess fossil fuel utilization and thus, a solution to avoid this problem is changing the sources of energy from non-renewable to renewable ones.

Food waste is abundant in all areas and is disposed of as waste. This food waste as landfills generates methanol gases and this case ignition during the very hot condition. This could cause a series of environmental problems by causing a fire that destructs the environment(Singh *et al.,* 2011). Converting this food waste into valuable products like ethanol is better than disposing of it as waste. Disposing food waste results in serious public and environmental health issues such as air, soil and groundwater contamination, disease-causing vectors, GHG emissions, water-born pollutants, waste leachate and odors when food waste is disposal landfill(Lin *et al.,* 2013).

In this study, food waste from the JIT student cafeteria has been used as a raw material to produce bioethanol. The amounts of waste food generated from the JIT student cafeteria is 360kg per day, 2520 kg per week and 919.8 tons per year. This figure shows as there is a large quantity of food waste is generated from JIT. Thus, it is important to convert this food waste into valuable fuel which is ethanol. pretreatment of this food waste is difficult in manual treatments, proximate analysis for food waste is very complex to analysis this was done by different technique, and acid hydrolysis is not good for the environment as well as for human being in order to alleviate such problem neutralization process was carried out.

1.3 Objective of the study

1.3.1 General objective

The general objective of this study is to optimize bioethanol production from food waste in the hydrolysis stage and to characterize the product obtained after the distillation step.

1.3.2 Specific objective

- To determine the proximate composition of the food waste sample from JIT student cafeterias such as moisture content, volatility content, ash content, fiber content, fat content, and protein content.
- To determine optimal values of temperature, acid concentration and time at the hydrolysis stage
- To characterize bioethanol by measuring density, viscosity, flammability, boiling point, pH and functional group analysis by using FTIR, etc.

1.4 Significance of the study

There are many types of wastes generated in different areas and this study was focused on food waste generated from the JIT student cafeteria. The way of disposing of food waste is not good for the environment and human beings. Food waste is disposed of as a landfill was changed to valuable products which were ethanol. Indirectly this conversion of food waste to ethanol might bring better environmental management. Converting this food waste to bioethanol is alleviating environmental pollution. The use of food waste as an alternative source for the production of ethanol can save food security. The ethanol produced can serve as a solvent for a different chemical solution. If the produced ethanol is further purified it can be blend with gasoline and used for transportation purposes. In addition to this using ethanol as fuel can reduce environmental problems. Ethanol has been found to provide significant environmental benefits when used in fuel blends to reduce smog emissions in vehicles. The significance of the study goes far for future researches as a base material to carry out further study. Furthermore, the study will serve as a reference for academicians, policymakers, and managers.

1.5 Scope of the Study

This study was carried out on JIT student cafeteria food waste. The samples were collected on Tuesday mooring at breakfast time and at lunchtime. This is also one factor that affects the yield of ethanol. The samples were different for seven days. If the sample were collected for seven days is different from one day, collecting the sample for seven days was difficult and beyond this study. For that reason, the sample was taken only for one day. During ethanol production, there were three optimization stages. Due to time limitation, from these three optimization stages, only the hydrolysis stage was optimized and three factors were considered. Many parameters are affecting the ethanol production process. Those were temperature, acid concentration and hydrolyzing time. In the fermentation stage, if temperature, pH and time of incubation were not constant it can affect the yield of ethanol. However, temperature pH and time of incubation were considered as constant for this study.

2. LITERATURE REVIEW

2.1 Biomass and food waste

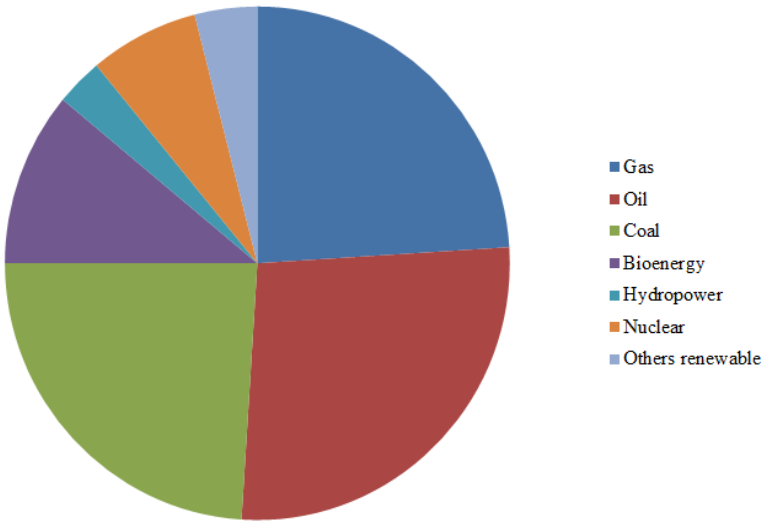
Biomass is plant matter such as trees, grasses, agricultural crops or other biological material. It can be used as a solid fuel or converted into liquid or gaseous forms for the production of electric power, heat, chemicals, or fuels. Food waste is a type of biomass and it uses as an alternative raw material to produce biofuels. To produce biofuels it must require different preconditions, and it has different economic, environmental and technological challenges (Saxena *et al.*, 2009). Food waste is a potential and cost-effective feedstock to produce fermentation products, but several challenges need to be addressed appropriately to achieve high product quality and yield. The composition of food waste depends upon the local eating habits, area, and eating periods this indicates that different foods have different compositions. Thus, the chemical composition needs to be determined before utilizing it as a resource to produce fermented products(Pham *et al.*, 2015). In comparison to other feedstock like corn, plant oils, and lignocellulose materials, food waste is more complex as it contains carbohydrates, amino acids, lipids, vitamins, phosphates, and nutrients. Food waste to various fermentation products is an emerging area of research, and therefore an in-depth understanding of all the aspects of food waste could help to overcome these challenges(Karmee *et al.*, 2016).

There are different challenges that have been encountered during the production of bioethanol from food wastes. From those challenges, one is technical challenges for each technology while converting food waste to fermentation products. The primary technical challenge is difficult to control process conditions that result in the production of harmful intermediate compounds causing low product yields and reducing the system stability. The high lipids and protein contents in food waste lead to the production of hydrogen sulfide, NH_3, and long-chain fatty acids during fermentation(Wyman *et al.*, 1999). The other technical challenges are scaling up of the technology, purification of end products and estimation of biomass that encourage the researchers to brainstorm to find the possible solutions(Waqas *et al.*, 2018). However, the recent advent of biochemical engineering resulted in the designing of a number of bioreactors with large-scale waste treatment capacity along with online monitoring of different process parameters including heat and mass transfer. Even though, product recovery and purification are still much

expensive. There are different methods to utilize biomass as energy sources such as thermal conversion, thermochemical conversion, bio-chemical and physiochemical (Singhania *et al.*, 2009).

2.1.1 World biofuel production and consumption

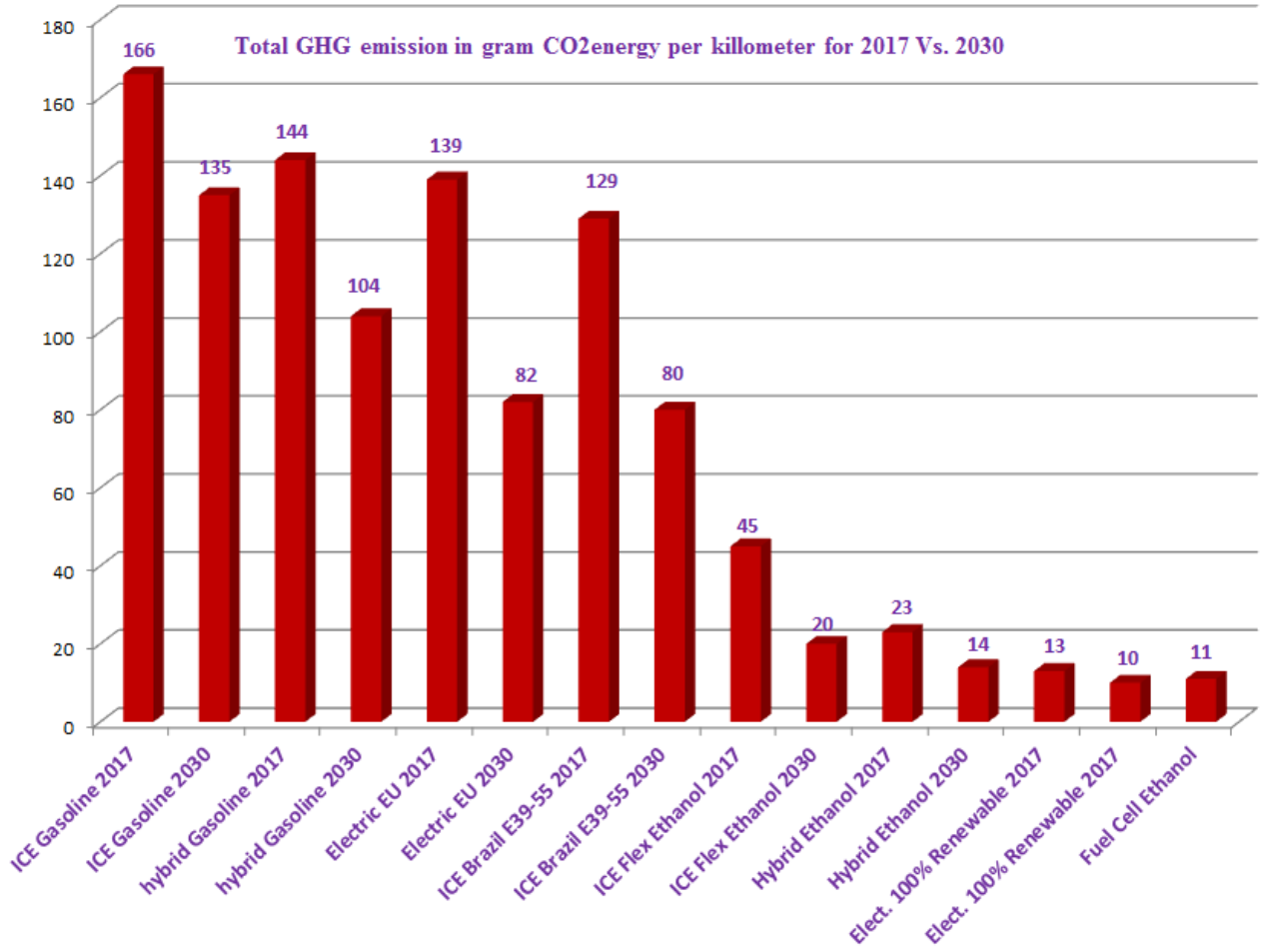
Bioethanol has long established itself as the world's number one biofuel and its market has continued to grow rapidly in recent years. The increasing use of bioethanol as a replacement for fossil fuels has already been pushed for years in Brazil and the USA(Hall *et al.*, 2009). Demand for biofuels was sustained by bioenergy obligatory blending and by the surge in demand for transportation fuels due to continued weak energy prices. Unfavorable price ratios of biofuels to conventional fuels resulted in limited demand for non-mandated use of biofuels, with the notable exception of Brazil where recent policy reforms in several states favor hydrous ethanol which can be used directly by their flex-fuel vehicle fleet. Despite low crude oil prices, policy decisions were favorable to biofuels in 2016 with developments such as mandate increases and differential taxation systems or subsidies enacted in several countries(Naylor *et al.*, 2017). The proportion of total transportation energy accounted for biofuels, including double counting for waste and residue-based biofuels, is expected to reach 6.4 percent by 2020 and to remain stable thereafter.



Source: World Energy Outlook (IEA)

Figure 2.1 **Type of world energy consumption**

From figure 2.1the world energy consumption is renewable and nonrenewable energy sources. The first most countries consume oil. The second most consumed energy type is coal and gases. The third most consumed type of energy is bioenergy such as biofuels and bi ethanol. Total world oil consumption is 27 percent, the energy consumption of coal and gas is 24 percent, the consumption of biofuel is 11 percent and the other is14 percent only.



Source: AEA, Brazilian Association of Automotive Engineering

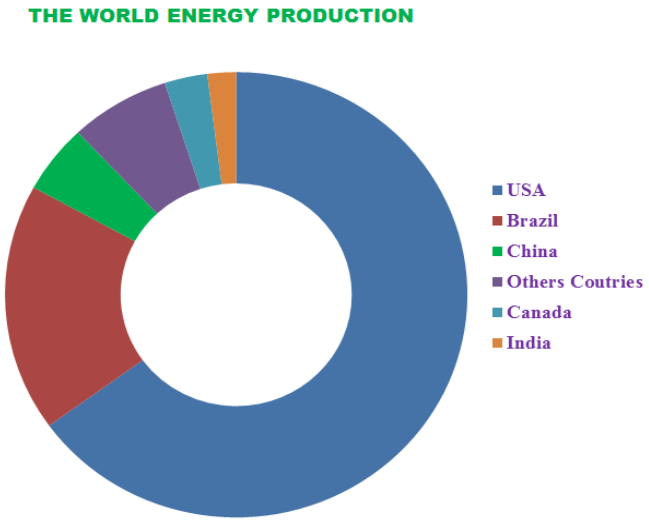
Figure 2.2 **Energy consumption predictions in 2030**

From above figure 2.2the energy consumed was release carbon dioxide when it burns. Carbon dioxide is the main source of gasses that cause global warming. All energy has its own index to produce carbon dioxide. Gasoline produces a high amount of carbon dioxide per liter compared to other types of energy sources. When pure gasoline and

hybrid gasoline are compared to pure gasoline produce a high amount of carbon dioxide than hybrid gasoline. The hybrid ethanol produces less amount of carbon dioxide. When we compare hybrid ethanol in 2017 and 2030 the amount of carbon dioxide emitted to the atmosphere is less in 2030 than in 2017. If pure ethanol is used for energy source it releases less amount of carbon dioxide than the hybrid.

2.1.2 World ethanol production trend and use

The output volume of the world ethanol industry exceeded 72.5 million tons. In 2013, North America accounted for the largest chunk of the world ethanol output. The region's production volume stood at over 43.3 million tons in the year 2013. In the same year, the top five ethanol or biofuel producing countries produce around ninety percent of world total ethanol production and consumption. Those countries produce 90 percent of ethanol produce namely the USA, Brazil, China, India, and Canada (Hebert, 2014). Over 2007 to 2010, the world ethanol market has exhibited considerable growth primarily due to its' expanding application area, namely renewable fuel. Furthermore, in 2011 and 2012 the growth in the global ethanol market slowed down given the unfavorable market conditions, especially in the US and Brazil. As of 2012, the world ethanol supply volume reached 69.5 million tons, registered. The amount of ethanol produced is less from the previous due to poor availability of the market and the raw material availability etc. In 2013, the ethanol market returned to the growth trend(Surriya *et al.*, 2015).



Source: From Wikipedia encyclopedia

Figure 2.3 Global production of ethanol in 2013

2.1.3 Ethiopia bioethanol production and consumption

Bioethanol production and consumption are increase day today. Ethanol production in Ethiopia is linked with sugar factories. The total identified irrigable land for sugar cane plantation in the country is about 700, 000 hectares, estimated at a potential to produce one billion liters of ethanol(WoldeGeorgis *et al.*, 2009). At present, the main supply line in the domestic market is dominated by two sugar factories (Fincha and Metehara) with the combination of their annual production capacity at around 11million liters. In order to transform this potential into reality, the government developed a strategic plan in 2007 considering jatropha as a principal feedstock for biodiesel production and sugarcane as a principal feedstock for bioethanol production. Among other things the strategy focused on establishing a biofuel program, encouraging feedstock development, motivating customer demand, improving environmental sustainability, awareness conception and promotion of biofuels, and renewing energy policy to incorporate bio-energy in detail. As a continuation of this endeavor, there have been repeated efforts to initiate using bioethanol for domestic use and particularly, blending 5 percent of ethanol with gasoline in the year of 2008 followed by 10 percent in the year of 2011 and to increase the percentage in the years to come was the plan set out(Yacob *et al.*,2013).

Table 2.1 Ethanol productions in Ethiopia

Year		Ethanol produced (liters)		
EC	GC	Fincha sugar factory	Metehara sugar factory	Total
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, 2013)*

Ethanol production in Ethiopia increase from year to year. Table 2.1 shows us the ethanol production increase from 2009 to 2015 this indicates demand for ethanol was increased. In 2009 and 2010 Metehara sugar factory did not produce ethanol. Metehara sugar factory starts producing ethanol in 2011 due to an increase in demand. Due to that reason, the yield of ethanol increased radically from 7 million litters to 13.5 million liters.

2.2 Conversion of biomass to biofuel

There are different methods of conversion to utilize biomass as energy sources such as thermal conversion, thermochemical conversion, bio-chemical and physiochemical.

2.2.1 Thermal-Conversion

In this process, there are three different ways of converting biomass to energy these are combustion, pyrolysis, and gasification. Combustion is the thermal conversion of organic matter with an oxidant by using oxygen to produce primarily carbon dioxide and water. The oxidant is in stoichiometric excess, i.e., complete oxidation. Pyrolysis is the thermal conversion (destruction) of organics in the absence of oxygen. In the biomass community, this commonly refers to lower temperature thermal processes producing liquids as the primary product and possibility of chemical and food byproducts. Gasification is the thermal conversion of organic materials at elevated temperature and reducing conditions to produce primarily permanent gases, with char, water, and condensable as minor products (Demirbas, 2004).

2.2.1.1Gasification

Gasification typically refers to conversion via partial oxidation using substoichiometric (insufficient) air or oxygen or by indirect heating to produce fuel gases (synthesis gas, producer gas). The product, or synthesis gas, is principally CO, H_2, methane, and lighter hydrocarbons, but depending on the process used, the product gas can contain significant

amounts of CO_2 and N_2, the latter mostly from the air. Gasification processes also produce liquids (tars, oils, and other condensates) and solids (char, ash) from solids feedstocks. The combustion of gasification-derived fuel gases generates the same categories of products as direct combustion of solids, but pollution control and conversion efficiencies may be improved. Electricity and heat can be produced by burning the synthesis gas in a steam boiler and turbine plant, a gas turbine or an internal combustion engine generator, or synthesis gases can be reacted to fuel products and other chemicals (Lattner and Jenkins 2009)

2.2.1.2 Pyrolysis

Pyrolysis means the thermal degradation of material usually without the addition of any air or oxygen. The process is similar to gasification but generally optimized for the production of fuel liquids or pyrolysis oils (sometimes called bio-oils if biomass feedstock is used). Pyrolysis also produces gases and a solid char product(Bridgwater *et al.*, 2000)

2.3 Anaerobic and Aerobic Digestion

2.3.1 Anaerobic Digestion

Anaerobic digestion is a process that microbial degrades organic matter in the absence of oxygen. Biodegradable organic matters, both soluble and particulate, are converted to carbon dioxide, methane and water. The anaerobic process also reduces and inactivates pathogens or harmful microorganisms(Ward *et al.*, 2008). The anaerobic process is a popular solid stabilization process, used in municipal wastewater treatment. A wide range of microorganisms, primarily prokaryotic, mainly bacteria and methanogens are involved in anaerobic digestion. The characteristic of the microbial community depends on the substrate with which the digester is fed. The conversions of complex organic materials into the simple matter are carried out by four types of microorganism: hydrolytic bacteria, fermentative acid genic bacteria, aceto-genic bacteria and methanogens (Shin *et al.*, 2010).

2.3.2 Aerobic digestion

Aerobic digestion involves the oxidation of biodegradable and microbial cellular matter by aerobic microorganisms resulting in the overall reduction in the mass of sludge and

generation of the finite amount of stabilized cell mass. The factors affecting the performance of aerobic digestion are solids retention time, temperature, pH, mixing, solids type and biosolids configuration (Merrylin *et al.*, 2014).

2.4 Saccharification

Cellulose saccharification is the process of turning polymeric lingo-cellulosic materials into fermentable sugars and can be accomplished by a number of processes including acid and enzymatic hydrolysis. Acid hydrolysis and enzymatic hydrolysis are currently the main two processes used to create fermentable sugars from cellulosic biomass. Acid hydrolysis processing breaks down the complex carbohydrates into simple sugars. Enzymatic hydrolysis processing uses a complex pretreatment processing stage to reduce the size of the material, making it more efficient than acid hydrolysis. In both processes, enzymes are used to convert the cellulosic biomass into fermentable sugars and then microbial fermentation (as in current corn-based systems) which is used to produce ethanol. As with current corn-based systems, carbon dioxide is produced as a co-product in this final stage of production(Alvira *et al.*, 2010).

2.5 FTIR Analysis

FTIR (Fourier-transform infrared spectroscopy) is used to determine the functional group of solid, liquid and gas based on the technique of absorption or emission of a sample. The liquid and a gaseous sample are simply put into FTIR. The Solid sample is tested by using reference that is a pellet.

2.5.1 Solid Sample Analysis

The test carried out on a solid sample which is called pellet formed by using a mold. The pellet is prepared from potassium bromide powder by using a compressor which used as a reference. The first step FTIR instrument is calibrated by using a potassium bromide (KBr) pellet. Then the sample of the pellet is inserted into the FTIR machine and allowed to scan and analyze the sample. Potassium bromide is a transparent and white powder so, light can easily pass through the sample. The FTIR analysis for solid starch bond stretching is determined by using different references.

The FTIR analysis observes the functional groups at different bond stretching vibration with the corresponding wavenumber. Thus, for O-H (hydrogen and oxygen bond) the stretching vibration is analyzed at wavenumber of 3300cm^{-1}, for CH2(one carbon and two hydrogen bond) the stretching vibration is analyzed at a wavenumber of 2947 cm^{-1}, for carbonyl (C=O) or double bond of oxygen and carbon the stretching vibration is analyzed at a wavenumber of 1740 cm^{-1}, for angular O-H bending of water molecules the stretching vibration is analyzed at a wavenumber of 1690 cm^{-1}, for anti-symmetric of the C-O-C bond (two carbon and one oxygen single bond) the stretching is analyzed at a wavenumber of 1152 cm^{-1}, for the C-O (carbon and oxygen single bond) the stretching vibration is analyzed at the wavenumber of the 1080 cm^{-1} and for Anhydroglucose ring O-C (oxygen and carbon single bond) the stretching vibration is analyzed at a wavenumber of 1010 cm^{-1} (Surovell *et al.*, 2001).

2.5.2 Liquid sample

The liquid sample is scanned and the functional group analyzed. For example to determine the functional group of ethanol first determine the bond stretch found in ethanol compounds. The O-H(oxygen and hydrogen bond) stretching vibration in ethanol ranges from 3500 cm^{-1} to 3200 cm^{-1} due to hydrogen-bonded. The C-H (carbon and hydrogen bond) bond stretching is analyzed at a wavenumber of 3100 cm^{-1} to 3000 cm^{-1}. The C-O(carbon and oxygen bond) stretching is analyzed at a wavenumber of 1260 cm^{-1} to 1050 cm^{-1} and C-C(carbon and carbon single bond) is stretching is analyzed at a wavenumber of 1102 cm^{-1}. Bond stretching for a similar bond in a different compound is different. Bond stretching for alkanes is C-H (carbon and hydrogen bond) stretch from 3000 cm^{-1} to 2850 cm^{-1}, for C-H(carbon and hydrogen bond) bend or scissoring from 1470 cm^{-1} to 1450 cm^{-1}, for C-H(carbon and hydrogen bond) rock methyl from 1370 cm^{-1} to1350 cm^{-1}, for C-H(carbon and hydrogen bond) rock methyl is seen only in long chain of alkanes from 725-720 cm^{-1}, for a ring the carbon bonds stretch from C-C (carbon and carbon single bond) stretch (in-ring) is ranged from 1600-1585 $cm^{-1}$and for C–C stretch (in-ring) is ranged from 1500-1400 cm^{-1}. FTIR analyze the compounds by using wavenumber (cm^{-1}) and transmittances (%). Wavenumber is on X-axis and transmittance is on Y-axis. The transmittance for ethanol analysis ranges from -10 to 150% and

wavenumber ranges from 5000 cm^{-1} to 400 cm^{-1}. Then the peak for each bond stretch is identified by using wavenumber and transmittance, but wavenumber is used for determining each stretch(Shiemke *et al.*, 1986)

2.6 Factors affect the yield of ethanol

The yield of ethanol is affected by different factors those are types of raw material, temperature, acid concentration, hydrolyzing time and size of the sample. These parameters have their own range to get an optimum yield of ethanol.

2.6.1 Type of raw material

The type of raw material is the main factor that affects the yield of ethanol. All raw materials have different starch or cellulose content. Corns, tomato, wheat, and other edible foods have high starch content. These foods contain high starch content from that raw material high yield of ethanol can be obtained. However, due to the food insecurity problem, these raw materials cannot be used as a raw material in a developing country. But America produces seventy percent of ethanol from corn. Due to the problem of food insecurity most country searches for other alternative raw materials to produce ethanol (Dhall *et al.*, 2013).

2.6.2 Temperature

The other factor that affects the yield of ethanol is temperature. The range of temperature in which the experiment was done can affect the yield of ethanol. The yield of ethanol is increased when the temperature increases. This is not true always, the temperature increases continuously the other side products will be formed. The yield of ethanol is increased when the temperature ranges from 80 ^{o}C to 120^{o}C. The yield of ethanol is continuously increased for food waste for the range of temperatures between 80 ^{o}C to 120^{o}C. As the temperature increase, the yield of ethanol may decrease this is due to other factors. During hydrolysis when the temperature is increase the yield of total reducing sugar is because it is converted products like ketoses and aldoses(Torija *et al.*, 2003).

2.6.3 Hydrolyzing Time

In another way, the hydrolyzing time can affect the yield of ethanol. Time can slightly affect the yield of ethanol. The hydrolyzing time range is ranged between 30 to 60 minutes within this range the yield of ethanol increased when the time is increased. This is true when the temperature and acid concentration is at a lower level(Brethauer *et al.*, 2010).

2.6.4 Acid concentration

Acid concentration is another factor that affects the yield of ethanol. There are two types of acid hydrolysis those are dilute acid hydrolysis and strong acid hydrolysis. Weak acid hydrolysis is preferable than strong acid hydrolysis this is due to strong acid hydrolysis is cost and it is difficult to safety purpose. For weak acid hydrolysis, the acid concentration ranged from 1% to 5%. When acid concentration increased the yield of ethanol is increased this is not always true. Sometimes as acid concentrations increase the yield of ethanol decreases. This is due to the effects of other factors (Martin *et al.*, 2007).

2.6.5 Particle Size

Particle size analysis for ethanol production is different for different raw materials. But the size of the sample also affects the yield of ethanol. When the size of a sample is small, the surface area of the sample increases. This enhances the yield of fermentable sugar and the yield of ethanol. In commercial ethanol production, the size of the sample ranged from 1.5mm to 3.5 mm (Binod *et al.*, 2012).

2.7 Hydrolysis

Acid hydrolysis is a crucial step for the ethanol production process to convert large polysaccharide to simple sugar by breaking bonds between each polysaccharide components. There are two types of acid hydrolysis those are strong acid hydrolysis and dilute acid hydrolysis. These two types of acid hydrolysis have their own advantages and disadvantages (Kumar *et al.*, 2009).

2.7.1 Strong acid hydrolysis

Strong acid hydrolysis is the process of hydrolyzing large polysaccharides like cellulose and other compounds to simple sugar. Strong acid hydrolysis is done by using concentrated acid. The acid concentration ranges from 45 to 50%. In strong acid hydrolysis sulfuric acid and hydrochloric acid are used. The advantage of strong acid hydrolysis is hydrolyzing large polysaccharides at lower temperature and pressure. This makes a higher conversion and lower side products. Concentrated acid hydrolysis for hydrochloric acid is performed at room temperature. The disadvantage of strong acid hydrolysis is corrosive, volatile, expensive and almost complete recovery is essential to make the process economical. The strong acid hydrolysis is chosen to get a high yield of simple sugar. Getting a high yield of sugar gives us a high yield of ethanol, by using the fermentation and distillation process. Strong acid hydrolysis has its own advantages and disadvantages in terms of costs, yields, material degradation, downstream processing and generation of process wastes. Strong acid hydrolysis is costly but it saves time and energy(Binder *et al.,* 2010).

2.7.2 Dilute acid hydrolysis

Dilute acid hydrolysis performs at the acid concentration of 1% to 5%. Sulfuric acid is generally considered as the most cost-effective means of the hydrolyzing process. Theoretical yields of hemicelluloses contain 80% to 95% sugars. Yields of glucose from cellulose are generally less than 50% but can approach 55% at elevated temperatures. Dilute acid hydrolysis is cheaper compared to strong acid hydrolysis. Dilute acid hydrolysis has better aspects with safety, not corrosive, less expensive and better in the economy. Its disadvantage is lower conversion and needs a high temperature to convert polysaccharides to simple sugar. It needs more time and temperature than strong acid hydrolysis(Kumar *et al.,* 2009).

$$\underset{\text{Starch}}{(C_6H_{10}O_5)_n} + \underset{Water}{nH_2O} \rightarrow \underset{Glucose}{nC_6H_{12}O_6} \quad \text{.....(2.1)}$$

2.7.3 Weak acid hydrolysis

Weak acid hydrolysis is different from strong acid hydrolysis and dilute acid hydrolysis. The weak acid hydrolysis has the nature of acidity which is weak. The process is known as weak acid hydrolysis which consists of sulfur dioxide combined with steam. It is particularly effective as a pretreatment for enzymatic cellulose saccharification. Sulfur dioxide is often used in combination with auto hydrolysis because it gives better sugar yields and helps to modify lignin for subsequent extraction or recovery(Ramos 2003).

2.8 Enzymatic Hydrolysis

Enzymatic hydrolysis is the process of converting polysaccharides to fermentable sugar by using a hydrolyzing enzyme called cellulases. The Enzymatic hydrolysis has many steps to convert large polysaccharides to fermentable sugar. The first step is transferring an enzyme that cultured in a medium to a bulk aqueous phase on the surface of polysaccharide. The second step is adsorption of an enzyme by polysaccharide compounds and to form an enzyme-substrate complex. By using these adsorbed enzymes the sample is converted to fermented sugar. The next step is transferring the hydrolysis products from the surface of the cellulosic particles to the bulk aqueous phase. After this, the enzyme will be separated from an aqueous solution of starches. The hydrolysis rate of cellulose is affected by crystallinity and depends on the accessible surface area of cellulose. These cellulose fiber parts composed of crystalline and amorphous regions. This affects the enzyme activity of adsorption. The external surface area is affected by the shape of the particle and the size of the particles. The size of a particle can affect the rate of hydrolysis and the adsorption of enzymes. The internal surface area depends on the capillary structure of the cellulose fiber. Enzymatic hydrolysis has its own advantages and disadvantages. Enzymatic hydrolysis helps to get high conversion relative to acid hydrolysis and high yield of ethanol. Its disadvantage is for all types of raw material it needs a different type of hydrolyzing enzyme. If there is a voltage fluctuation during enzyme culturing the enzyme will cease out. It needs highly sophisticated technology to culture those enzymes (Sticklen 2008).

2.9 Fermentation

Increased yield of ethanol production by microbial fermentation depends on the use of ideal microbial strain, appropriate fermentation substrate and suitable process of technology. According to an ideal microorganism used for ethanol production must have rapid fermentative potential, improved flocculating ability, enhanced ethanol tolerance, and good thermo-tolerance. Although no microbial strain has all these desirable qualities, few yeast strains have been found to possess appreciable characteristics for ethanol production (Brooks 2008). Various strains of indigenous yeasts capable of producing ethanol have been isolated from different local sources such as molasses, sugar mill effluents and local fermented pineapple juice. In most of these studies, the preferred candidate for industrial production of ethanol has been Saccharomyces cerevisiae. This yeast also has the ability to produce ethanol which is not contaminated by other products from the substrate (Wong *et al.*, 2012).

$$C_6H_{12}O_6 \rightarrow 2\,C_2H_5OH + 2\,CO_2 \ldots\ldots\ldots\ldots\ldots (2.2)$$

2.10 Ethanol

Cellulosic ethanol processes can be differentiated primarily by the method of hydrolysis employed. Hydrolysis is a pretreatment method that has been investigated in depth during acid hydrolysis processes, enzymatic hydrolysis, and steam explosion. With the possible exception of acid recycling and recovery, acid processes are technologically mature. But enzymatic processes are projected to have a significant cost advantage once improved (Alvira *et al.*, 2010). Steam explosion yields less sugar and releases more material that inhibits fermentation. After hydrolysis, the sugars can undergo microbial fermentation producing ethanol and CO_2. Ethanol inhibits microbial growth, essentially halting the process when ethanol concentration is near 12%. Ethanol must be separated from the fermentation broth and concentrated by conventional distillation technology and dehydrated to yield fuel-grade anhydrous ethanol. Wet-ethanol fuels (ethanol-water mixtures that reduce the need for full distillation and dehydration) are being investigated.

The remaining liquid broth is recycled or sent to a wastewater treatment facility for appropriate management (Ishola 2014).

2.11 Media preparation

The yeast (saccharomyces cerevisiae) is cultured in a medium. The medium is prepared from different compounds. Those compounds are peptone water, dextrose, magnesium sulfate, urea, and distilled water at different proportions. The medium preparation step includes, first in 100ml of distilled water add 10g/l of magnesium sulfate secondly add 20 g/l of peptone water in the solution thirdly add 5 g/l of urea and 20 g/l of dextrose in 200mL of sample solution prepared (Damtew 2008).

2.12 Properties of ethanol

Ethanol is a colorless and liquid at room temperature. Ethanol is produced from different sources from those the first one is biomass having the following properties. Pure ethanol has the molecular formula of CH_3CH_2OH and molecular mass of 46.068 g/mol. pure ethanol has a density of 0.789 g/cm³. The boiling points and melting points of ethanol is 78.4°C and −114.3 °C respectively. Ethanol is fully miscible in water and having a viscosity of 1.1 cP at 20 °C and having a flashpoint of 286.15 K (13 °C or 55.4 °F) (Haverhals, 2008).

2.13 Uses of Bioethanol

The main use of ethanol is as a motor fuel, fuel additive and used as an organic solvent. Ethanol and other alcohols can be used to power motor vehicles instead of gasoline. In almost all cases the ethanol is mixed with gasoline. Ethanol has the advantages of being renewable, cleaner-burning and produces less GHG (Altintas *et al*., 2002). 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 the potential to supply the existing chemicals industry and household uses. However, the most prevalent use of bioethanol in Ethiopia is as a transport fuel in spark-ignition engine vehicles and the current amount of ethanol fuel blended with gasoline which is 10 percent 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).

3. MATERIALS AND METHODS

3.1 Materials and chemicals

3.1.1 Equipment and instruments

During the experiment different equipment was used to convert biomass to bioethanol within the laboratory. Also, different characterizing equipment was used during product characterization. Digital ovens with a model number DHG-9203A and serial number PCD-E3000 used to dry a sample and to measure moisture content. Alcoholmeter with a model number of used to determine ethanol percent in a product. Autoclave with a model number of YX-12LM and with a serial number 8419200000 used to sterilization and hydrolysis of food waste sample during break down of large component to small. Balances with a model number SF-550 used to weight samples. Incubator with a model number of BJPX-1102C used to convert fermentable sugar to ethanol. Distillation was used for the separation of a solution by the equipment of fractional distillation with a model number AD01used for purification of ethanol or separate ethanol from a solution. Densitometer with a model number of DST-3000 used to measure the density of the sample and the product is in a liquid form. Digital pH meter with a model number of PH-920 used to measure the pH of the hydrolyzed solution before and after fermentation. Sieves with a model number of BK-TS200 used to sieve the crushed sample of a different sized sample. Viscometer with a model number of NDJ-5S was used to determine the fluidity of the product. Magnetic stirrer with a model number of HS-17 was used to mix the solution. Aluminum foil was used to put a sample in an oven. The computer was used to document preparation and data analysis. Crushers were used to crush the dried sample to reduce the size of waste foods. The fine cloth was used for the filtration of the sample during hydrolysis and fermentation. The glove was used for safety purposes. Graduated cylinders of different volumes were used to measure the volume of solution and sample. Mortar and pestle were used to reduce the size of the sample. Plastic bags were used to collect and transport samples. Platters were used to put a sample during the experiment. Volumetric flask was used to handle the solution. Small beakers were used during pH testing of the products as well as different solution preparation. Stationary materials like paper, pen were used to draft, record data's during the experiment and data analysis.

3.1.2 Chemicals

Sulfuric acid (H_2SO_4) was used during the hydrolysis process initially with a concentration of 98% and it was diluted. Dry instant yeast (saccharomyces cerevisiae) were cultured and used when in the fermentation process. Ammonia solution with a concentration of 28% was used to neutralize the solution after hydrolysis without hydrolyzing it. Dextrose ($C_6H_{12}O_6$) or glucose was used during yeast cultured. Distilled water (H_2O) that deionized water was used during the hydrolysis process and yeast culture. Hydrochloric acid (HCl) was used during pH adjustment. Magnesium sulfate ($Mg_2SO_4.7H_2O$), peptone water and urea were used to yeast culture.

3.2 Methods

Food waste was easily available in different areas like households, restaurants, and food processing factories (Parizeau *et al.*, 2015). JIT students' cafeteria was the main area for this research to collect a sample. JIT student cafeteria has a variety of food wastes like injera, bread, rice, meat, lentil, legume, etc. These all were in the mixed form and disposed to the food waste bin. The plastic bag was used to take a sample from the waste food bin and transported to a sample preparation section. This transported sample was prepared in the laboratory for further process. To separate the different components of food waste, screening was held on. After screening the size of food waste was reduced in order to increase the surface area of the sample. To reduce the moisture content a sample was placed in an oven at a temperature of $65^{O}C$ for three days. Then mix this sample and grind it. Finally, place it in a dry place. The amounts of waste food generated from the JIT student cafeteria are 360kg per day, 2520 kg per week and 919.8 tons per year.

Figure 3.1 Food waste disposal area around JIT student cafeteria

3.2.1 Sample collection and preparation

3.2.1.1 Sample acquisition

The sample collected from the waste bin was transported to the sample preparation section. The sample was taken on breakfast and lunchtime. The samples collected during these times contained injera, bread, rice, and potato as a major component, and for analysis, they were separated into the individual component by screening.

Figure 3.2 Sample of the food waste

3.2.1.2 Sample preparation for potato and rice

The sample was transported from the waste disposal area to the sample preparation section. Then Sample was sorted based on its variety. Sample separated into four parts potato, rice, injera, and bread. These four components were placed into four different platters. From potato and rice, it was easy to remove impurities. The potato and rice were washed by distilled water until all adhering dirty matters were removed. Next, wash these samples by hot water in order to reduce the salts, fat content, and protein content from food waste. Then sodium hydroxide was used to wash the sample of potato and rise. Treatment of food waste by sodium hydroxide was helped to reduce the fat content and protein content into an insignificant level. The oil and other impurities removed from the potato and rice then the size was reduced and the weight of the sample was measured and kept in an oven at a temperature of 70^{o} C for three days. Like the potato, the rise was also washed with distilled water and then kept in an oven at a temperature of 70^{o} C for three days.

3.2.1.3 Sample preparation for injera and bread

The Sample of injera was separated manually and impurities were removed by hands. Then injera and bread were washed by distilled water until all adhering dirty matters were removed. Next, wash these samples by hot water in order to reduce the salt's contents, fat content, and protein content from food waste. When this sample was washed by hot water the bread and injera have formed a solution and dissolved within hot water. After one day the bread and injera residues were sediment and the hydrophobic oil was suspended on the water then it is removed easily. Sodium hydroxide was used to wash the sample of injera and bread. This Treatment of food waste by sodium hydroxide was helped to reduce the fat content and protein content into an insignificant level. The injera and bread were dried by using sunlight for two days in order to reduce the moisture content. The reduced injera and bread mixture was weighed and placed into an oven at a temperature of 70^{o} C for three days. There was not any specific time set for drying the sample. The dryness of the sample would check by visual judgment on dried samples as they were easily breakable and crushable. The samples were taken out from their respective oven driers. After checking the dryness, the sample was weighed and crushed.

3.2.1.4 Sample proportioning

The sample was proportioned by quantification and mixing of the components in predetermined percentages. The proportions of these components were determined on the real basis of their availability. All samples were available in the JIT student cafeteria throughout the year. All samples were proportioned equally at a percentage of 25.

3.2.1.5 Sample grinding

After drying, the samples were weighed and mixed in the proportions 25% (equally). The mixture was then crushed (ground) by using mortar and pestle in or to get a different size. The maximum particle size of the ground mixed sample was 2 mm. The grounded sample was kept far away from the availability of moisture and allowed to stay at room temperature.

3.2.1.6 Sample screening

The dried and grinded sample was screened based on its size. As the sample had different sizes, it was performed by using different size sieve. The required size of the sample was 2mm; the sample which less than and greater than 2mm was rejected from the analysis.

3.3 Proximate analysis

The proximate analysis was used to determine the composition of a sample. In proximate analysis moisture content, protein content, fat content, fiber content, and ash content were determined. In the proximate analysis, different equipment and chemicals were used that were not listed in materials and methods due to its much in number.

3.3. 1 Moisture content and volatile content

A 100g of food waste was measured and placed into the digital oven at a temperature of 65^{o}C for three days. To determine the moisture content the equation 3.1.1 was used. A crucible was weighed empty, and then 1.5 g sample was put in it. The sample and the crucible were placed in a muffle furnace for 7 min at 950 ^{o}C. The crucible was removed from the furnace and placed in a desiccator to cool, then was reweighed. The percent volatile matter content was determined using the formula of 3.1.2.

$$\%Mosture\ content = \left[\frac{w_1 - w_2}{w_1} \right] \times 100\% \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3.1.1)$$

Where; w_1 is a sample before dry, w_2 is a sample after dry

$$\%\text{Volatile content} = \left[\frac{w_1 - w_2}{w_1} \right] \times 100\% \text{.....(3.1.2)}$$

Where W_1=original weight of the sample and W_2=weight of the sample after cooling

3.3.2 Ash content

A 2.5 g of the material was weighed and transferred to a relatively broad ash-producing dish that has been ignited in an oven for 30 minutes at 100^{o}C and cooled in desiccators for 1 hour to reach ambient temperature. The sample was weighed after it removed from the oven. The sample was then burned in a muffle furnace at 550^{o}C for 1 hour. Then the sample was withdrawn from the furnace and allowed to cool and moisten with a few drops of de-ionized water. The de-ionized water was evaporated on a hot plate. Then after, it was ash for 30 min at 550^{o}C and cooled to ambient temperature. Some drops of de-ionized water and 5 drops of concentrated nitric acid (HNO3) were added and evaporated on a hot plate. Finally, the ashes in the muffle furnace were kept for 30 min at the same temperature and cooled in desiccators for 45 to 60 minutes. The cooled ash sample was weighted for further ash analysis. The percent of ash content was determined from the following equation.

$$\%Ash\ content = \left[\frac{w_3 - w_2}{w_1} \right] \times 100\% \text{.....(3.2)}$$

Where; W_3 is the weight of ashes sample and crucible, W_2 is the weight of crucible, and W_1 is the weight of the pre-ashes sample.

3.3.3 Fiber content

Two g of the sample was considered as W_3. This 2g sample was poured into a 600 ml beaker and 200 ml of 1.25 % H_2SO_4 were added to this beaker. And after this, the solution was boiled for 30 minutes, by placing a watch glass over the mouth of the beaker and keeping the level constant with distilled water. After 30 min, 20 ml of 28% KOH were added to the mixture and boiled gently for 30 minutes while stirring occasionally. The sample was then filtered through vacuum filtration which had been coupled with the

sintered glass crucible. Then the solution from the beaker was poured into the sintered glass crucible. The beaker walls were rinsed with hot distilled water several times and then finally washed with 1% H_2SO_4 and 1% NaOH. The filtrate was then dried using dry crucible (W_1) for 2 hours in an oven at 130^oC and cooled for 30 minutes in a desiccator and then weighed (W_2) and the same crucible was transferred to muffle furnace at 550-600^oC for 30 minutes afterward. Then the sample was withdrawn, cooled in a desiccator and weighed (W_2). The fiber content was estimated using the following equation as shown below:

$$\%Fiber\ content = \left[\frac{w_2 - w_1}{w_3} \right] \times 100\% \text{.....(3.3)}$$

Where; W_1 is crucible weight before drying, W_2 is crucible weight after drying and W_3 is sample dry weight.

3.3.4 Fat content

A clean aluminum cup with boiling chips that have been dried at 92 ^oC for an hour was weighed and then kept in desiccators for 30 minutes to cool. 3.5 g of sample was weighed accurately and cover with fat-free cotton and was attached with a magnetic ring to hang the thimble to the extraction chamber. 70 ml of diethyl ether were added to the aluminum cup and the extraction was allowed to happen for 4 hours. Then the aluminum cup was removed from the extraction unit and placed on a drying oven at 92^oC for 30 minutes. While kept on desiccators to cool for 1 hour. The aluminum cup was weighed immediately after withdrawal from the desiccators. The percent of fat content for the starch sample was calculated according to the following equation.

$$\%Fat\ content = \left[\frac{w_f}{w_s} \right] \times 100\% \text{.....(3.4)}$$

Where; the weight of fat (w_f) = weight of aluminum cup after extraction-weight of the aluminum cup before extraction, w_s is the weight of the sample.

3.3.5 Protein content

A starch sample (0.5 g) was placed in a 500 ml digestion flask. For 6 ml of acid mixture, 2 parts of concentrated sulphuric acid and 1 part of concentrated orthophosphoric acid was added. The flask was placed on a heater and allowed to react and digested at 420℃ for 1 hour. As soon as the violent reaction ceased, the heat was increased and the destruction was continued until the content appeared light green. It was then cooled and diluted with distilled water. After cooling, the material was distilled by steam distillation with 40% of sodium hydroxide and the ammonium (NH_4^+) was released in the form of ammonia (NH_3) from the solution. Finally, the condensed NH_3 was trapped by 1% boric acid and titrated against 0.1M standard HCl and the analyte was referred to as a crude protein. Since the method determines the nitrogen in the components of all protein. The protein content was determined as a mean of three measurements, and reported by multiplying percent nitrogen by 6.25. Based on this the protein content was determined as insignificant.

3.3.6 Determine the starch or carbohydrate

The amount of starch found in the wet sample was determined based on the above values of moisture content, ash content, fiber content, fat content, and protein content. The amount of carbohydrate present was determined by using the following formula below:

$$\%\text{Carbohydrate} = \left[1-\left(\%\text{fat} + \%\text{protein} + \%\text{fiber} + \%\text{moisture} + \%\text{total ash} + \text{volatile content}\right)\right]\times 100 \quad (4.5)$$

3.4 Dilute acid hydrolysis

The pretreatment was mainly meant for sterilization of the biomass before hydrolysis. The autoclave was selected for hydrolysis because autoclave has many advantages those were for sterilization purpose, it was easy to control the temperature as well as pressure by using different valves, also to prevent contamination during operation of hydrolysis because it performs a batch operation, it is better to get a high yield of fermentable sugars during hydrolysis. Before diluting acid hydrolysis the autoclave was used for steam pretreatment. Each 60g sample was soaked in 500ml distilled water. Then the samples were heated at 121 ^{O}C for 15 minutes and the pressure was abruptly released until it declined to 0.5bar. When the temperature reached 100^{O}C, the pressure valve of the

autoclave was closed to avoid delayed boiling in the sample vessel. This accidental pressure release enabled a partial decrystallization of the carbohydrate biomass. After this, different acid concentrations were prepared based on the procedure. There were five different acid concentrations of 0.5%, 1%, 3%, 5% and 6% were prepared. The temperatures have been adjusted at five different values, those were 93^{o}C, 100 ^{o}C, 110 ^{o}C, 120 ^{o}C, and 127^{o}C. The time was adjusted at five different points that were 33 minutes, 40 minutes, 50 minutes, 60 minutes and 67 minutes. The hydrolysis was carried out in autoclave and a total of twenty experiments were done in this stage. In this experiment, there weren't constant factors. The One-factor-at-a-time, in which experimental factors were varied one at a time, with the remaining factors held constant, was formerly regarded as the only correct way to conduct research. The method provides the effect of a single variable at selected fixed conditions of other variables. However, for such estimation, it was necessary to assume that, the effect would be the same at other settings of the other variable over the range of current interest.

Figure 3.3 Hydrolysis processes in an autoclave

3.5 Procedure for hydrolysis step

The first step was to prepare the acid solution to the predetermined strength or concentration with acid concentrations of 0.5%, 1%, 3%, 5% and 6% volume to water. The concentration of sulfuric acid was originally at 98% by volume to water. It was concentrated and diluted to an acid concentration of 0.5%, 1%, 3%, 5% and 6%. The dried, crushed and screened samples were added into dilute acid. The samples were prepared 10% V/W to the dilute acid solution. It was added to the glass vessel. Water was added to the autoclave for steam production and to control temperature during hydrolysis. Then the prepared solution in the volumetric flask was put into the autoclave with the closed vessels. After this, the autoclave was closed carefully and the power was supplied

to autoclave. The autoclave was performed at a specific temperature for a time range and the pressure was adjusted at one bar. In the hydrolysis step, three factors were considered. Those were temperature, acid concentration and hydrolyzing time. When the time was completed the autoclave power was shut down and after a few minutes, when autoclave temperature was decreased. Then the sample was taken from the autoclave when a sample in the solution was being converted to simple sugar. The temperature of the sample was maintained at room temperature. Then the sample was filtered with fine cloth and it was closed in a vessel in order to prevent contamination.

Table 3.1 Design expert analysis for hydrolysis step

Std	Run	Temperature (Factor A)	Acid concentration (Factor B)	Hydrolyzing time (factor C)	Yield of ethanol (Response)
1	6	100.00	1.00	40.00	-
2	1	120.00	1.00	40.00	-
3	2	100.00	5.00	40.00	-
4	5	120.00	5.00	40.00	-
5	17	100.00	1.00	60.00	-
6	16	120.00	1.00	60.00	-
7	12	100.00	5.00	60.00	-
8	7	120.00	5.00	60.00	-
9	4	93.00	3.00	50.00	-
10	20	127.00	3.00	50.00	-
11	15	110.00	0.50	50.00	-
12	14	110.00	6.00	50.00	-
13	19	110.00	3.00	33.00	-
14	11	110.00	3.00	67.00	-
15	8	110.00	3.00	50.00	-
16	9	110.00	3.00	50.00	-
17	10	110.00	3.00	50.00	-
18	13	110.00	3.00	50.00	-
19	3	110.00	3.00	50.00	-
20	18	110.00	3.00	50.00	-

3.6 Media preparation section

Before fermentation, the dry instant yeast (saccharomyces cerevisiae) was grown in a medium cultured. The medium was prepared from different components like peptone water (in powder form), distilled water, urea, dextrose (in liquid form) and magnesium sulfate ($Mg_2SO_4.7H_2O$). The medium was prepared from 25 g/L of magnesium sulfate, 50 g/L peptone water, 12.5 g/L of urea and 50 g/L of dextrose. These were prepared in 250 ml of distilled water. Then 8g of saccharomyces cerevisiae was measured and added into the prepared medium. This solution was shacked with a magnetic stirrer and added into an incubator at a temperature of 30^oC for three days. After three days the yeast was being grown and added into a sample based on its proportion.

3.7 Fermentation step

The fermentation step was carried out in an incubator. The sample solution from the hydrolysis stage was cooled and filtered. The fermentation stage was carried out at a constant temperature of 30^OC. First, the hydrolyzed sample was filtered by using a fine cloth and this was used to remove impurities. Before saccharomyces cerevisiae added in the solution, the medium was maintained to neutral by using an ammonia solution. This filtered sample was diluted and the pH was adjusted to six to seven. This helped to maintain neutral conditions for microorganisms to grow. Then the hydrolyzed and filtered samples were shacked to mix properly. Then the cultured saccharomyces cerevisiae was added into hydrolyzed, filtered and neutral sample solution. Saccharomyces cerevisiae was added into the sample based on its proportion listed above. Then the sample was placed into the incubator at a temperature of 30^oC for three days. The fermentation process was carried out anaerobically which means with the absence of oxygen. After three days the sample was taken and maintained at room temperature. Then sample solutions were placed on stationary tables in order to sediment a solution for one day. Then the sediment samples were filtered and prepared for the distillation section.

Figure 3.5 Fermentation processes

3.8 Distillation step

The distillation step was carried out by using fractional distillation. In the first step, the heater was used as a heat source to separate ethanol from other impurities. In the second step, the distillation was performed by using a water bath as a heat source. The temperature of the heater was not maintained at a constant temperature. However, by using a heater, the distillation was carried out for one and a half hours. Finally, by using a water bath, the temperature was maintained to79 ^{o}C to purify the ethanol in a better way.

Figure 3.6 Distillation processes

3.9 Process optimization

The process optimization was focused on the optimization of the hydrolysis step. The points discussed under result and discussion part relays on the hydrolysis stage experiments which relate with optimum temperature, acid concentration and hydrolyzing

time. In hydrolysis steps three factors were varied. Those were temperature, acid concentration and hydrolyzing time. The acid concentration, hydrolyzing time and temperature of the process was used to determine the maximum possible amount of fermentable sugar produced during this process. In the fermentation process, the hydrolyzed sample was converted to ethanol by using yeast. In the fermentation stage, all factors were maintained to be constant.

3.10 FTIR procedures to test a solid sample

The molding material was washed by using concentrated ethanol in order to avoid contamination that affects the results during FTIR analysis. The pellet was made by using the mold and compressing machine. The compressing machine was performing at a force of 25 kN. The reference pellet was performed from KBr to calibrate the FTIR machine when it was scanned. The small amount of solid sample and KBr were mixed to prepare a solid sample pellet. This mixed powder of KBr and solid sample was added into molds to perform the small-sized pellet with very fine thickness. This fine thickness was helped to transfer lights easily. Then compress these molds by using a compressing machine at 25kN. After this, the FTIR machine was calibrated by using KBr pellets. Then the sample pellet was added into the FTIR machine and the result was obtained.

Figure 3.7 Pellet and FTIR analyzing machine

3.12 Bioethanol characterization

The produced ethanol was characterized by using a different characterizing machine. The characterizations were helped to check the quality of ethanol produced. Bioethanol produced from waste food was compared with ethanol from the literature review. In characterization section density, viscosity, flammability, functional group, alcohol content, and others were determined.

4. RESULT AND DISCUSSION

There are four types of engineering experiments that are a comparative experiment, screening and characterizing experiments, modeling experiments, and optimization experiments. A comparative experiment is performed only when the one variable change at a time and all other factors are held constant. By changing one factor is possible to determine the response. Screening experiment is performed when several factors are selected from which one has highly affected the response and gives out the rank. The modeling experiment is a mathematical model and relates output with the factor. That means the dependent variable (response) was related to an independent variable by a mathematical equation. The fourth one is the optimization experiment this type of experiment which helps the researcher to determine the set point of the process factors that optimize the process response. The experiment was analyzed by using a design expert. In this research central composite experiment was used. In the hydrolysis step, three factors were studied. Those are temperature, acid concentration and hydrolyzing time.

Table 4.1 The experimental points of factorial design

SNO	Factors	Point 1	Point 2	Point 3	Point4	Point 5
1	Temperatures(^{o}C)	93	100	110	120	127
2	Acid concentration (%)	0.5	1.00	3.00	5.00	6.00
3	Hydrolyzing time(minutes)	33	40	50	60	67

4.1 Experimental results obtained from laboratory

The experiments were performed in the laboratory by the combination of different factors like temperature, acid concentration and hydrolyzing time. The temperature was performed at five different points those were 93^{o}C, 100 ^{o}C, 110 ^{o}C, 120 ^{o}C, and 127 ^{o}C. Acid concentration was performed at a sulfuric acid concentration of 0.5 %, 1.00%, 3.00%, 5.00%, and 6.00%. The hydrolyzing time was controlled to 33 minutes, 40 minutes, 50 minutes, 60 minutes and 67 minutes. To analyze the experiment the design expert was used. This design expert was used to determine the effects of each factor and the interaction effects of those factors.

Table 4.2 Results obtained from laboratory experiments

Std	Run	Temperature (Factor A)	Acid concentration (Factor B)	Hydrolyzing time (factor C)	Yield of ethanol (Response)
1	6	100.00	1.00	40.00	18.85
2	1	120.00	1.00	40.00	28.28
3	2	100.00	5.00	40.00	25.72
4	5	120.00	5.00	40.00	21.6
5	17	100.00	1.00	60.00	24.8
6	16	120.00	1.00	60.00	30.6
7	12	100.00	5.00	60.00	25.12
8	7	120.00	5.00	60.00	16.41
9	4	93.00	3.00	50.00	18.1
10	20	127.00	3.00	50.00	19.2
11	15	110.00	0.50	50.00	28.02
12	14	110.00	6.00	50.00	24.66
13	19	110.00	3.00	33.00	23.32
14	11	110.00	3.00	67.00	24.6
15	8	110.00	3.00	50.00	24.01
16	9	110.00	3.00	50.00	23.8
17	10	110.00	3.00	50.00	22.8
18	13	110.00	3.00	50.00	22.6
19	3	110.00	3.00	50.00	23.2
20	18	110.00	3.00	50.00	24.2

4.2 The experimental results analyzed by using ANOVA

The results obtained from the laboratory experiments were analyzed by ANOVA software. There were three main factors considered namely temperature, time and acid concentration. These three factors might be significant or non-significant effects on a yield of ethanol. The level significant (α) value was 0.05(5%). If the probability value (p-value) was greater than α-value the factors had non-significant effects on a yield of ethanol. When P-value was less than α-value, the factors had significant effects on a yield of ethanol. Based on this table 4.3 was obtained from ANOVA software.

Table 4.3 Significance of each factor analyzed by design expert

ANOVA for Quadratic model Response 1: yield of ethanol Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	233.28	9	25.92	99.98	< 0.0001	Significant
A-temperature	1.60	1	1.60	6.16	0.0325	
B-acid concentration	33.50	1	33.50	129.22	< 0.0001	
C-time	1.87	1	1.87	7.21	0.0229	
AB	101.39	1	101.39	391.08	< 0.0001	
AC	8.49	1	8.49	32.74	0.0002	
BC	26.21	1	26.21	101.09	< 0.0001	
A^2	38.34	1	38.34	147.88	< 0.0001	
B^2	26.25	1	26.25	101.25	< 0.0001	
C^2	0.8800	1	0.8800	3.39	0.0952	
Residual	2.59	10	0.2593			
Lack of Fit	0.3878	5	0.0776	0.1759	0.9603	not significant
Pure Error	2.20	5	0.4410			
Cor Total	235.87	19				

In this section, the discussion was focused on the significance of single factor and interaction effects. The selected model was Quadratic model and the model was significant and lack of fit model was not-significant. This shows that the selected model was accepted. All three factors had significant effects on a yield of ethanol produced. The temperature had p-values of 0.0325 this indicates that temperature had a significant effect on a yield of ethanol. The p-value of the temperature was less than the α-value. When the temperature was changed from lower value to higher value the yield of ethanol was changed. The acid concentration had a p-value of <0.0001 this was shown in tables 4.3. This means that the acid concentration p-value was less than α-value. This indicates the acid concentration had significant effects on a yield of ethanol produced. The third factor was time, the p-value of time was 0.0229 and this p-value was less than the value of α. This means an effect of time had significant effects on a yield of ethanol. The interaction effects of temperature and acid concentration p-value were < 0.0001. The p-value of AB was less than α-value. The interaction effects of temperature and acid concentration had a significant effect on a yield of ethanol. The second interaction effect was temperature and time p-value which had 0.0002. The interaction effects of temperature and time p-value

were less than α-value, this means the interaction effect between temperature and time had significant effects on a yield of ethanol. The third interaction effects were time and acid concentration these interaction effects have p-value was < 0.0001. The interaction effects of the p-value were less than the value of α. The interaction effects of time and acid concentration had a significant effect on a yield of ethanol. The interaction effect of temperature (A^2) p-value was < 0.0001 and this was less than α-value. Doubling the value of temperature had significant effects on a yield of ethanol. The interaction effects of acid concentration (B^2) p-value were < 0.0001 which was less than the value of α. When the effects of acid concentration were doubled, the yield of ethanol was significantly affected. The interaction effect of time (C^2) had a p-value of 0.0952. This indicates that its value was greater than α-value. When the value of time doubled it had no-significant effects on a yield of ethanol.

4.3 The effects of a single factor on the yield of ethanol

The three factors namely temperature, acid concentration and hydrolyzing time were positively and negatively affect the response. In this study, the value of α (level of significance) was 5%. Based on this the effect of each factor was analyzed and discussed.

4.3.1 Effects of temperature on yield of ethanol

At a low level of acid concentration and time as the temperature increases the yield of ethanol also increases proportionally, but after a certain point as the temperature increased the yield of ethanol was constant and became a maximum value. In this experiment, the temperature had positive effects on the yield of ethanol. The following Figure 4.1a shows the relationship between temperature and ethanol yield at a low level of acid concentration and hydrolyzing time.

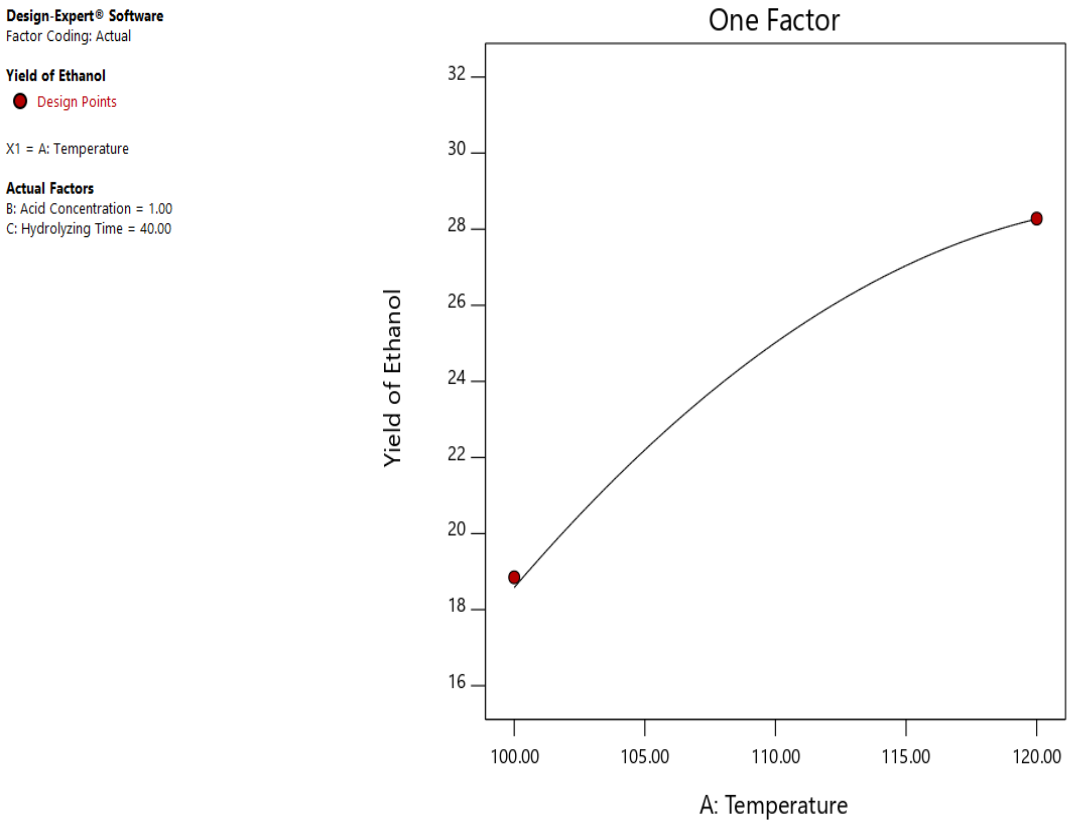


Figure 4.1a Effects of temperature on the yield of ethanol

At a high level of acid concentration and hydrolyzing time as the temperature was increased from low-level value to high-level values, the ethanol yield was decreased. In figure 4.1b when the value of temperature has increased the yield of ethanol was decreased. This shows that at a high level of acid concentration and hydrolyzing time the temperature has negative effects on the yield of ethanol. The acid concentration was at a lower level the yield of total reducing sugar was increased when the temperature was increased. This reaction was endothermic reaction due to that increase of temperature was increase the yield of total reducing sugar as well as the yield of ethanol.

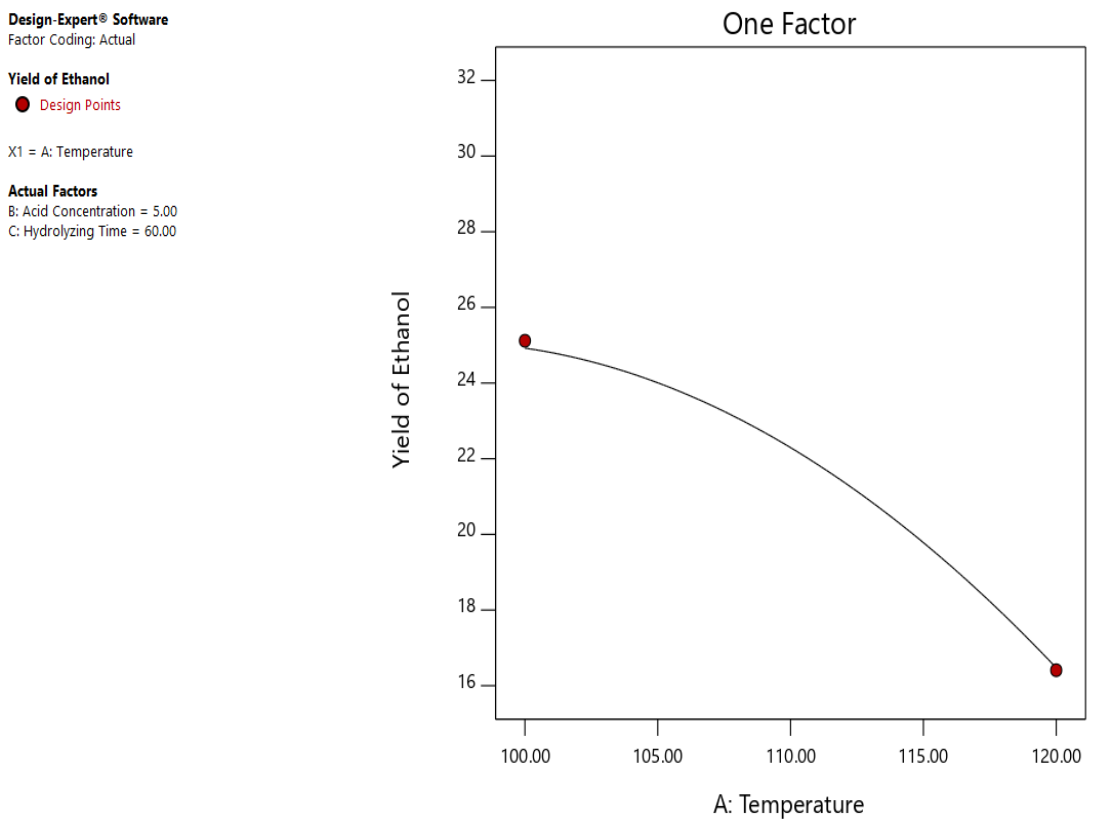


Figure 4.1b Effects of temperature on the yield of ethanol

4.3.2 Effects of acid concentration on yield of ethanol

As the percent of acid concentration was increased the yield of ethanol was also increased at the low level of hydrolyzing time and temperature. When the temperature and hydrolyzing time were at a low level the acid concentration had positive effects on the yield of ethanol. This relationship between acid concentration and ethanol yield was represented in a figure 4.2a. When the temperature increased the yield of total reducing sugar was decreased when the acid concentration was at a high level. From the literature reviewed the acid concentration is between 47-50% hydrolysis process was carried out at room temperature during this condition the hydrolysis temperature has increased the yield of total reducing sugar was decreased. This reaction is an exothermic reaction due to the reasons the temperature decreased favors the increase in the yield of total reducing sugar or the yield of ethanol. In this experiment, the acid concentration was at a high level the yield of total reducing sugar was decreased when the temperature was increased. This is due to the contacting patterns between the molecules cause to shift from endothermic to the partially exothermic reaction.

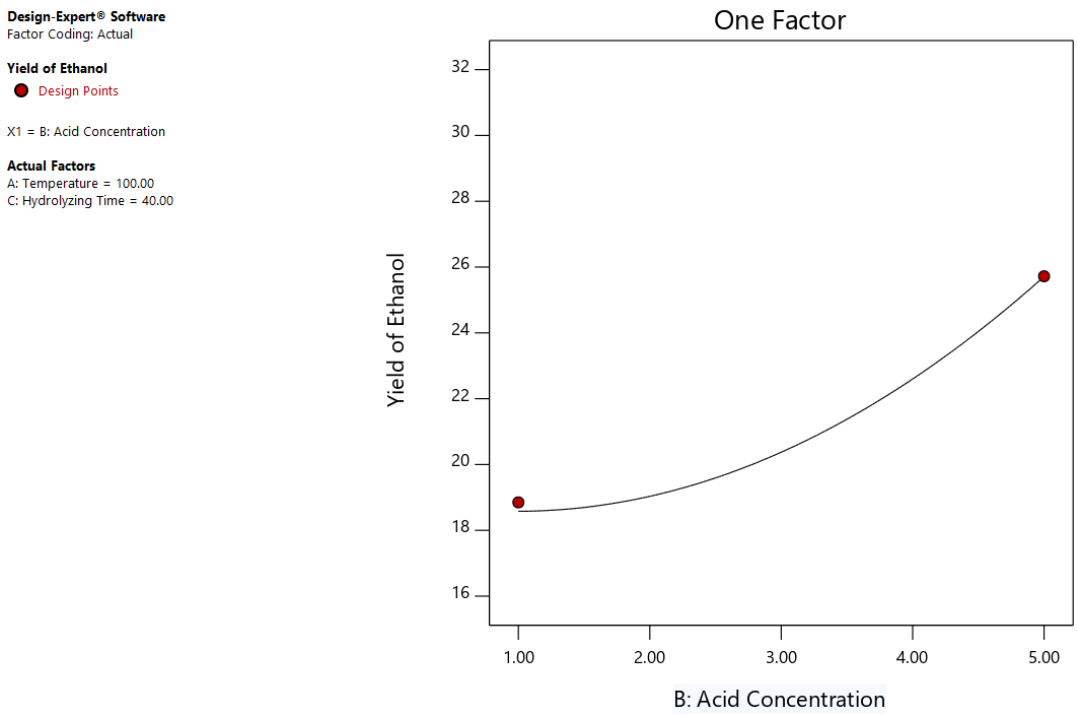


Figure 4.2a Effects of acid concentration on the yield of ethanol

At a high level of temperature and hydrolyzing time, as the percent of acid concentration was increased the yield of ethanol was decreased. When the temperature and hydrolyzing time was at a high level, the acid concentration had negative effects on a yield of ethanol. The relationship between acid concentration and ethanol yield was represented in figure 4.2b. In order to increase the ethanol yield, there must be the time and temperatures were at a low level when acid concentration was at a high level. If the acid concentration was at a low level the value of temperature and time was obtained at a high level to get much ethanol yield.

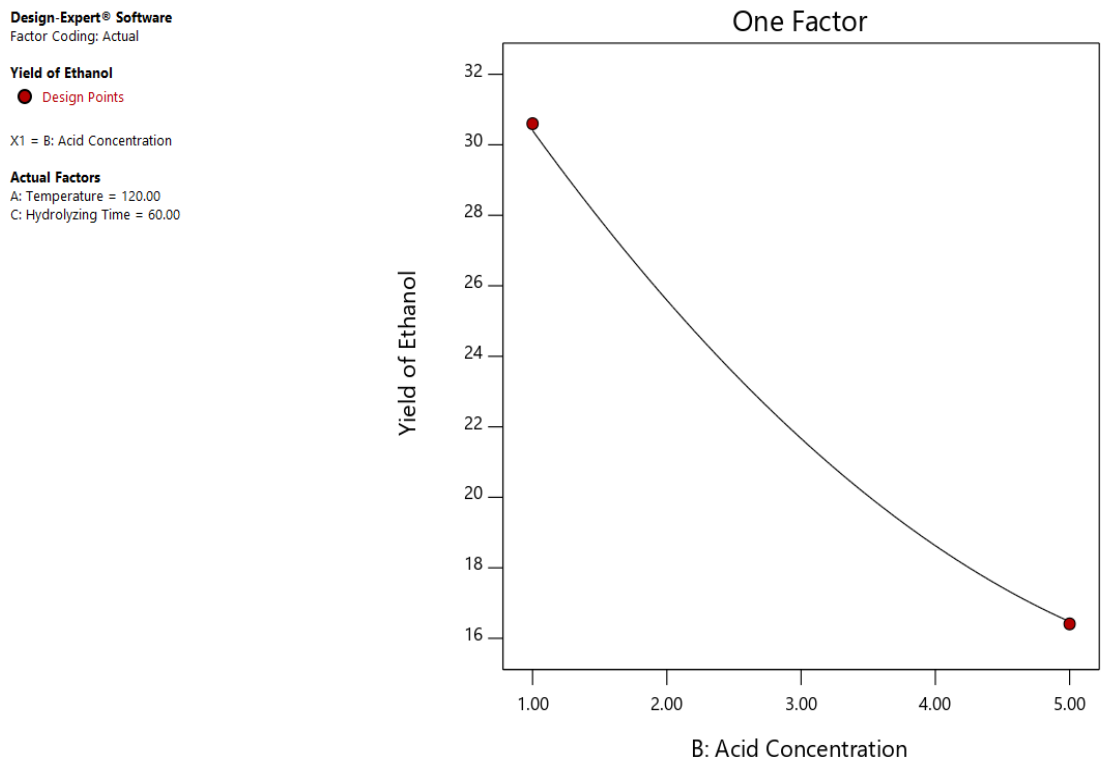


Figure 4.2b Effects of acid concentration on the yield of ethanol

4.3.3 Effects of hydrolyzing time on the yield of ethanol

In this experiment, the yield of ethanol was increased as hydrolyzing time increased, when temperature and acid concentration was set at a low level. This relation was shown that hydrolyzing time had positive effects on a yield of ethanol produced at the low level of temperature and acid concentration. This relationship between hydrolyzing time and ethanol yield produced was represented on a figure 4.3a.

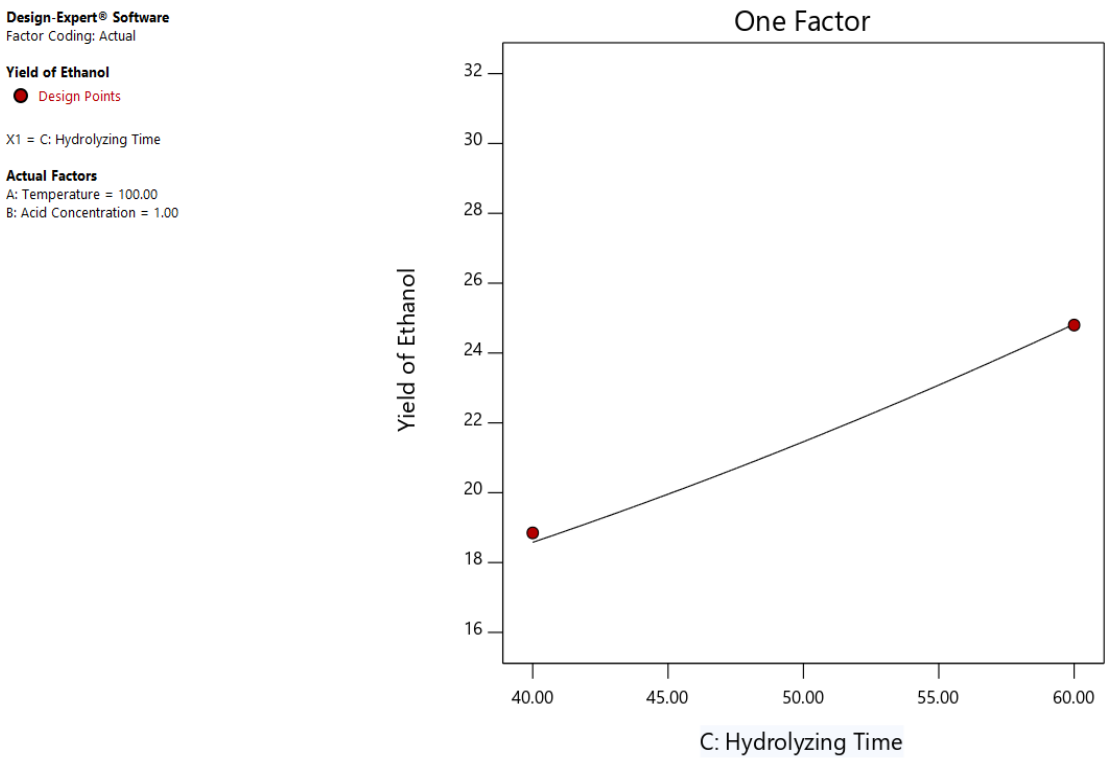


Figure 4.3a Effects of time on the yield of ethanol

At a high level of temperature and acid concentration, the yield ethanol was decreased when the value of hydrolyzing time increased. Hydrolyzing time had a negative effect on the yield of ethanol produced when the temperature and acid concentration was at a high level. This relationship represented in figure 4.3b. The relationship between hydrolyzing time and ethanol yield depends on the level of temperature and acid concentration. At a high level of temperature and acid concentration, a low amount of ethanol was produced when the time was set at a high level.

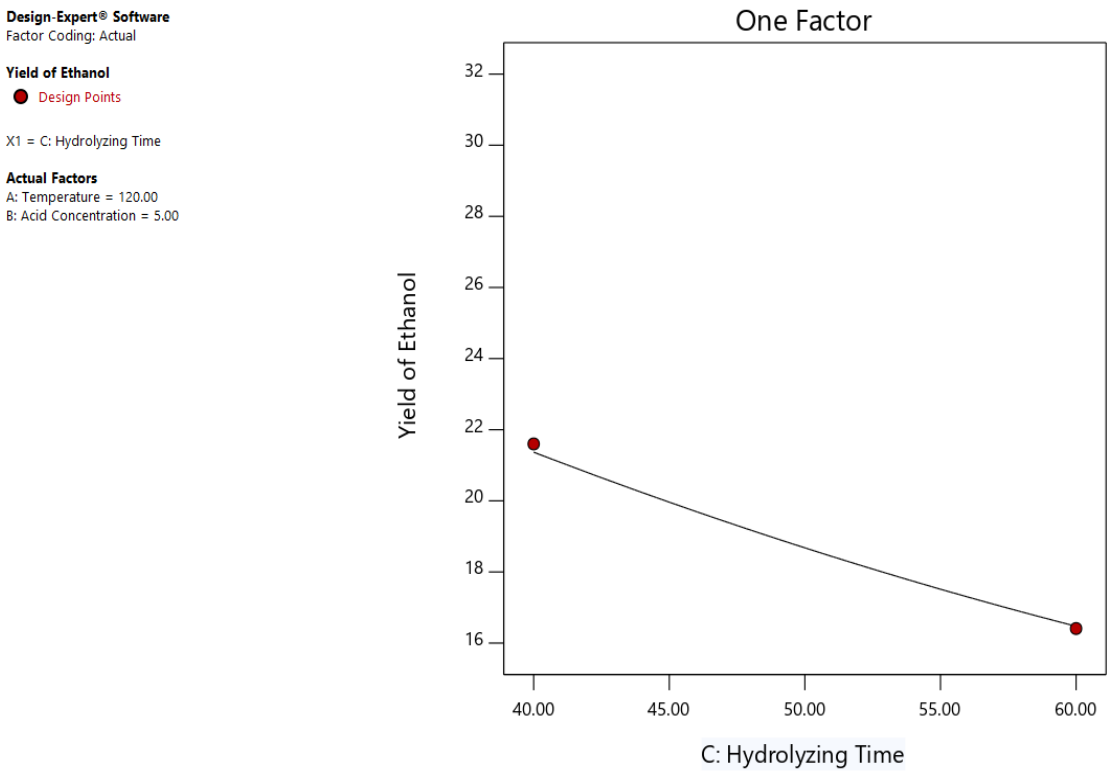


Figure 4.3b Effects of time on the yield of ethanol

At a high level of temperature and acid concentration, the yield ethanol was decreased when the value of hydrolyzing time increased. Hydrolyzing time had a negative effect on the yield of ethanol produced when the temperature and acid concentration was at a high level. This relationship represented in figure 4.3b. The relationship between hydrolyzing time and ethanol yield depends on the level of temperature and acid concentration. At a high level of temperature and acid concentration, a low amount of ethanol was produced when the time was set at a high level.

4.4 Interaction effect of the parameters on a yield of ethanol

The previous section discussion depends on single-factor effects on a yield of ethanol. The single factors had positive and negative effects on the yield of ethanol obtained. Thus it depends upon the level of two factors. In this section, the effects of interaction parameters on the yield of ethanol were discussed at a low level and high level of a single factor.

4.4.1 Interaction effects of temperature and acid concentration on a yield of ethanol

At a low level of hydrolyzing time, as the temperature and acid concentration were increased the yield of ethanol was decreased. The interaction effects of temperature and acid concentration had a positive relationship with the yield of ethanol obtained at a low level of hydrolyzing time. The relation is shown in fig 4.4a.

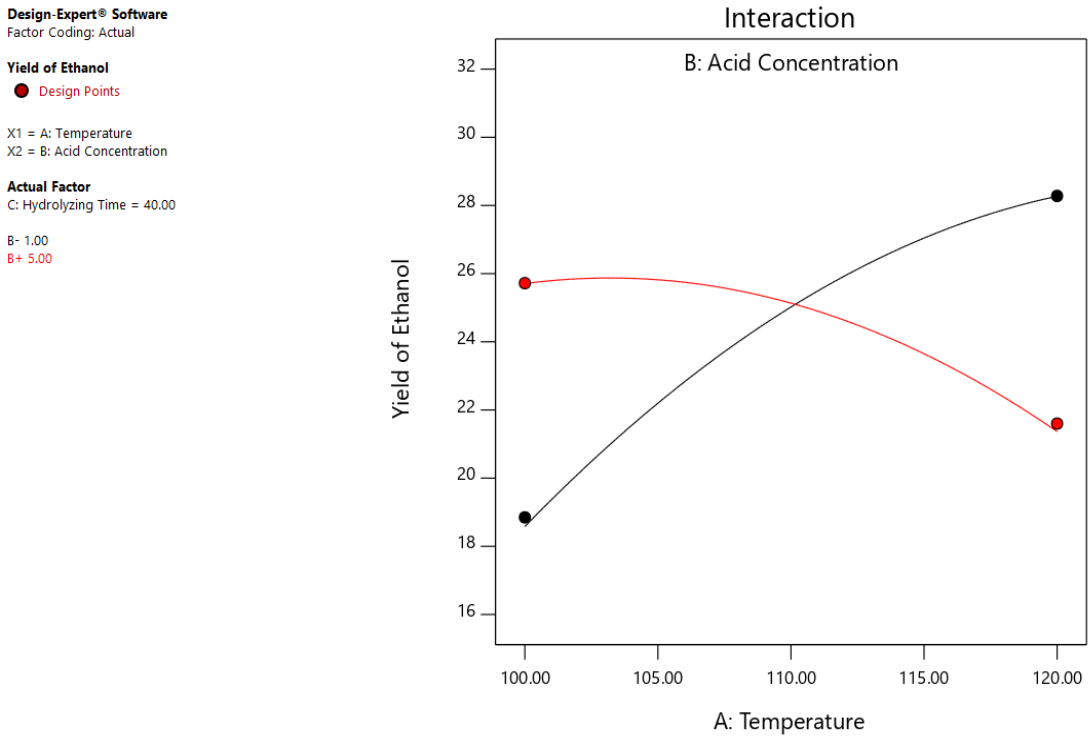


Figure 4.4a Interaction effects of temperature and acid concentration

Similarly, as the interaction effects of temperature and acid concentration were increased the yield of ethanol was decreased at a high level of hydrolyzing time. This was shown in fig 4.4b, for the low level of hydrolyzing time as the yield of ethanol was decreased due to the interaction effects of temperature and time. The interaction effects of temperature and acid concentration had negative effects on a yield of ethanol obtained. But in figure 4.4a the yield of ethanol was decreased slowly when the temperature and acid concentration was increased. But in the case of 4.4b, the yield of ethanol was decreased faster than the previous figure 4.4a and also the yield ethanol obtained was smaller than the figure 4.4a.

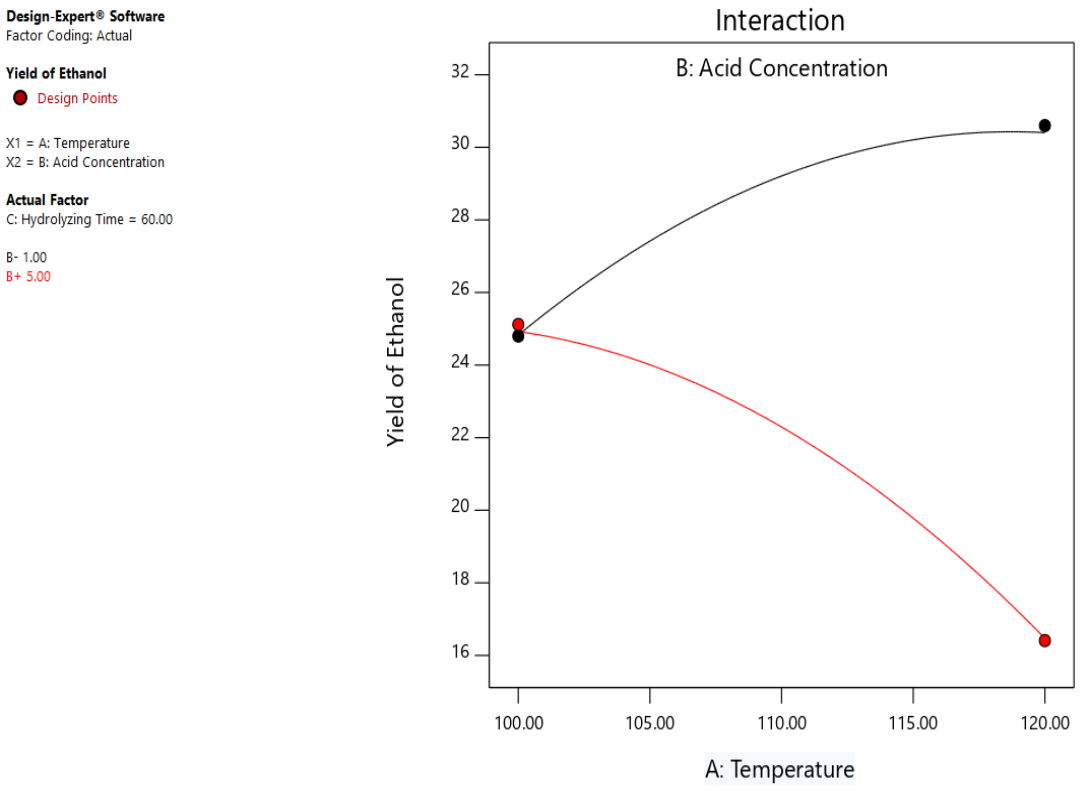


Figure 4.4b Interaction effects of temperature and acid concentration

4.4.2 Interaction effects of temperature and hydrolyzing time on a yield of ethanol

The interaction effects between temperature and hydrolyzing time were increased as the yield of ethanol was increased at a low level of acid concentration. This interaction effect has favored the yield of ethanol obtained. As temperature and hydrolyzing time were increased at the same time the yield of ethanol was increased at a low level of acid concentration. When the acid concentration was kept at a low level, both factors were increased from low value to high value which favors the production of ethanol. The relationship between interaction effects of both time and temperature as shown in fig 4.5 below.

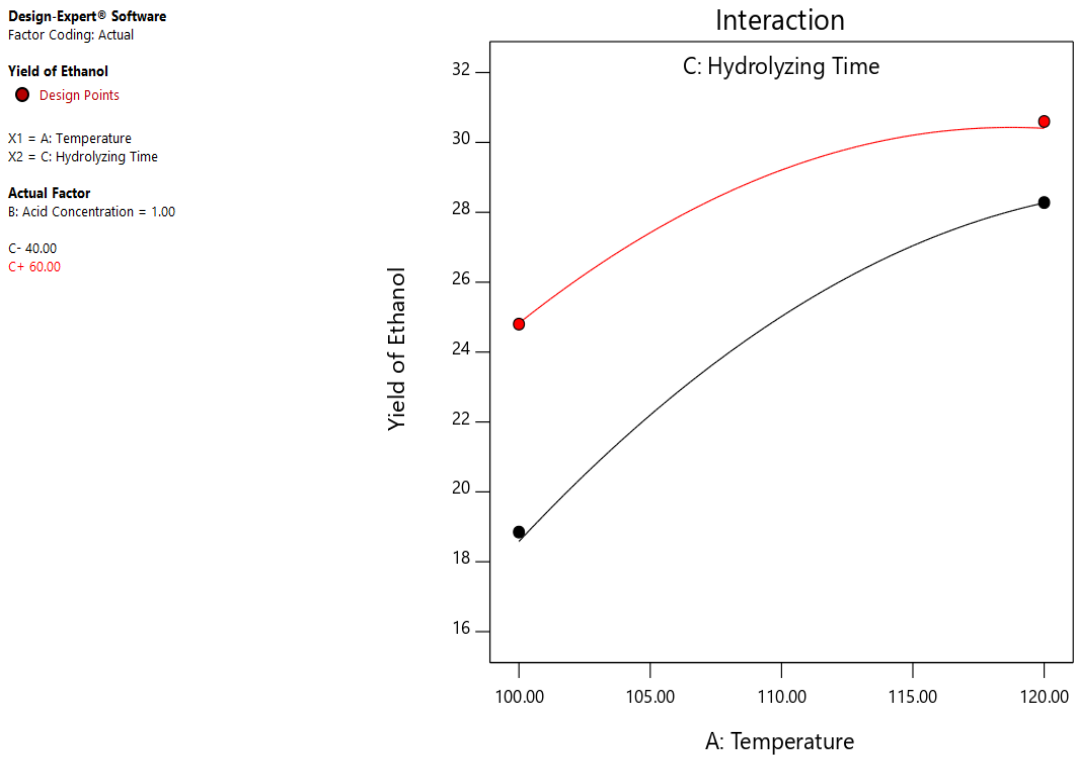


Figure 4.5a Interaction effects of temperature and hydrolyzing

When the acid concentration was kept at a high value, the interaction effects had negative effects on a yield of ethanol obtained. The interaction effects of temperature and time had negative effects on a yield of ethanol. At the same time when the value of temperature and time were increased from low value to high value, the yield of ethanol was decreased at a high level of acid concentration. At a high level of acid concentration, the interaction effects of temperature and time had a negative effect on the production of ethanol. There was a negative relationship between ethanol yield and interaction effects which were shown in figure 4.5b.

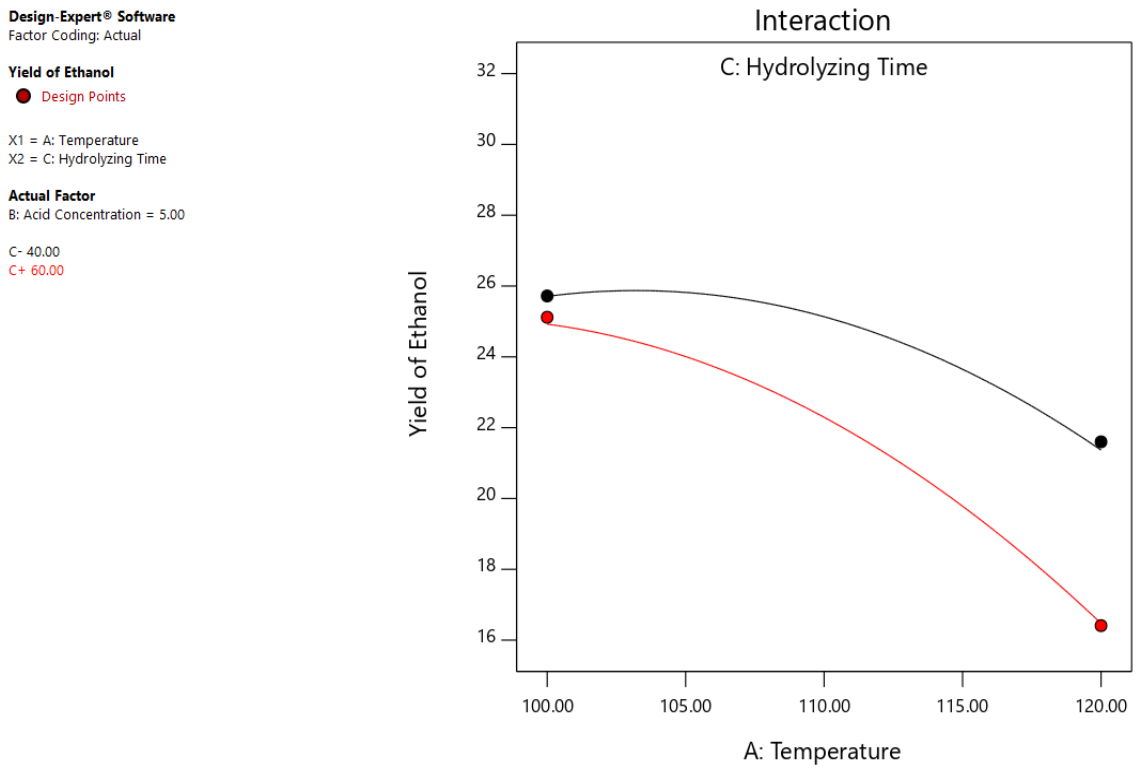


Figure 4.5b The interaction effects of temperature and hydrolyzing

4.4.3 Interaction effects of acid concentration and hydrolyzing time on a yield of ethanol

The interaction effects of acid concentration and time had negative effects on a yield of ethanol at high value of temperature. When both acid concentration and time were increased from its lower value to higher values the yield ethanol obtained was decreased. At a higher value of temperature as the values of acid concentration and time were increased the yield of ethanol was not favored. This relation was shown in figure 4.6a.The ethanol yield was decreased from left to right on a diagram.

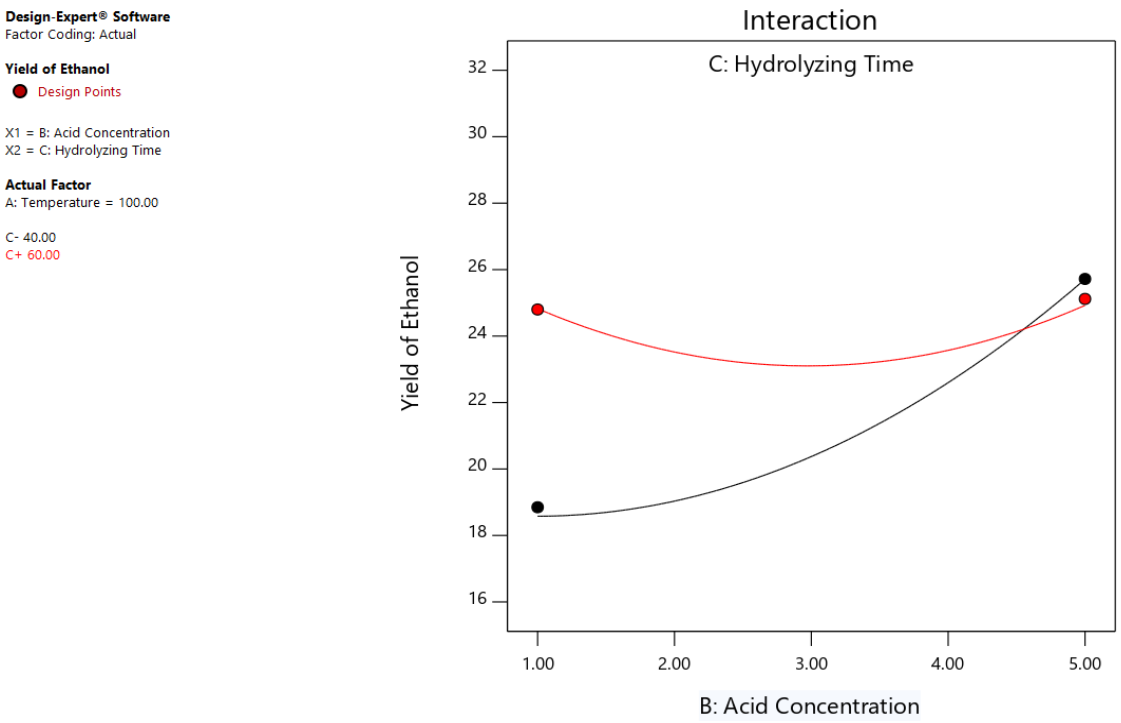


Figure 4.6a Interaction effects of acid concentration and hydrolyzing time

At a lower level of temperature, the interaction effects of acid concentration and time had negative effects on a yield of ethanol produced which was shown on a fig 4.6b. Initially, the yield of ethanol was decreased and it became its lower value. After that, the yield of ethanol has become rose from left to right. The interaction effects of acid concentration and time had negatively affected the yield of ethanol at a certain point. After this, the yield of ethanol was increased due to the interaction effects of acid concentration and time.

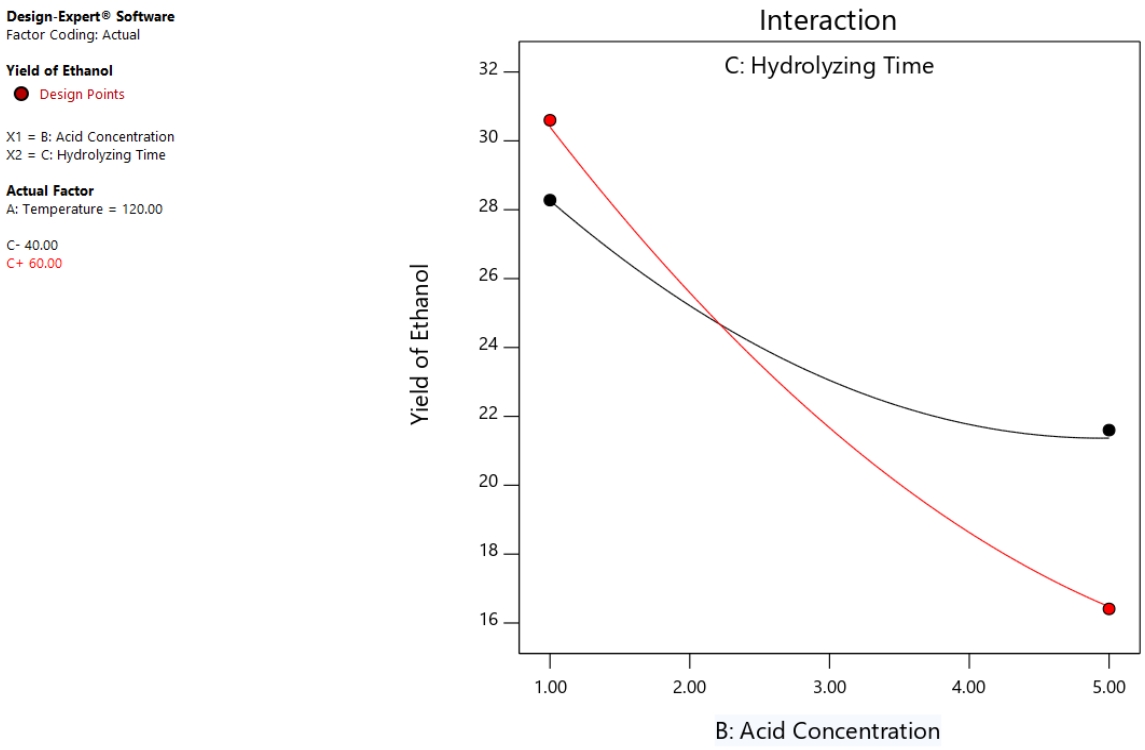


Figure 4.6b Interaction effects of acid concentration and hydrolyzing time

4.5 The interaction effects of the factor itself (A^2, B^2, and C^2) on a yield of ethanol

In this section, the interaction effects of factors itself affect the yields of ethanol. A^2 represents the interaction effects of temperature. It had similar effects with a temperature (A) and also had a positive relationship with the yield of ethanol. B^2 represents the interaction effects of acid concentration (B) which had similar effects with acid concentration. C^2 represents the interaction of time which had similar effects with time(C).

4.6 Model equation analyzed by design expert software

The model equation was the equation used for the best fitting of experimental results (actual values) with the predicted values. This model equation was developed by selecting the significant factors from the above discussion and takes the coefficients of that factors calculated and analyzed by ANOVA software.

Table 4.4 Coefficients of each factor

Coefficients in Terms of Coded Factors

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	23.40	1	0.2052	22.95	23.86	
A-temperature	0.3403	1	0.1372	0.0347	0.6460	1.0000
B-acid concentration	-1.70	1	0.1494	-2.03	-1.37	1.02
C-time	0.3684	1	0.1372	0.0627	0.6740	1.0000
AB	-3.56	1	0.1800	-3.96	-3.16	1.0000
AC	-1.03	1	0.1800	-1.43	-0.6289	1.000
BC	-1.81	1	0.1800	-2.21	-1.41	1.000
A^2	-1.60	1	0.1312	-1.89	-1.30	1.01
B^2	1.77	1	0.1758	1.38	2.16	1.02
C^2	0.2417	1	0.1312	-0.0506	0.5341	1.01

The coefficient of temperature was found to be 0.3403; the coefficient of acid concentration was found to be -1.70, the coefficient of time was found to be 0.3684. The interaction effects between temperature and acid concentration (AB) were found to be -3.56. The interaction effect between temperature and time (AC) was found to be -1.03. The interaction effect between time and acid concentration (BC) was found to be -1.81. A^2 was found to be -1.60, B^2 was found to be 1.77, C^2 was found to be 0.2417 and the intercept was found to be 23.40. The model equation was represented as follows;

$$\text{Yield of ethanol}(Y) = 0.3403\times A - 1.70\times B + 0.3684\times C - 3.56\times(AB) - 1.03\times(AC) - 1.81\times BC - 1.60\times(A^2) + 1.77\times(B^2) - 0.2417\times(C^2) + 23.40 \quad \text{.....(4.1)}$$

All factors and interaction effects had a significant effect on the yield of ethanol. C^2 hadn't affected the yield of ethanol therefore C^2 was removed from the equation and finally, the equation reduced to:

$$\text{Yield of ethanol}(Y) = 23.4 + 0.3403 \times A - 1.70 \times B + 0.3684 \times C - 3.56 \times (AB) - 1.03 \times (AC) - 1.81 \times BC - 1.60 \times (A^2) + 1.77 \times (B^2) \quad \text{.....(4.2)}$$

Finally,

$$\text{Yield of ethanol}(Y) = 23.40 + 0.34A - 1.7B + 0.37C - 3.56AB - 1.03AC - 1.81BC - 1.6A^2 + 1.77B^2 \text{.....} (4.3)$$

Based on the above equation temperature had a positive slope, this means that the temperature had a positive effect on the yield of ethanol. The slope of acid concentration was shown that negative value. When the acid concentration was increased from its lower value to higher value the yield of ethanol was decreased. Therefore, acid concentration had a negative effect on a yield of ethanol. The slope of time in the model equation was positive. When the time has increased the yield of ethanol was also increased proportionally, this means that the time had a positive relationship with the yield of ethanol. The interaction effects of temperature and acid concentration had a negative slope, which means a negative relation on a yield of ethanol. The interaction effect of temperature and time had a negative relation with the yield of ethanol. In a similar way, the interaction effect of time and acid concentration had a negative slope and had a negative relation with the yield of ethanol. A^2 had a negative slope and there was a negative relation with the yield of ethanol. B^2 had a positive slope and a positive relation with the yield of ethanol. Therefore temperature, time and B^2 favor the production of ethanol when those factors were increased from lower value to higher value. When all interaction effects and A^2 were increased from lower value to higher value the yield of ethanol was decreased.

4.7 The actual and predicted values

The value of R^2 was used to determine how close the value of actual value with the predicted value. The model equation precisely represents the actual value of 98.9%. The actual value was highly precise with the predicted value.

Table 4.5 Fit of statistic data

Fit Statistics			
Std. Dev.	0.5092	R^2	0.9890
Mean	23.49	Adjusted R^2	0.9791
C.V. %	2.17	Predicted R^2	0.9737
		Adeq Precision	39.2641

The value of the actual was highly closed with the predicted value. The inclined straight line represents the value of predicted value and dotes on a straight line represents the actual value. The actual values were highly close with the predicted value and R^2 was 0.989.

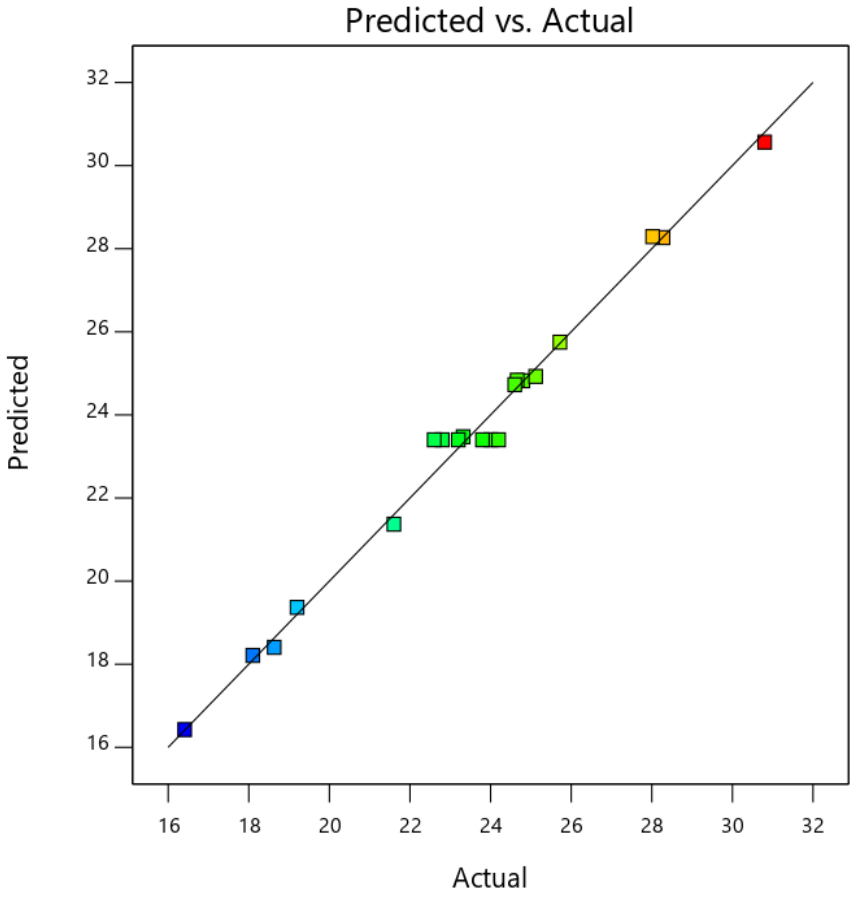


Figure 4.7 Actual vs. predicted values

2.8 Residual plots

The residual plots for normal, temperature, acid concentration and time were shown on a figure 4.8, 4.9a, 4.9b, and 4.9c respectively. The plot had specified ranges. This shows that the experiments were done on specified ranges.

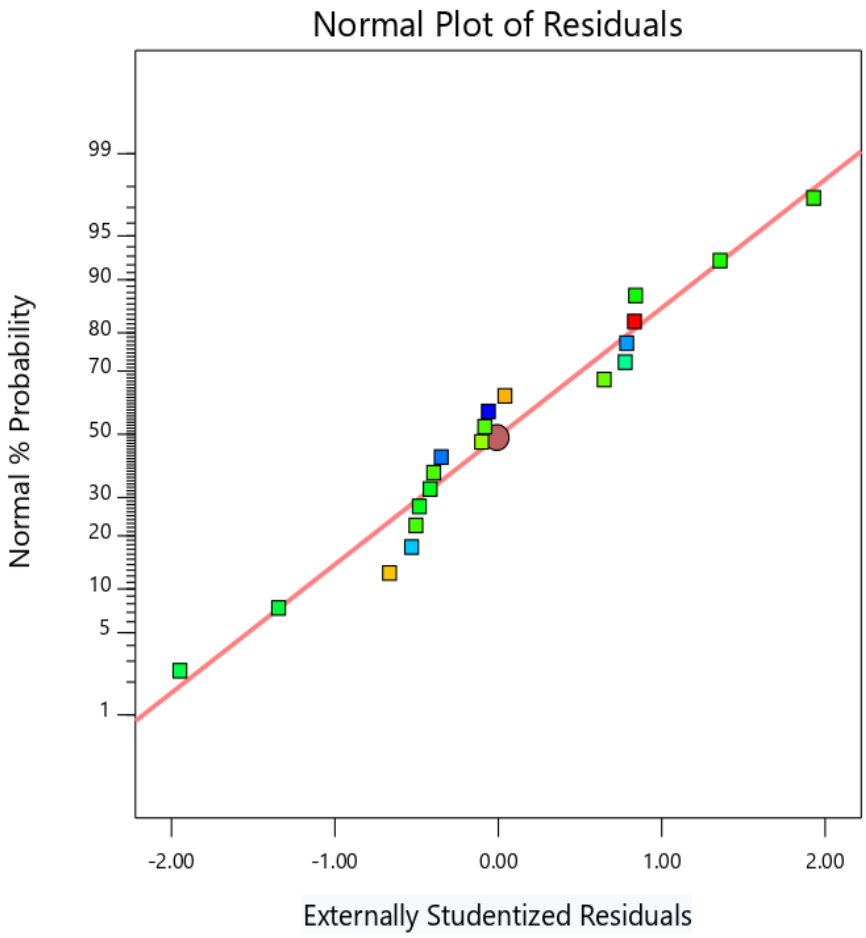


Figure 4.8 Normal plots vs. residuals

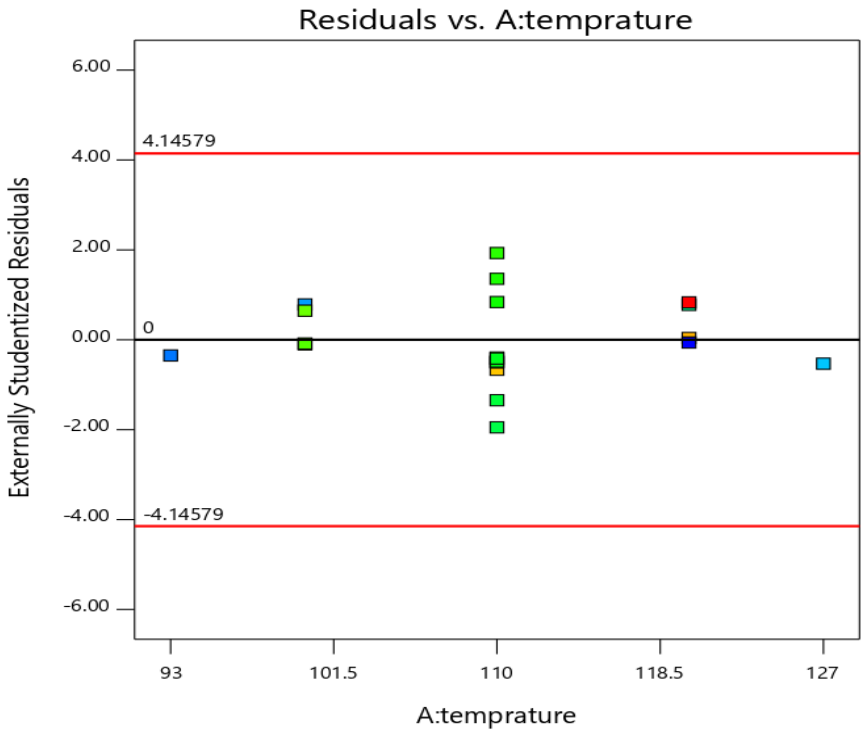


Figure 4.9a Residual vs. temperature

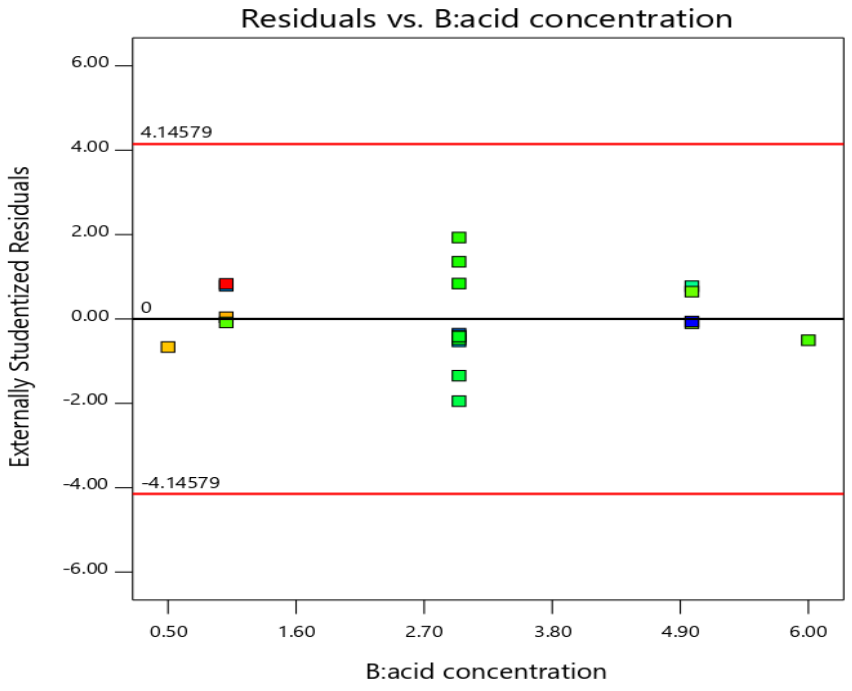


Figure 4.9b Residual vs. acid concentration

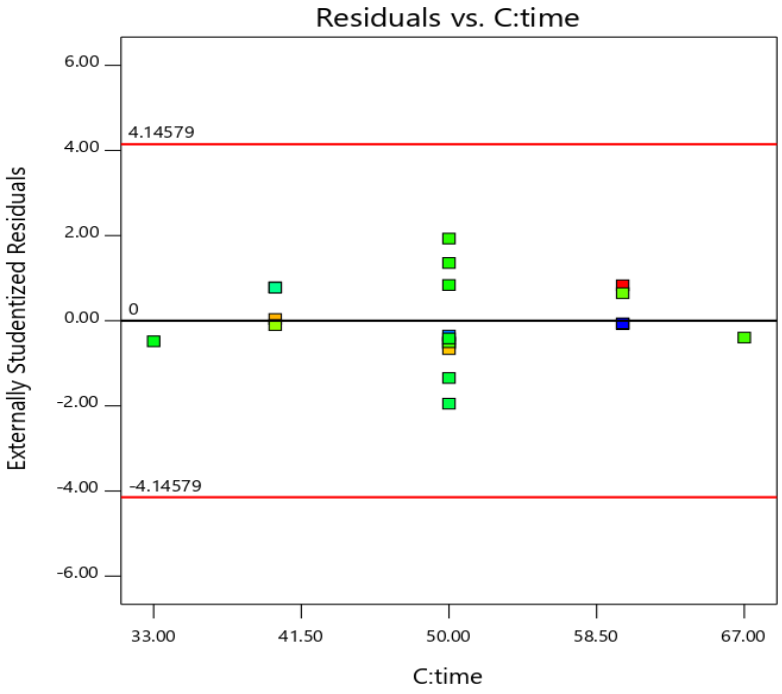


Figure 4.9c Residual vs. residual vs. time

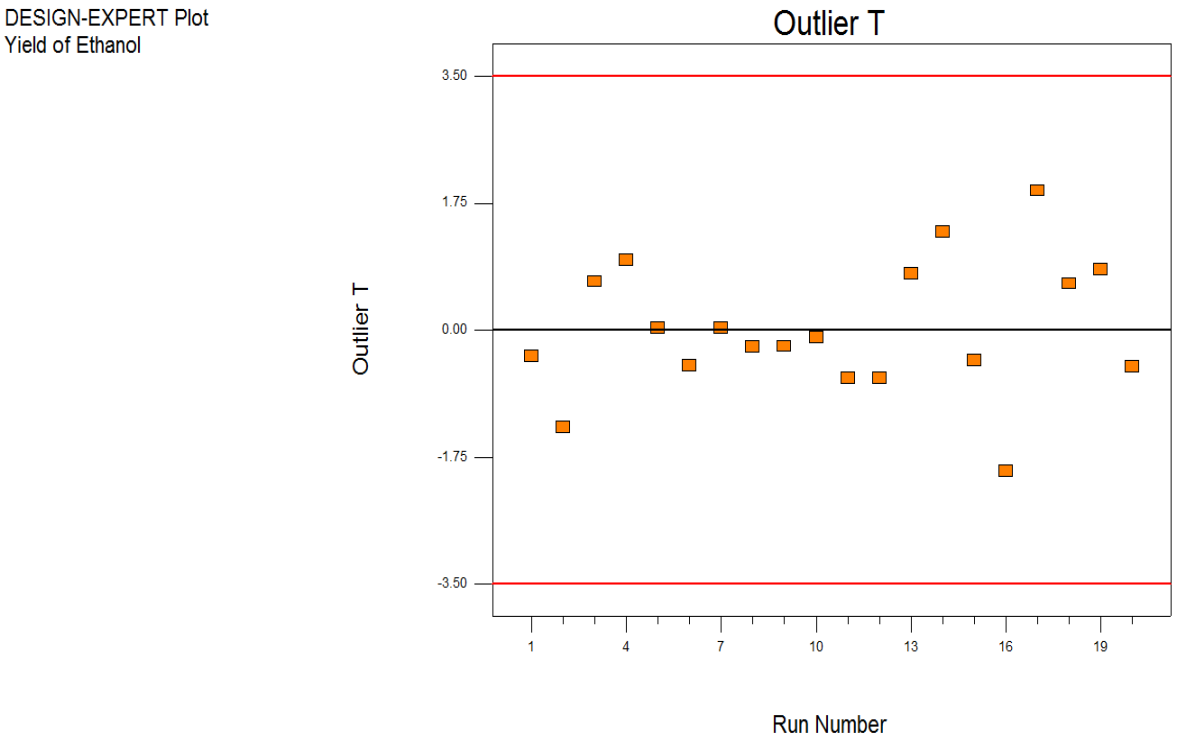


Figure 4.9d Diagnostics

From figure 4.9 d, the diagnostics diagram has unstructured this indicates the experiment was done randomly. Randomization was better during the experiment in order to get better results.

4.8 Determining optimum points for ethanol yield

The optimum value of temperature, acid concentration and time were determined. Each parameter had its own range for a point in which the maximum yield of ethanol was obtained. The maximum yield of ethanol was determined by using a combination of three-factor and interaction effects of each factor. In the previous section, the yield of ethanol was discussed by changing two factors and one was kept constant. To determine the effects of temperature and acid concentration, the time was constant at higher-level value or to the lower level value. Similarly, in order to determine the effects of temperature and time, the acid concentration was kept constant and constant for the temperatures. The effects of three parameters were determined for the production of the yield of ethanol.

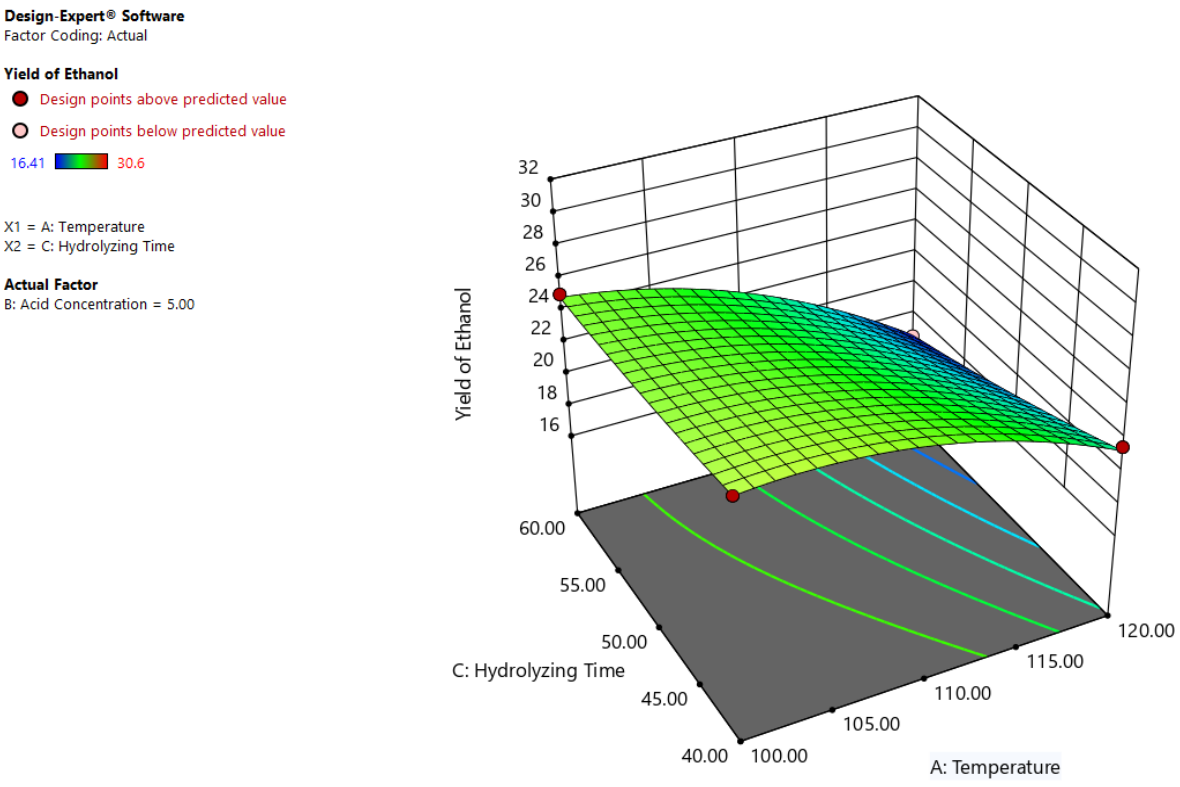


Figure 4.10a Minimum yield of ethanol

For a low level of temperature and time, the yield of ethanol obtained was maximum when acid concentration was set at a high level as shown in Figure 4.10a. When the acid concentration was at 5% and both parameters were increased, the yield of ethanol was decreased. Therefore, the yield of ethanol was minimum at a high level of acid concentration. At this point, the yield of ethanol was obtained to be 16.41 ml/g.

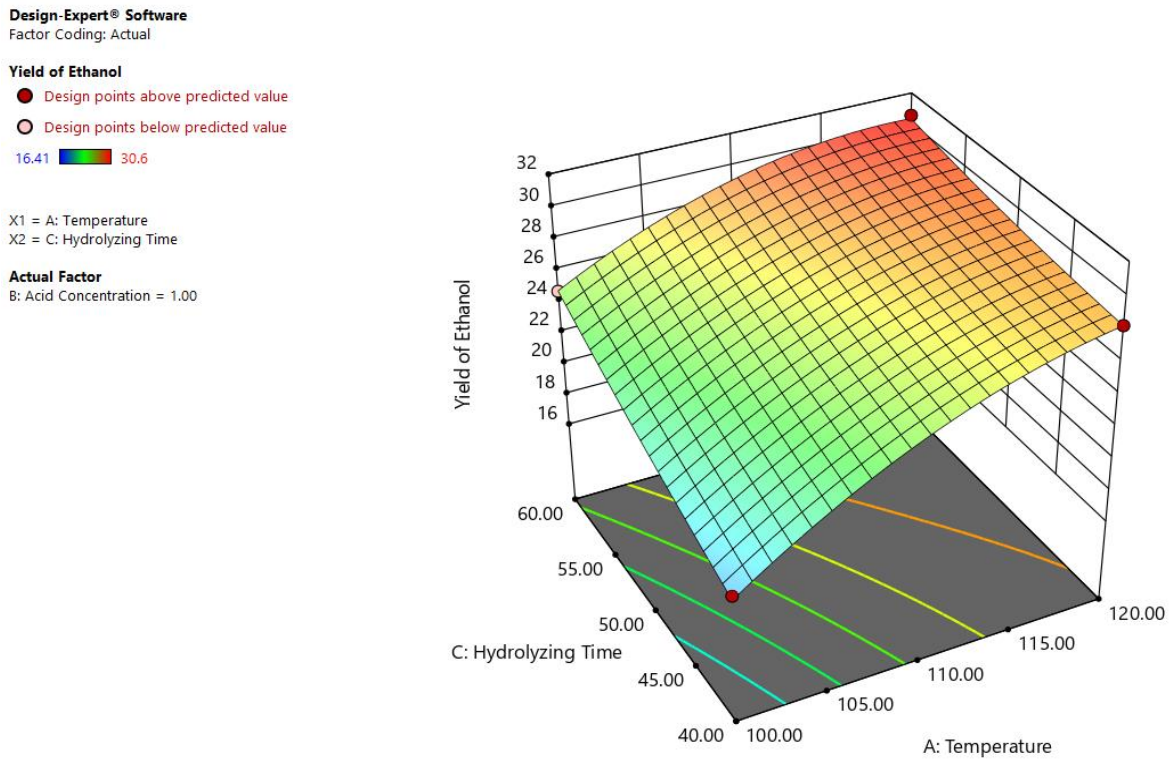


Figure 4.10b The optimum yield of ethanol

In figure 4.10b the yield of ethanol was maximum at the high level of temperature and time when acid concentration was at a lower level. The yield of ethanol was optimum when the acid concentration, time and temperature were set at 1%, 1 hour and 120^{o}C respectively. Therefore the yield of ethanol was optimum at a high level of temperature and time when the acid concentration was at a low level. At this point, the yield of ethanol was obtained to be 30.6 ml/g.

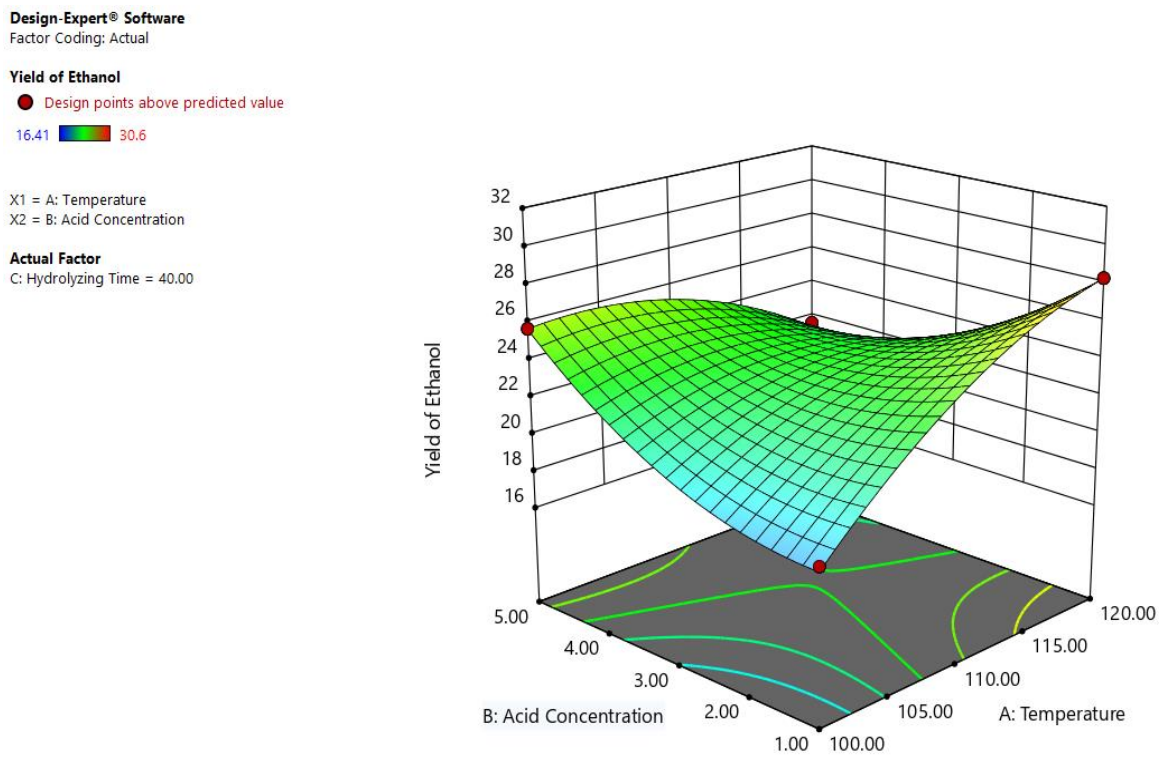


Figure 4.11a The yield of ethanol at a lower level of time

In figure 4.11a the yield of ethanol was determined by varying temperature and acid concentration at a lower level of time. The yield of ethanol was obtained 21.6 ml/60g when time, acid concentration and temperature was set at 40 minutes, 5% and 120^{o}C respectively.

In figure 4.11b the yield of ethanol was determined by varying temperature and acid concentration when the time was at a high level. The yield of ethanol was obtained 16.41ml/60g when time, acid concentration and the temperature was set at 60 minutes, 5% and 120^{o}C respectively. The yield of ethanol was obtained 24.8 ml/60g when time, acid concentration and temperature was set at 60 minutes, 1% and 100^{o}C respectively. The yield was maximum at 1% of acid concentration and at a temperature of 100^{o}C when the time was at 60 minutes.

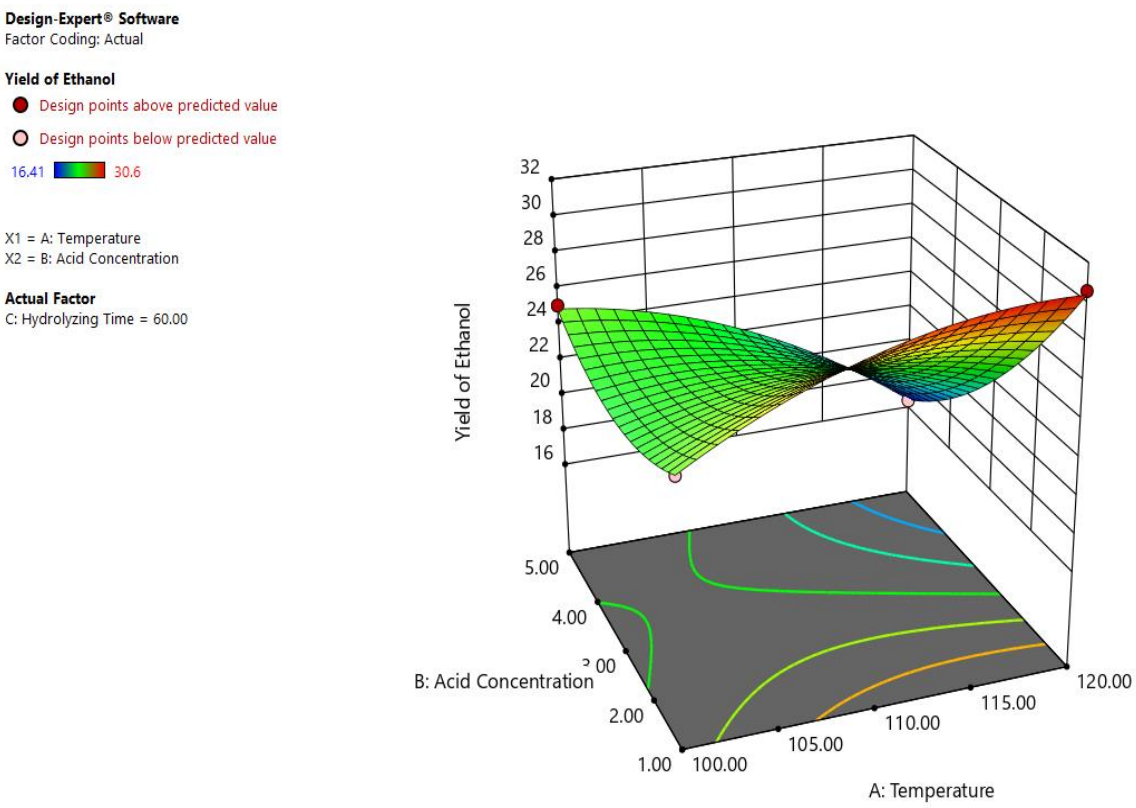


Figure 4.11b The yield of ethanol at a high level of time

The figure 4.12a the yield of ethanol was determined by varying acid concentration and time when the temperature was kept constant at a lower level. The yield of ethanol was obtained 25.12 ml/60g when time, acid concentration and the temperature was set at 60 minutes, 5% and 100^{o}C respectively. The yield of ethanol was obtained 18.36 ml/60g when time, acid concentration and the temperature was set at 40 minutes, 1% and 100^{o}C respectively. In this section maximum yield (25.12 ml/60g) was obtained at the acid concentration of 5% and a time of 60 minutes when the temperature was kept at 100^{o}C. The minimum yield of ethanol (18.63ml/60g) was obtained at 1% of acid concentration and time of 40 minutes when the temperature was kept at 100 ^{o}C.

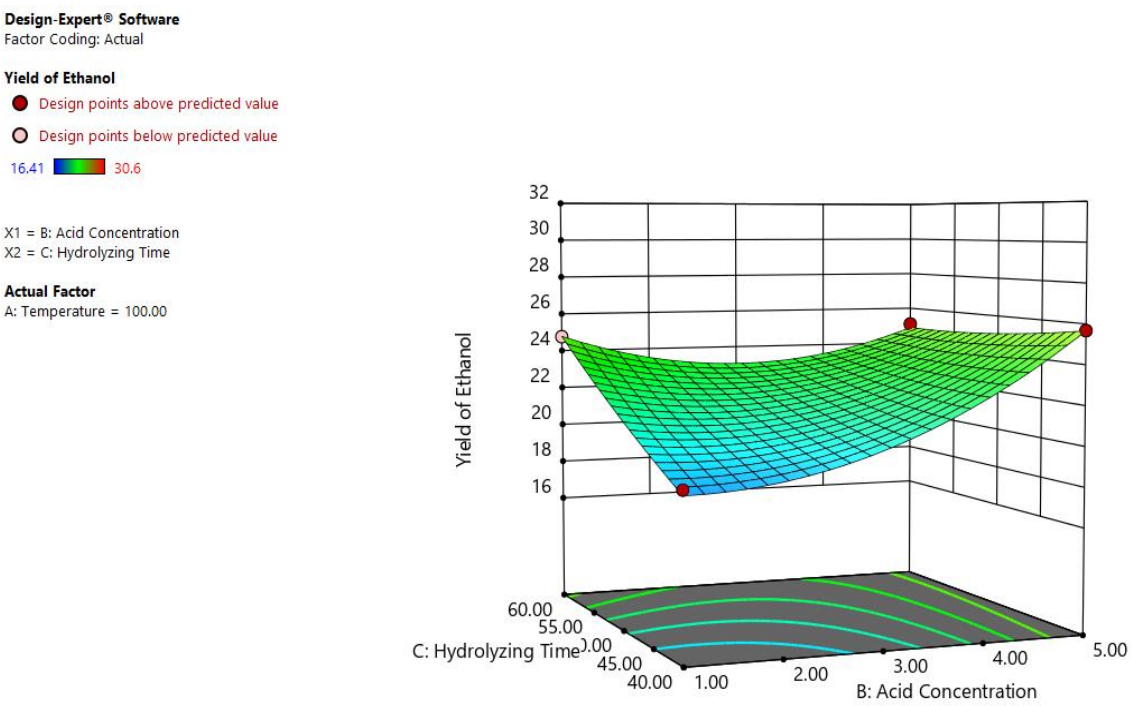


Figure 4.12a The yield of ethanol at a lower level of temperature

The figure 4.12b the yield of ethanol was determined by varying acid concentration and time when the temperature was kept constant at a lower level. The yield of ethanol was obtained 16.41 ml/60g when time, acid concentration and the temperature was set at 60 minutes, 5% and 120^{o}C respectively. The yield of ethanol was obtained 28.28 ml/60g when time, acid concentration and the temperature was set at 40 minutes, 1% and 120^{o}C respectively.

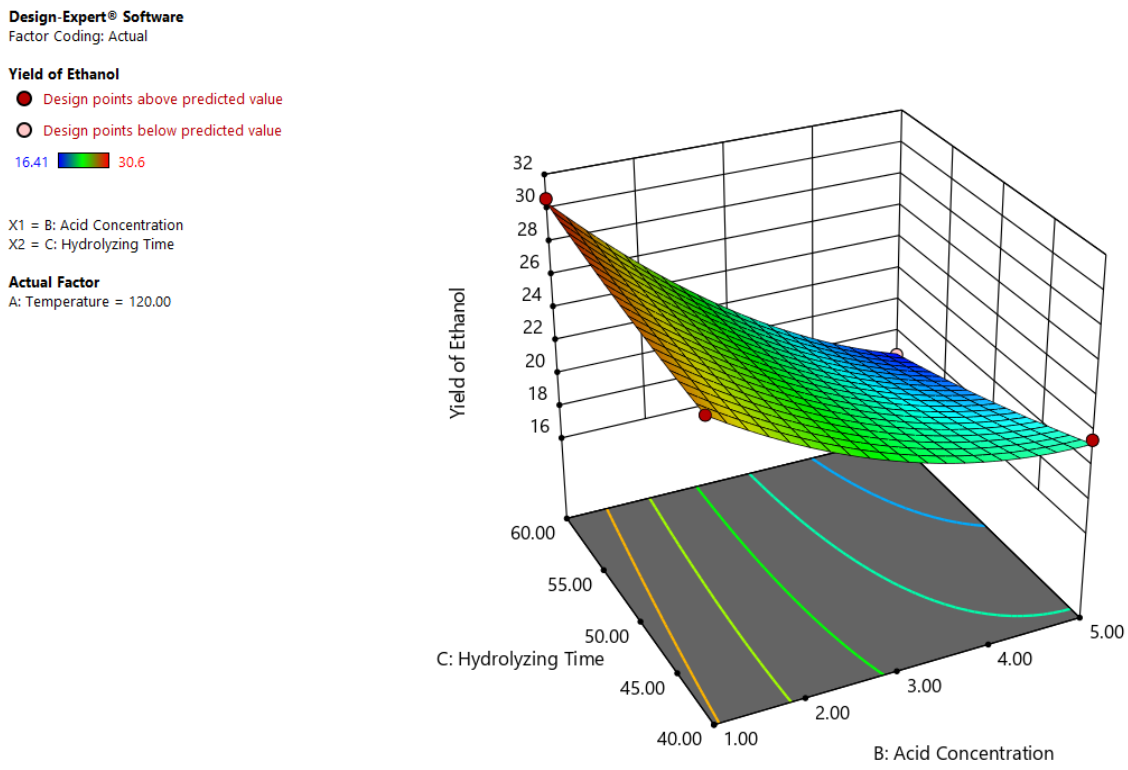


Figure 4.12b The yield of ethanol at a high level of temperature

Table 4.6 The yield of ethanol at a lower and higher level of factors

Std	Run	Temperature (Factor A)	Acid concentration (Factor B)	Hydrolyzing time (factor C)	Yield of ethanol (Response)
1	6	100.00	1.00	40.00	18.85
2	1	120.00	1.00	40.00	28.28
3	2	100.00	5.00	40.00	25.72
4	5	120.00	5.00	40.00	21.6
5	17	100.00	1.00	60.00	24.8
6	16	120.00	1.00	60.00	30.6
7	12	100.00	5.00	60.00	25.12
8	7	120.00	5.00	60.00	16.41

Therefore, based on the above 3-D diagram and table 4.6 the maximum yield of ethanol was obtained 30.6 ml/60g. This yield of ethanol was obtained at a higher level of temperature, at a higher level of time when the acid concentration was kept 1%. This

maximum yield of ethanol was obtained at temperature, time and acid concentration of 120^{o}C, 1% and time of 60 minutes respectively. The above discussion was focused on the higher and lower level of the factor but it determines the yield of ethanol at the middle-level value by setting the optimization within the range. In table 4.7 the optimum yield of ethanol was obtained 30.581 ml/60 g and desirability was 0.985. There were 64 desirable or optimum points were obtained. But the selected points were the desirability of 0.985 from those selected was approximately at the temperature of 119^{o}C, the acid concentration was 1% and the time was 60 minutes with the ethanol yield of 30.581 ml/60 g.

Table 4.7 Optimum point and Desirability

64 Solutions found from those the selected was 5 with the desirability of 0.985					
No-	temperature	acid concentration	Time	yield of ethanol	Desirability
1	118.984	1.000	60.000	30.581	0.985
2	119.068	1.000	60.000	30.581	0.985
3	118.899	1.000	60.000	30.581	0.985
4	119.224	1.000	60.000	30.580	0.985
5	118.971	1.000	60.000	30.580	0.985

4.9 Characterization of food waste

4.9.1 Proximate analysis

Proximate analysis was determined for moisture content, ash content, fiber content, fat content and protein content, etc. The results of each were calculated on the basis of the hundred. The results of each analysis have their own formula and laboratory procedures.

4.9.1.1 Moisture content and Volatile content

The moisture content of food waste was obtained 41.5 g using equation 3.1.1. Based on this result food waste contains around 41.5% moisture. The volatile content of the food

waste was determined by using equation 3.1.2 was 30.2%. This shows that food waste contains a high amount of moisture content and volatile content compering with solid content the sum of both accounts 71.7%. Therefore, it needs a long time to dry.

4.9.1.2 Ash content

The ash content indicates that the amount of ash available in a sample. The ash content of the sample was calculated in equation 3.2. The ash content of the sample was performed by using the furnace. The result of ash content was obtained was 2.58%. Hence the food waste contains a small amount of ash. The amount of ash in the sample was high the amount of fermentable sugars obtained was low. But in this analysis the ash content is very low this did not affect the amount of fermentable sugar during hydrolysis.

4.9.1.3 Fiber content

The fiber content is the amount of fiber present in a sample. From equation 3.3 the amount of fiber was obtained 4.5%. This result shows that food waste contains around 5% of fiber. This fiber was not converted to fermentable sugar during hydrolysis. As the amount of fiber was high the yield ethanol was low. The cellulose had contained a high amount of fiber but food waste contains a few amounts of fiber. The amount of fiber was removed from the sample when it was filtered after the hydrolysis process.

4.9.1.4 Fat content

The fat content indicates the amount of fat or oil present in a sample. From equation 3.4 the amount of fat content obtained was 1.2%. Hence the amount of fat content was small in food waste samples. If the fat content was high in food waste it may be converted to other side products during the hydrolysis process. The fat content was high it enhances the production of fatty acid during hydrolysis processes. This also inhibits the total yield of ethanol production and inhibits the growth of yeasts. This 1% of fat was insignificant effects during the hydrolysis process.

4.9.1.5 Protein content

An insignificant amount of protein was present in a food sample. In food waste sample the protein content obtained was insignificant. If the amount of protein content was high it might affect the results during the hydrolysis process and there might be the formation

of another side product. The amount of protein present in food waste was high it causes for the production of ammonium, sulfides this inhibits the formation of ethanol yield.

4.9.1.6 Determine the starch or carbohydrate content of a sample

The amount of starch found in the food waste sample was determined from equation 3.5. The amount of starch found in a sample was depended upon the moisture content, volatile content, ash content, fiber content, fat content, and protein content. The amount of carbohydrate present in a sample was determined by equation 3.5 and the result was 20.02%. Hence the food waste had contained a high amount of moisture content and a high amount of carbohydrate content. The food waste was reaching in starch content. When moisture content and volatile content were removed from food waste the starch content was compared with other components thus its value was high. This high amount of starch content was yield a high total reducing sugar during hydrolysis.

4.9.2 Functional group analysis for a solid sample

FTIR was used to analyze a solid sample bond stretch and to determine the sold sample had contained starch or not. This was analyzed by a bond stretch of each bond found in a sample analyzed by wavenumber. The functional group was determined by a bond stretch. The O-H (hydrogen and oxygen bonds) was vibrated at a wavenumber of 3300cm^{-1}, the −CH2 (one carbon and two hydrogen bond) was stretched at a wavenumber of 2947 cm^{-1}, the carbonyl (C=O) or double bond of oxygen and carbon was stretched at wavenumber of 1740 cm^{-1}, the angular O-H bending of water molecules was stretched at wavenumber of 1690 cm^{-1}, the anti-symmetric of the C-O-C bond (two carbon and one oxygen single bond) was stretched at a wavenumber of 1152 cm^{-1}, the C-O (carbon and oxygen single bond) was stretched at the wavenumber of the 1080 cm^{-1} and Anhydroglucose ring O-C (oxygen and carbon single bond) was stretched at wavenumber of 1010 cm^{-1}. The above bond stretch was found in the pure solid starch sample. Based on the results obtained from the above the food waste sample was contained starch. This shows that the food waste sample reached in starch. Therefore, food waste was used as an alternative source to produce bioethanol. The starch content of this solid sample was having the same peak with the starch content of the pure. All peaks found in figure 4.13

was found in pure starch peak. Therefore, this starch analyzed was similar to the literature reviewed.

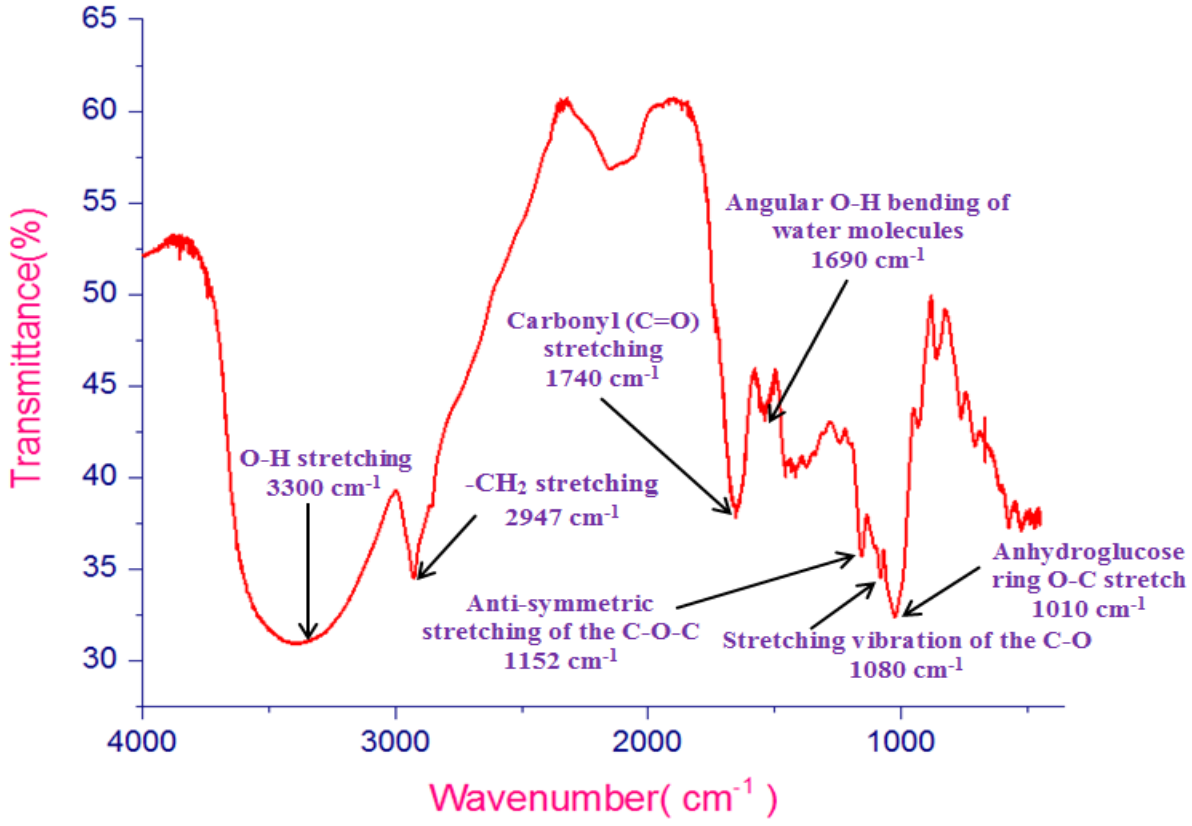


Figure 4.13 Solid sample FTIR analyses

4.10 Functional group analysis for Bioethanol

FTIR used to determine the functional group of solid, liquid and gases. Ethanol was a liquid at room temperature and its functional group was determined by FTIR. The functional group of ethanol was analyzed using FTIR (Fourier-transform infrared spectroscopy). The FTIR determines the vibration of each stretch based on the absorbance of each bond. The vibrations of all bonds in a compound were ranged in FTIR based on its absorbance. The X-axis was wavenumber and Y-axis was the percent of transmittance. The O-H stretch of hydrogen bond was ranged from 3500-3200 cm^{-1}, the C-O stretch 1260-1050 cm^{-1}, the C-H stretch from 3100-3000 cm^{-1} and the C-C bond

stretch around 1100 cm^{-1}. In this study, FTIR analysis for ethanol produced from waste food was determined based on its wavenumber range. The O-H bonds vibration stretch on 3300 cm^{-1}, the C-H bond stretch on 3050 cm^{-1}, the C-O bonds stretch on 1080 $cm^{-1}$and the C-C bond stretch around 1180 cm^{-1}. Based on FTIR analysis ethanol produced from waste food had the same FTIR analysis reading with pure ethanol commercially available. The FTIR analysis of bio-ethanol produced compared with standard ethanol the O-H stretch for pure ethanol was 3391 cm^{-1} and for ethanol produced was 3300 cm^{-1}, for C-H stretch for pure ethanol was 2961 cm^{-1} and for ethanol produced was 3050 cm^{-1}, for C-C stretch for pure ethanol was 1102 cm^{-1} and for ethanol produced was 1180 cm^{-1}, for C-O bonds stretch of pure ethanol was 1055 cm^{-1} and C-O bonds stretch on 1080 cm^{-1} for ethanol produced in this study. This shows ethanol produced in this study was almost similar wavenumber readings with commercially available ethanol of 98%.

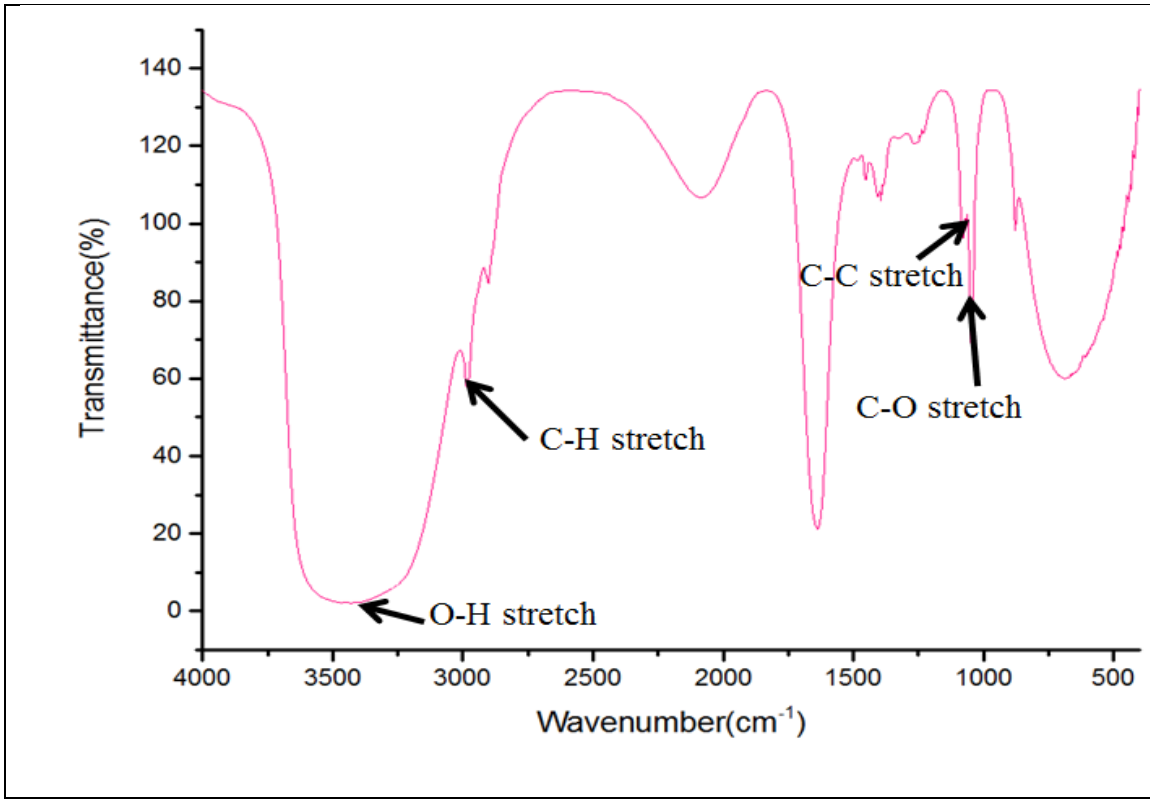


Figure 4.14 FTIR analysis of ethanol produced

4.11Density and purity of ethanol

The density of ethanol was measured by using different equipment like Pycnometer, densitometer, etc. The density of ethanol was measured by using the above equipment was obtained 0.802 g/ cm^3. The density of pure ethanol was 0.789g/cm^3. Hence ethanol produced from food waste was denser than pure ethanol. The difference between densities of ethanol produced from waste food and pure ethanol was 1.5%. This difference was resulted due to the existence of water as a mixture of produced ethanol. The purity of ethanol was measured by using alcoholmeter. The ethanol produced had a purity of 70%. The purity of ethanol depends on the efficiency of distillation. In the first and second steps, the distillation process was carried out by using a heater as a heat source. In the first step distillation process the ethanol obtained was 35% and 65% was other impurities, in the second step the amount ethanol obtained was 40 percent and 60 percent was water. In the third and fourth steps, the water bath was used as a heat source. In this case, the temperature of the water bath was set at 79^o C and the amount of ethanol obtained was 62 percent and in the fourth step the temperature of the water bath was maintained at 78.4 ^{o} C and the ethanol obtained was seventy percent.

4.12 Viscosity, pH, flammability and boiling points of ethanol

Ethanol produced from food waste had the following properties. Ethanol produced was flammable, less in viscose, had a neutral property and have a low boiling point compared with water. Ethanol has a hydroxyl group that makes slightly basic properties. The pH of ethanol was measured by using pH meter. Ethanol produced has a pH of 6.67 which was almost neutral. The pH of 100% pure ethanol has a pH of 7.33. Hence the ethanol produced from waste food has been comparable pH with pure ethanol. This difference resulted due to the existence of a water mixture in produced ethanol and there may be some acids were present that were added during the hydrolysis process. The viscosity of ethanol was measured by using viscometer and ethanol produced from food waste has a viscosity of 1.2cP. This viscosity obtained was almost the same result compering with pure ethanol the pure ethanol has a viscosity around 1.1 this deviation was resulted due to impurities present within ethanol. The ethanol produced has boiling points of 79^oC which had some deviation with pure ethanol. The bioethanol produced having the flammability of 15^oC but the pure ethanol has the flammability ranged between 10 to 12

^{o}C. This deviation was occurred due to impurity or water present within bioethanol. When the amount of water present in bioethanol was increased the flammability of ethanol was decreased and the temperature for the flammability was increased. The pure ethanol has a boiling points78^{o}C this difference was happened due to some amounts of water and impurities within a liquid ethanol.

5. CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

This study shows the direction for the use of food waste as an alternative raw material for bioethanol production. In this research, the yield of ethanol was affected by three different factors those were temperature, acid concentration and hydrolyzing time. These three factors can affect the yield of ethanol negatively or positively, it depends on the combination of each factor and the levels each parameter. Based on the experimental results obtained and model equation, the yield of ethanol affected positively by temperature, time, B^2. The yield of ethanol affected negatively by acid concentration, the interaction effects between temperature and acid concentration, the interaction effects between temperature and time, the interaction effects between acid concentration and time, and A^2. This model equation represents 98.9% accurate, which means a good model equation. The maximum amount of ethanol was obtained 30.6 mL from 60g of the food waste sample. This was obtained at a temperature of 120^oC, acid concentration of 1% and hydrolyzing time of 60 minutes. At this combination of the parameter, the highest desirability and its value were obtained as 0.985. When the temperature was above 120 ^oC, the yield of ethanol was decreased due to the conversion of simple sugar to other side products. Based on FTIR analysis the product obtained shows as ethanol since it contains the functional group that found in ethanol. The FTIR analysis shows that as the products had the C-H, C-C, C-O, and OH bonds based on wavenumber. Ethanol produced had contained O-H stretch at a wavenumber of 3300 cm^{-1}, C-H stretch at a wavenumber of 3050cm^{-1}, C-C stretch at a wavenumber of 1180 cm^{-1} and C-O stretch at a wavenumber of 1080 cm^{-1}. Therefore, the sample solution contains a functional group of ethanol. The ethanol produced from food waste was characterized for density, viscosity, flammability, boiling points, pH and had the corresponding numerical value of 0.802g/cm^3, 1.2 cP, 15 ^oC, 79^oC and 6.67 respectively. Ethanol produced in this experiment was used as solvents for different chemicals, to wash laboratory equipment to prevent contamination and it's used for blended with fuel if it further purified.

5.2 RECOMMENDATIONS

- In this research, food waste was taken in one day only. For further study, it is better to take a sample for one week. If a sample will be taken for one week the variety of food waste might increase and it needs complex proximate analysis may be needed.
- Manual pretreatment was difficult if possible other methods or systems should be designed in order to get a dry sample easily. In this area, it needs another research.
- In this study, three factors were considered those were temperature, acid concentration and time. There is another factor that affects the yield of ethanol during the hydrolysis process. To increase the yield of ethanol more than three factors should be considered to get the maximum yield of ethanol.
- In this research, only the hydrolysis stage was optimized and only five data points were taken but to get good results it should better to optimize fermentation and distillation stages.

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APPENDIX

Yield and density of ethanol

Std	Run	Factor A	Factor B	Factor C	Density of ethanol g/mL=g/cm^3	Yield of ethanol (g)	Yield of ethanol (mL)	Predicted value
1	6	100.00	1.00	40.00	0.801	15.10	18.85	18.85
2	1	120.00	1.00	40.00	0.803	22.71	28.28	28.27
3	2	100.00	5.00	40.00	0.801	20.65	25.72	25.71
4	5	120.00	5.00	40.00	0.804	17.37	21.6	21.37
5	17	100.00	1.00	60.00	0.800	19.84	24.8	24.38
6	16	120.00	1.00	60.00	0.802	24.54	30.6	30.41
7	12	100.00	5.00	60.00	0.800	20.10	25.12	24.93
8	7	120.00	5.00	60.00	0.802	13.16	16.41	16.47
9	4	93.00	3.00	50.00	0.803	14.53	18.1	18.27
10	20	127.00	3.00	50.00	0.803	15.42	19.2	19.32
11	15	110.00	0.50	50.00	0.801	22.44	28.02	28.29
12	14	110.00	6.00	50.00	0.801	19.75	24.66	24.84
13	19	110.00	3.00	33.00	0.790	18.42	23.32	23.53
14	11	110.00	3.00	67.00	0.800	19.68	24.6	24.68
15	8	110.00	3.00	50.00	0.802	19.26	24.01	23.40
16	9	110.00	3.00	50.00	0.803	19.11	23.8	23.40
17	10	110.00	3.00	50.00	0.802	18.29	22.8	23.40
18	13	110.00	3.00	50.00	0.804	18.17	22.6	23.40
19	3	110.00	3.00	50.00	0.802	18.61	23.2	23.40
20	18	110.00	3.00	50.00	0.800	19.36	24.2	23.40

Where;

Factor A-temperature

Factor B- acid concentration

Factor C-time

Percent of conversion to ethanol based on 60g sample prepared

Std	Run	Factor A	Factor B	Factor C	Yield of ethanol (g)
1	6	100.00	1.00	40.00	15.10
2	1	120.00	1.00	40.00	22.71
3	2	100.00	5.00	40.00	20.65
4	5	120.00	5.00	40.00	17.37
5	17	100.00	1.00	60.00	19.84
6	16	120.00	1.00	60.00	24.54
7	12	100.00	5.00	60.00	20.10
8	7	120.00	5.00	60.00	13.16
9	4	93.00	3.00	50.00	14.53
10	20	127.00	3.00	50.00	15.42
11	15	110.00	0.50	50.00	22.44
12	14	110.00	6.00	50.00	19.75
13	19	110.00	3.00	33.00	18.42
14	11	110.00	3.00	67.00	19.68
15	8	110.00	3.00	50.00	19.26
16	9	110.00	3.00	50.00	19.11
17	10	110.00	3.00	50.00	18.29
18	13	110.00	3.00	50.00	18.17
19	3	110.00	3.00	50.00	18.61
20	18	110.00	3.00	50.00	19.36

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The relationship between Concentrations versus Absorbance of a Standard simple sugar

Run	Glucose solution ,g/ml	Absorbance, %
1	0	0
2	0.001	0.007
3	0.002	0.0014
4	0.003	0.047
5	0.004	0.08
6	0.005	0.198
7	0.006	0.316
8	0.007	0.412
9	0.008	0.508
10	0.009	0.6265
11	0.01	0.745
12	0.02	1.661

The absorbance, concentration of total reducing sugars in the solution and yield of ethanol obtained during the experiments

Std	Run	Factor A	Factor B	Factor C	Abs. of glucose (%)	Total reducing sugar (g/ml)	(Response)
1	6	100.00	1.00	40.00	0.882	0.0175	18.85
2	1	120.00	1.00	40.00	1.641	0.0491	28.28
3	2	100.00	5.00	40.00	1.423	0.0394	25.72
4	5	120.00	5.00	40.00	1.128	0.0305	21.6
5	17	100.00	1.00	60.00	1.323	0.0350	24.8
6	16	120.00	1.00	60.00	1.962	0.0578	30.6
7	12	100.00	5.00	60.00	1.423	0.0394	25.12
8	7	120.00	5.00	60.00	0.772	0.0248	16.41
9	4	93.00	3.00	50.00	0.872	0.0269	18.1
10	20	127.00	3.00	50.00	0.892	0.0273	19.2
11	15	110.00	0.50	50.00	1.641	0.0491	28.02
12	14	110.00	6.00	50.00	1.323	0.0350	24.66
13	19	110.00	3.00	33.00	1.282	0.0321	23.32
14	11	110.00	3.00	67.00	1.323	0.0350	24.6
15	8	110.00	3.00	50.00	1.301	0.0342	24.01
16	9	110.00	3.00	50.00	1.290	0.0324	23.8
17	10	110.00	3.00	50.00	1.168	0.0310	22.8
18	13	110.00	3.00	50.00	1.168	0.0310	22.6
19	3	110.00	3.00	50.00	1.276	0.0319	23.2
20	18	110.00	3.00	50.00	1.309	0.0321	24.2

The concentration of the glucose can be calculated by using absorbance

$$C = \left[\frac{Abs - b}{m} \right]$$

$$C = \left[\frac{0.882 - (-0.1714)}{60} \right]$$

$$= \frac{1.0534}{60}$$

$$= 0.0175$$

By following these steps do for all data

The yield of reducing sugar can be calculated by using the formula below

$$\%Y = \left(\frac{C \times V}{M} \right) \times 100$$

$$= \left(\frac{0.0175g / ml \times 500ml}{60g} \right) \times 100$$

$$= \left(\frac{8.75g}{60g} \right) \times 100\%$$

$$= 14.6\%$$

The maximum ethanol yield obtained was

$$\%Y = \left(\frac{C \times V}{M} \right) \times 100$$

$$= \left(\frac{0.0600g / ml \times 500ml}{60g} \right) \times 100$$

$$= \left(\frac{30g}{60g} \right) \times 100\%$$

$$= 50\%$$

To obtain the maximum amount of total reducing sugar was 30 g from 60g of food waste sample.

$$\left(\frac{60g \times 50\%}{100\%}\right) = 30g$$

Then to determine the total ethanol obtained from the total reducing sugar was

$$\left(\frac{24.54\text{g}}{30g}\right) \times 100\%$$

$$81.8\% \approx 82\%$$

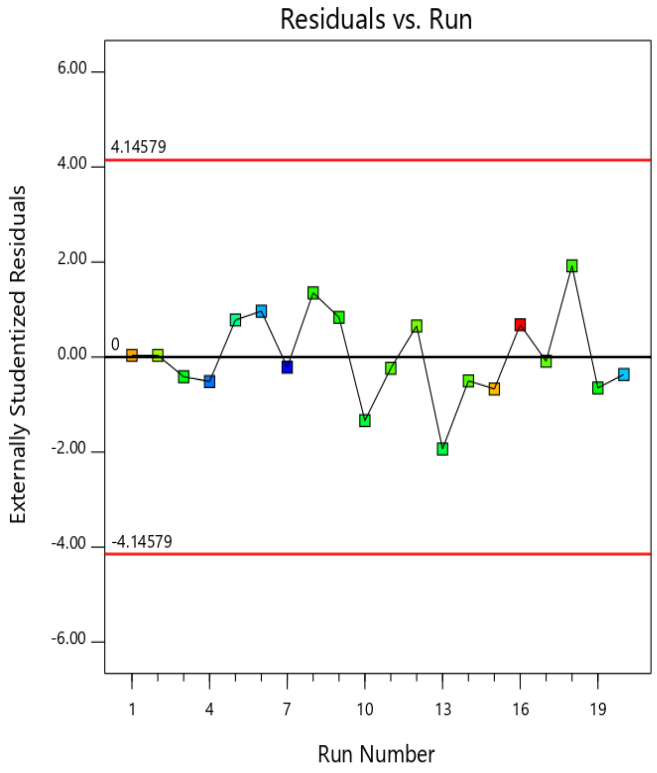
64 Solutions found

Number	temperature	acid concentration	Time	yield of ethanol	Desirability
1	118.984	1.000	60.000	30.581	0.985
2	119.068	1.000	60.000	30.581	0.985
3	118.899	1.000	60.000	30.581	0.985
4	119.224	1.000	60.000	30.580	0.985
5	118.971	1.000	60.000	30.580	0.985
6	119.512	1.000	60.000	30.577	0.984
7	118.458	1.000	60.000	30.576	0.984
8	119.656	1.000	60.000	30.574	0.984
9	119.860	1.000	59.993	30.568	0.984
10	118.968	1.000	59.918	30.567	0.984
11	119.958	1.000	60.000	30.566	0.984
12	117.913	1.000	60.000	30.562	0.983
13	118.017	1.007	60.000	30.529	0.981
14	117.025	1.000	60.000	30.519	0.980
15	119.998	1.000	59.383	30.465	0.977

Design-Expert® Software

Yield of Ethanol

Color points by value of
Yield of Ethanol:
16.41 30.6

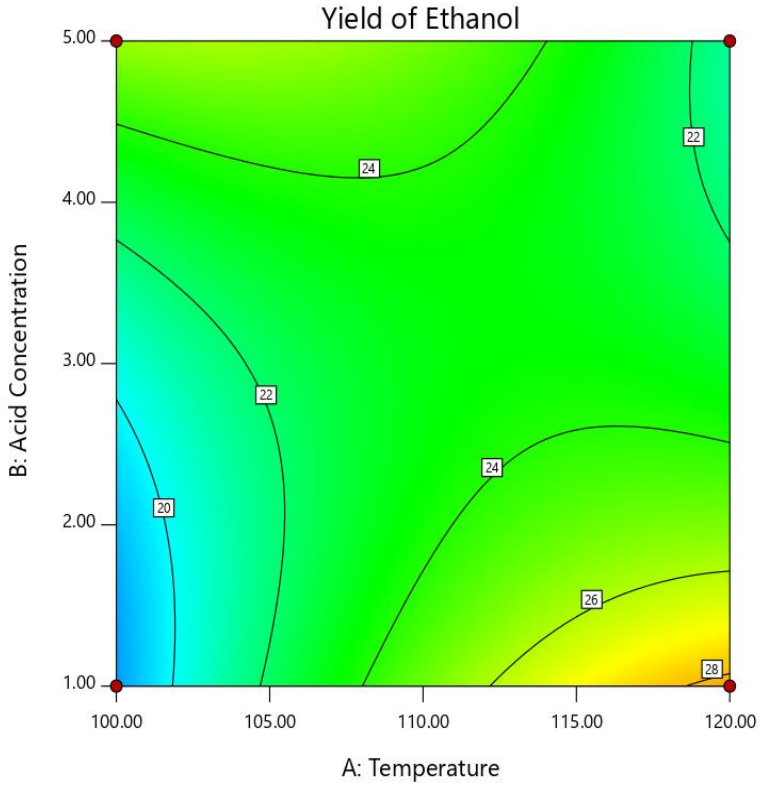


Design-Expert® Software
Factor Coding: Actual

Yield of Ethanol
Design Points
16.41 30.6

X1 = A: Temperature
X2 = B: Acid Concentration

Actual Factor
C: Hydrolyzing Time = 40.00

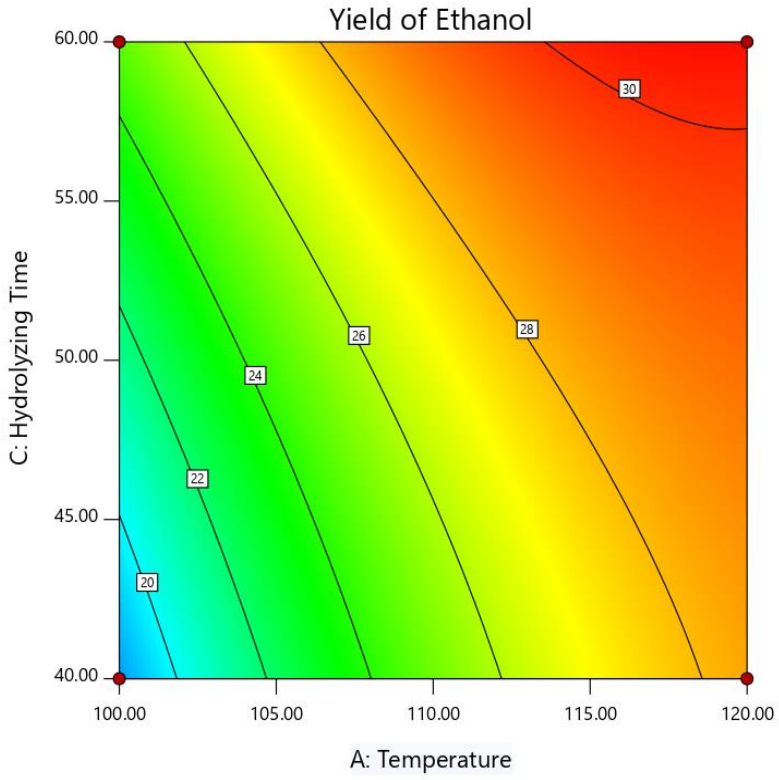


Design-Expert® Software
Factor Coding: Actual

Yield of Ethanol
Design Points
16.41 30.6

X1 = A: Temperature
X2 = C: Hydrolyzing Time

Actual Factor
B: Acid Concentration = 1.00

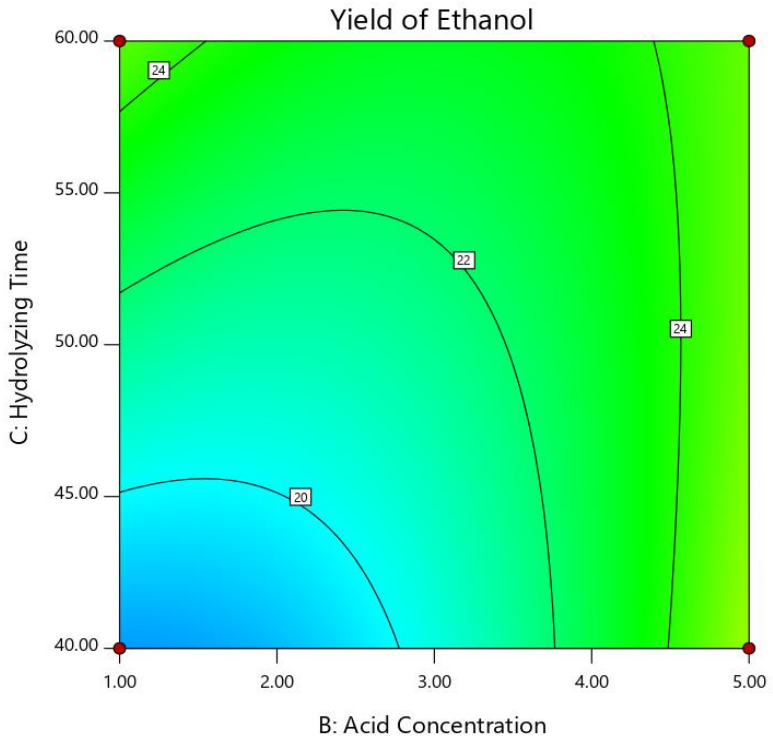


Design-Expert® Software
Factor Coding: Actual

Yield of Ethanol
Design Points
16.41 30.6

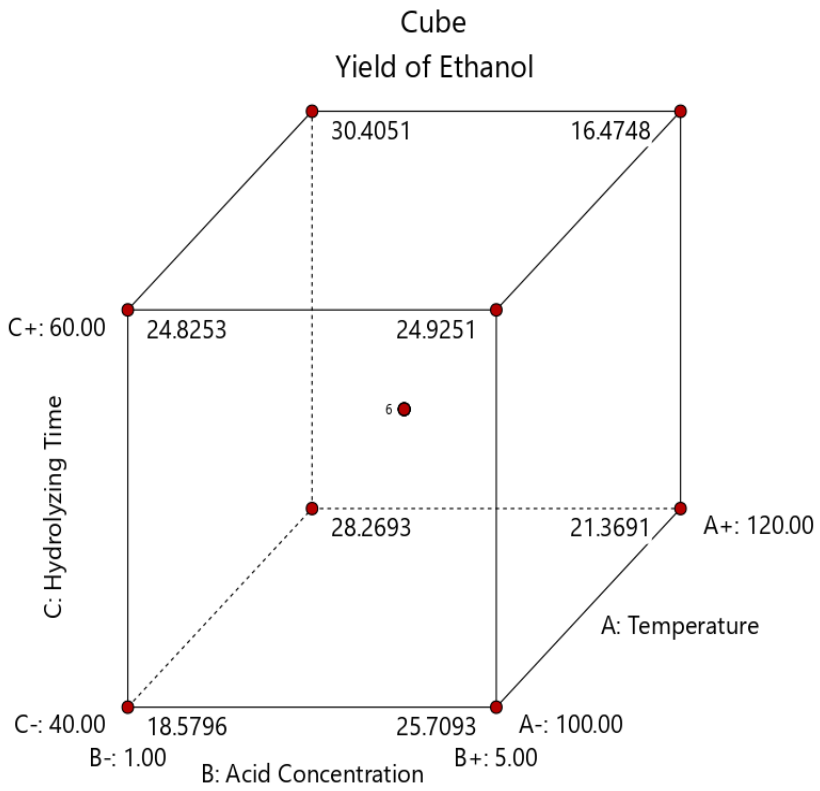
X1 = B: Acid Concentration
X2 = C: Hydrolyzing Time

Actual Factor
A: Temperature = 100.00



Design-Expert® Software
Factor Coding: Actual

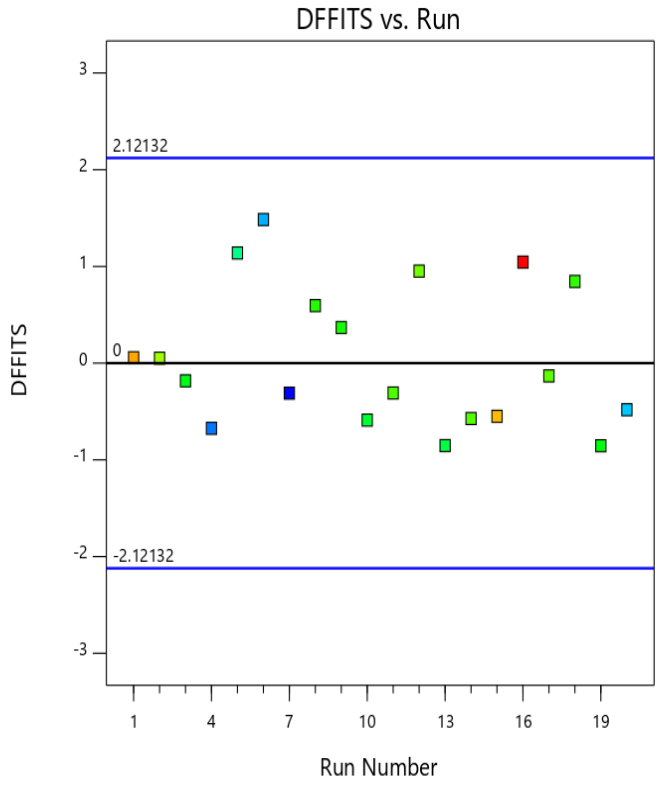
Yield of Ethanol
X1 = B: Acid Concentration
X2 = C: Hydrolyzing Time
X3 = A: Temperature



Design-Expert® Software

Yield of Ethanol

Color points by value of
Yield of Ethanol:
16.41 30.6







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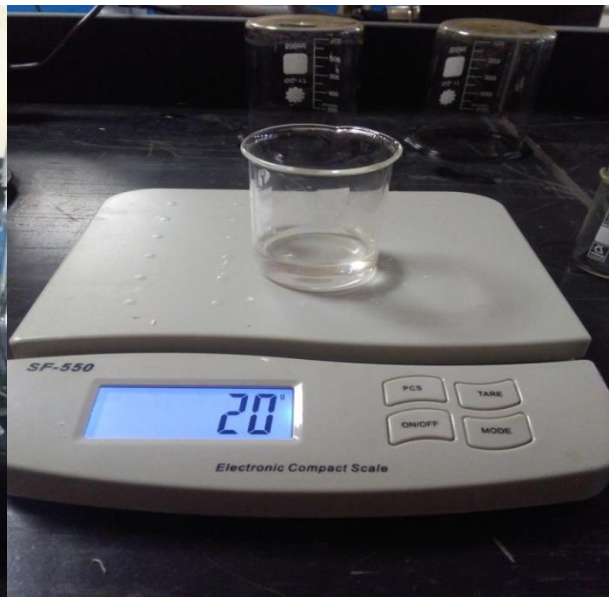
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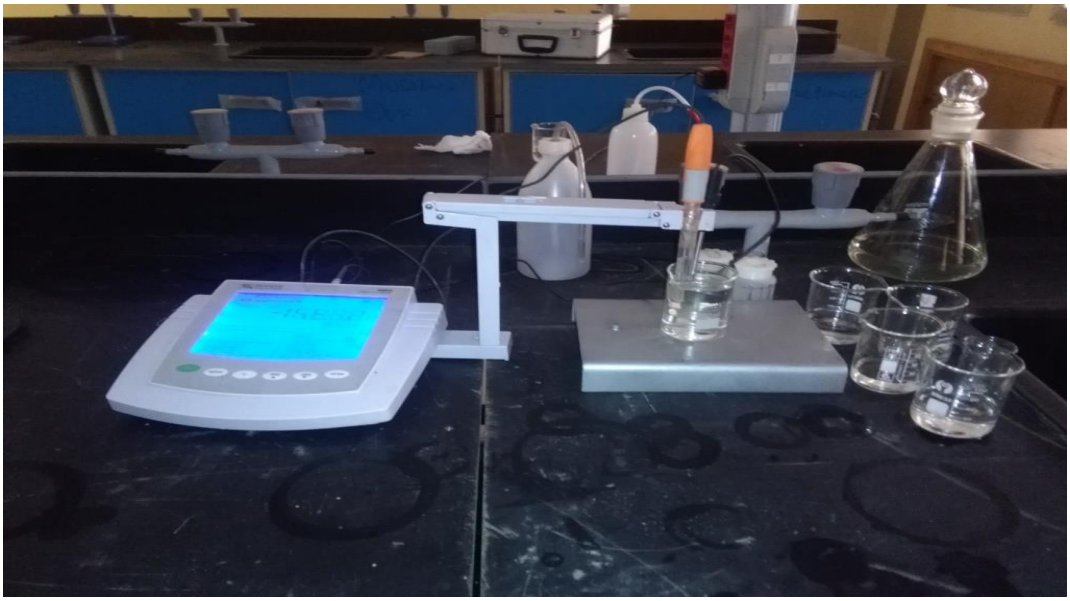


CBR Tester