ADDIS ABABA UNIVERSITY

ADDIS ABABA INSTITUTE OF TECHNOLOGY (AAiT)

SCHOOL OF CHEMICAL AND BIO-ENGINEERING

VALORIZATION OF COFFEE HUSK TO BIO-ETHANOL USING SHF METHOD

BY:

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SUPERVISED BY: PROF. DR. ING. BELAY WOLDEYES

JULY, 2018

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A THESIS IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CHEMICAL ENGINEERING UNDER PROCESS ENGINEERING STREAM.

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ADDIS ABABA INSTITUTE OF TECHNOLOGY (AAiT)

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JULY, 2018

DECLARATION

I, the under signed, declare that the thesis covers my own work. In agreement with internationally accepted practices, I have dually acknowledged and refereed all materials used in this work.

Gebreegziabher Tadesse

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ABSTRACT

Coffee husk considers as one of the agricultural waste lignocellulosic biomass that contain high amount of cellulose. Furthermore, the aim of this work was, valorization of coffee husks to bioethanol by using separate hydrolysis and fermentation methods which is critically used as replacements for renewable sources of energy and to minimize environmental pollutions too. This method was accomplished by four major experimental procedures, which were pretreatment, hydrolysis, fermentation and distillation processes. There were three parameters and 20 lab experiments exposed in the laboratory work depending on the H_2SO_4 concentration, hydrolysis temperature and time in the hydrolysis step. The effect of these parameters was analyzed using design expert soft war by CCD design. The results were show that the content of sugars increased as the acid (H_2SO_4) concentration increased from 1% - 3 % and decreased as the acid concentrations increased from 3 % up to 5%. At the optimal acid concentration (3%; H_2SO_4), hydrolysis temperature (130 ℃) and hydrolysis time (70min), the sugar yield gotten was 46.75% from the coffee husk. This sample was further investigated to produce bioethanol and the ethanol yield from this experiment was 51.03%. Based on central composite design ANOVA was carried out to determine the numerical significance of the quadratic response surface model, in which the p-value of the model was less than 0.0001, which shows the model was statistically significant. Consequently, based on this result, the designated model was adequate to fit the data of response variable. Characterization of the bio-ethanol produced was accomplished by FTIR analyzer.

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LIST OF ACRONOYMS

AFEX	Ammonia Fiber Explosion/Expansion
ANOVA	Analysis of Variance
BD	Bulk Density
CH	Coffee Husk
CCD	Central Composite Design
CO_2	Carbon dioxide
db	dry base
FC	Fixed Carbon Content
FTIR	Fourier Transform Infrared spectroscopy
GHG	Greenhouse Gas
LCW	Lignocellulose Waste
MC	Moisture Content
RSM	Response Surface Methodology
SHF	Separate Hydrolysis and Fermentation
SSF	Simultaneous Sacharifcation and Fermentation
SDA	Sugar Development Agency
VM	Volatile Matter content
wb	weight base

1. INTRODUCTION

1.1. Background

Bioethanol as energy has been used throughout man's long history. Ethanol was one of the most common lamp illuminants used in 1850s and approximately 90 million gallons ethanol was produced in the United States (Matsakas et al.,2014). But due to the tax burden on ethanol to assist in financing the civil war and the cheaper price of kerosene, it quickly replaced ethanol as the premier illuminant in 1861. Then in 1906, the alcohol tax was lifted, which transformed the interest in ethanol and in 1908, Henry Ford designed the automobile car „Model T" to run on ethanol. By 1914, the production of ethanol had recovered slightly and reached 10 million gallons (Ocean et al., 2002). But in 1919, due to the development of petroleum as fuel, the use of ethanol as fuel decreased again (Mushimiyimana & Tallapragada, 2016). This veto was ended in 1933 and by the early 1940s the production of ethanol rebound again when it was used during World War II for fuel and to make synthetic rubber. During this period, about 600 million gallons of ethanol was produced annually in the U.S (Tesfaw & Assefa, 2014). At the end of World War II, demand for ethanol diminished and continued to decline for the next two decades, mostly due to inexpensive petroleum imports (Lin et al., 2014). Currently first-generation bioethanol production processes utilize more simply degradable biomass feed stocks such as cereals (corn or grain). Conversely, the utilization of these agricultural crops absolutely for energy production is heavily conflicting with food and feed production (Sarkar et al., 2012). Second generation bioethanol can be produced from lignocellulosic biomass. These wastes (LCW) states that plant biomass wastes that are composed of cellulose, hemicellulose and lignin. These are assembled into different categories such as wood residues, grasses, waste paper, agricultural residues (including straw, coffee husk, peelings, cobs, stalks, nutshells, nonfood seeds), food industry residues and municipal solid wastes (Sarkar et al., 2012). At present, the second-generation bio-products such as bioethanol, biodiesel and methane from lignocellulose biomass are progressively been produced from wastes rather than from energy crops because the latter plays for land and water with food crops that are already in high demand.

Ethiopia is one of the coffee producer countries in the world. Jimma zone produces about 70% of coffee in Ethiopia. Coffee generates large amount of coffee by-products/residues during processing which are considered as the major solid wastes (Sime et al., 2017). For every 2kg of coffee beans produced, approximately 1 kg of husks are generated. In Ethiopia 192000

metric tons of coffee husk are generated per year as by product (Sime et al., 2017). This solid residue (coffee husks) uses as a supplement for animal feed, direct use as fuel and as fermentation for the different production of a diversity of products (enzymes, citric acid and flavoring substances) and soon. However, the amounts of coffee husk generated is high, there is still a need to find other alternative uses for this solid residue. Valorization of this coffee husk to some valuable products such as bio-ethanol can be used for environmental pollution prevention as well as for alternative source of energy. So, valorization of this waste indicates that excellent potential of residue utilization for valuable product production that it does not involve costs related to raw material growth. The development of fuels (bioethanol) from these coffee husk has many advantages in terms of energy and environmental issues. This bioethanol fuel has received increased attention in recent years due to its in reducing of greenhouse gas emissions and for decreasing global reliance on petroleum products (Navya et al., 2012). The process of converting these biomass residues like coffee husk to bioethanol generally involves four major aspects: effective pretreatment, hydrolyzing of cellulose, fermentation of reducing sugars (glucose), downstream processing or distillation (Kumar et al., 2016).The bioethanol produced through fermentation of sugars (in this case from sugar containing organic residues such as coffee husk) is a renewable energy source than fossil fuels. Thus the increase of the energy production (mainly from non–renewable energy sources) increases petroleum price, and environmental impacts caused by fossil fuels (Sarkar et al., 2012). This is due to the limitation of oil reserves, fluctuation of oil price, the increased concern about the global warming and climate change caused by the increment of the greenhouse gas emissions, and the awareness to promote rural economics (Sahu, 2014). Bioethanol, in spite of its lower heating value than gasoline, it has become as one of the most important renewable fuels in the worldwide markets, due to its economic and environmental benefits (Ohimain et al., 2012). Bioethanol is widely used as a biofuel due to the following reasons:

(1) bioethanol has high oxygen content and octane number; (2) bioethanol is non-poisonous; and (3) bioethanol is environmentally approachable since it decreases pollutant emissions such as carbon monoxide, Sulphur and nitrogen oxides (Methodology, 2014). Saccharomyces cerevisiae has been the most usually used microorganism for the bioethanol production by the fermentation of different feedstock rich in sugars (Sahu, 2014). From the economic view point, coffee husk is an agricultural waste which can be used as a raw material for the production of ethanol due to its high sugar content. So, this bioethanol is developing as an important biofuel

for the transportation sector as replacement for petroleum fuels, a way of preventive the greenhouse gas emissions (Cutzu & Bardi, 2017).

A number of factors like hydrolysis and fermentation temperature, hydrolysis and fermentation time, and acid concentration in the hydrolysis step affect the valorization of coffee husk to bioethanol. Based on some literatures, the recommended value for optimum operating conditions this research is: hydrolysis temperature 90-200℃, sulfuric acid concentration 0.5–8%, and reaction time 15-2000 min. So, for this experimental work the dilute acid hydrolysis procedure was started by adding of 1%, 3%, and 5% (v/v) diluted sulfuric to the solid component from pretreatment steps and the coffee husks were hydrolyzing in the autoclave at three levels of temperature (120, 130, and 140℃), time of (30, 50, and 70min). But in response surface CCD (central composite design) understands to five levels of experiments with two center points for each. In this work, coffee husk residue was used as the feedstock to produce bioethanol. In addition to this, optimization of acidic hydrolysis is performed, with expectation of yielding the optimum quality of the produced reducing sugar. Response surface methodology based on the Central Composite experimental design(CCD) was then used to analysis and to optimize the experimental operating parameters of the hydrolysis processes. Quadratic regression models were developed in this study and used to predict the reducing sugar yield from hydrolysis. The bioethanol produced is the characterized and measured by FTIR analyzer. The objective of this work is to valorize coffee husks to valuable product bioethanol which can use as critically alternative renewable sources of energy for transportation sector instead of petroleum fuels and as pollution protection sector.

1.2. Statement of The Problem

Coffee produces large amount of coffee by-products/coffee husk during dry methods and based on the information studied in Ethiopia indicates more than 192,000 tons of coffee husk was releasing in to the environment since 2014/15. Most of these husks were disposed to the environment and this represents a serious environmental problem mainly due to the high content of tannins and phenolic compounds in the coffee husk. Some people burn the generated coffee husk, some also used as animal feed while others disposes it on the field. it is great important that converting to value added product such as bioethanol instead of disposing to rivers or simply burning of these coffee husks. In other case, in earlier time, the world economy has been controlled by technologies that depend only on fossil energy, such as petroleum, coal, or natural gas to produce fuels, chemicals, materials and power. Since these fossil fuels are not renewable sources of energy this can cause energy limitation as well as environmental pollution by increasing concentration of CO_2 in the atmosphere and concerns over global warming. So, an alternative renewable energy source is required to solve such problem. One of the most effective, feasible way and non-food competitive feedstock raw material that is needed for the production of alternative renewable source of energy can be valorization of coffee husk residues to bio ethanol. Accordingly, valorization of this coffee husk to valuable product (bioethanol) can not only solve environmental pollution but also as renewable source of energy to replace the use of fossil fuel which not renewable sources. Consequently, coffee processing by-products coffee husk presents as interesting characteristics for energy and environmental applications by valorizing it to bioethanol. The main objective of this work is to valorize these coffee husks to valuable product bioethanol using separate hydrolysis and fermentation methods in laboratory scale. As this work will scale up to large company it is critically importance to solve many problems like shortage of renewable sources of energy and in minimizing environmental pollution around the coffee growing areas.

1.3. Objectives

1.3.1. General objectives

The general objective of this thesis was: Valorization of coffee husk to bioethanol using separate hydrolysis and fermentation (SHF) methods.

1.3.2. Specific objectives

(1) Characterization of raw material (coffee husk).

(2) Characterization of the reducing sugars(glucose) produced from coffee husk using benedicts solution.

(3) Optimization of the parameters for maximum yield using numerical optimization.

(4) Characterization of the result (bio-ethanol) produced from coffee husks by using FTIR method.

1.4. Significance of The Study

Due to wide and continuing over-utilization, fossil fuels are quickly being depleting (Matsakas et al., 2014). If consumption goes in this rate the fossil fuel reserve will be depleted and continuous burning of the fossil fuels increases release of greenhouse gasses to the atmosphere and causes global warming by increasing the amount of CO_2 (Matsakas et al., 2014). Reducing the use of fossil fuels would considerably reduce the amount of CO_2 produced, as well as reduce the intensities of pollutants (Matsakas et al., 2014). As concern about global warming and dependence on fossil fuels grows, the pursuit for renewable energy from non-food competitive feedstock raw material is desirable for the production of alternative fuel energy sources such as bioethanol that extremely reduce CO_2 emissions which becomes a matter of widespread consideration. The results of this study will give awareness to produce bioethanol highly contributes in the additional fossil fuel and it has also great significance in terms of promising the environmental pollution protection by valorizing of coffee husk residues to a renewable form of energy source such as bio-ethanol. This work is economically feasible way in which the raw material can be locally existing, abundant and no economic value. Since ethanol is one of the best tool in which we have to fight air pollution from vehicles, valorization of coffee husk to bioethanol is very important and statically feasible project. And there is no fuel available at balance today that matches ethanol's ability to improve overall environmental quality compared to fossil fuels such as gasoline.

1.5. Scope of the Study

Coffee husk consisted of known amount of cellulose (53%), hemicellulose (10%) and lignin (11%) (Ballesteros et al., 2014). This high amount of cellulose in the coffee husk can uses for different product productions. Consequently, in this study coffee husk was used for bioethanol production by destroying the hemicelluloses and lignin content in pretreatment and hydrolysis steps. The pretreatment is needed to release the hemi-cellulose and lignin from the cellulose and at the same time to reduce cellulose crystallinity and to increase cellulose porosity. Hence, the present study was introduced in determining the acidic pretreatment techniques in changing the physical structure of coffee husk in order to increase the cellulose digestibility. The pretreated coffee husk was hydrolyzed using dilute acid hydrolysis to produce glucose. Following hydrolysis, the identification of the reducing sugar of the hydrolyzed coffee husk was conducted. Operating parameters which may affect the hydrolysis of cellulose (produced from coffee husk) into glucose such as acid concentration, hydrolysis temperature and time were analyzed. Response Surface Methodology (RSM) based on Central Composite Design (CCD) was applied to determine the best combination of the affecting parameters in the hydrolysis step to study the effect of the parameters and these parameters were optimize using numerical. The experiment with maximum yield of glucose was fermented to produce ethanol and this produced ethanol was separated using rotary distillation. The main scope of this study was to valorize coffee husk residues to bioethanol using separate hydrolysis and fermentation method. The produced bioethanol from fermentation broth was concentrated by rotary distillation approach and further analyzed by FTIR.

2. LITERATURE REVIEW

2.1. Over View

Coffee is one of the most popular and important beverages in the world. It grows commercially in rapid way over the last years. In general, around 25 million small producers rely on coffee for their living (Sarkar et al., 2012). Ethiopia is one of the coffee producer countries in the world. Jimma zone produces about 70% of coffee in Ethiopia. Coffee generates large amount of coffee by-products/residues during processing which are considered as the major solid wastes (Sime et al., 2017). For every 2kg of coffee beans produced, approximately 1 kg of husks are generated. In Ethiopia 192000 metric tons of coffee husk are generated per year as by product (Sime et al., 2017). There has been some significant investigation of coffee husk as a substrate for ethanol production however it's not clearly done by the method of separate hydrolysis and fermentation method and therefore its potential can be estimated from other often-used substrates like sugarcane bagasse. It is estimated that in the later year sugarcane bagasse in Ethiopia had the leading potential for ethanol production. Since Ethiopia being a leading producer of coffee it's also possible to utilize this coffee husk to bioethanol in a very high amount instead of sugar cane for another application.

2.2. Current Over view of Bioethanol production

2.2.1. Worldwide current status of bio-ethanol production

Current study indicates that; the most dominated bio-fuel is bio ethanol. Its worldwide production shows an upward trend with a higher increase over the last 20 years (Navya et al., 2012). Since 2005, global production ability for bio-ethanol fuel was showed around 45 billion liters per year. This shows approximately 15% annual growth between the years of 2000 and 2005 (Tesfaw & Assefa, 2014). After one year in 2006 this value was enlarged to 49 billion liters. Brazilian and Americans was produce 75% of the total world ethanol productivity with 40% Brazilian and 35% American, followed by Asia/Pacific and Europe/Africa with respective values of 15 and 10%(Production, & Peel, 2012). Based on the current status fuel ethanol production will be forecast to have the strongest increase in the Brazilian and Americans, where the production rate is estimated to rise to around 75 and 80 billion liters by 2020 (Journal, & Qureshi, 2014). This indicates around 45 billion liters increase in the estimation period than the current production rate. In Asia it was increase to 10 billion liters throughout the same period, and in Europe, there is a strategy of bio-fuels share in the transportation sector, and the

production will rise highly. Therefore, the total world ethanol production in 2020 is forecast to reach over 120 billion liters (Sarkar et al., 2012). Commonly the

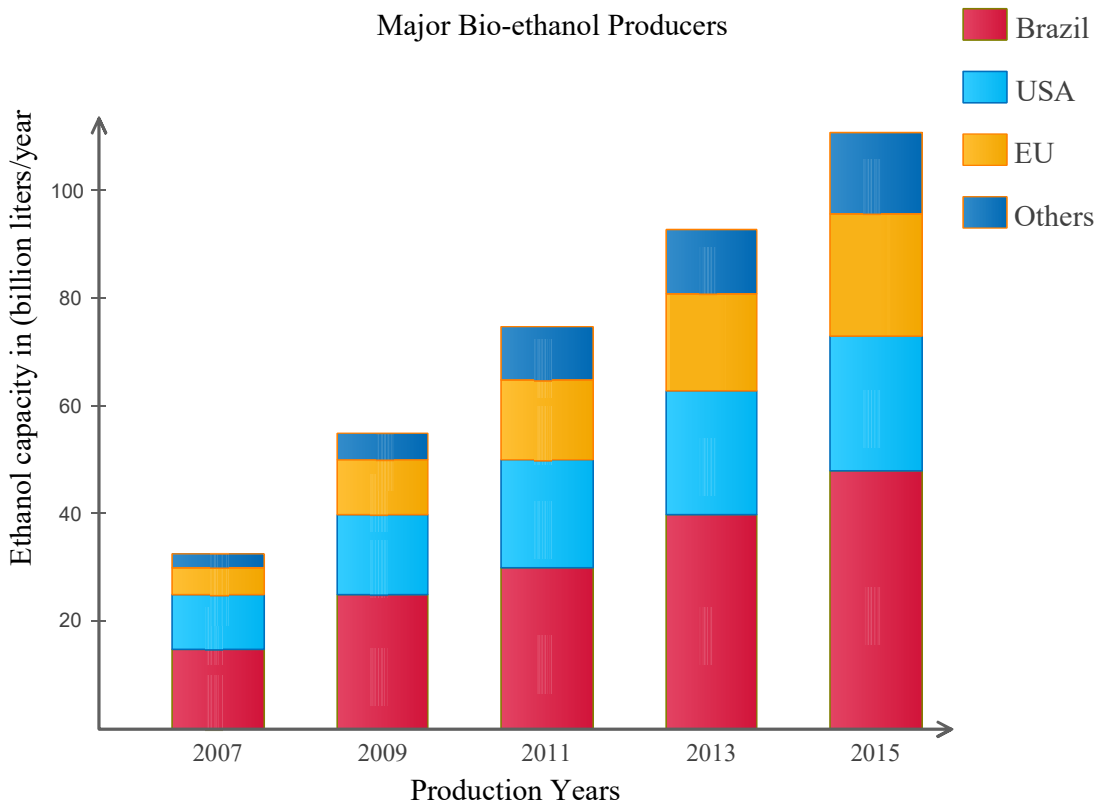

Figure 2. 1: Annual bioethanol production by main producers

After some years in 2010, production of fuel ethanol touched as 76 billion liters per year, with an increase of 10 percent over 2009. The United States and Brazil accounted for 88 percent of over-all ethanol production in 2010 (Sarkar et al., 2012). The increasing of sugar prices combined with adverse weather conditions in a major producing region, occasioned in a reduction of Brazil's ethanol production rate from 27.1 billion liters to 26.3 billion liters from in the years of 2009 up to 2010 (Journal et al., 2014). This is because of majority the ethanol produced in Brazil is from sugar cane. In recent years, significant global trade in fuel ethanol has appeared, with Brazil being the leading exporter of that ethanol. However, Brazilian ethanol export degenerated by almost 31 percent in 2010 due to the increment of sugar can price, as a result the international demands for bioethanol declined in great part (Gasmalla, & Man, 2012).

2.2.2. Current Bio-ethanol in Ethiopia

One of the methods of producing bio-ethanol is microbial transformation of lignocellulosic material through fermentation process (Mushimiyimana & Tallapragada, 2016). This production process can be consists conversion of biomass to fermentable sugars, fermentation of sugar to ethanol and the processes of the separation and refining of the ethanol (Tesfaw & Assefa, 2014). In the international current mindfulness for the use of bioethanol to replace petroleum and generation of power along with sugar mill and other plants can have led to setting up of number of ethanol plants and co-generations (Sime et al., 2017). In Ethiopia there are different sugar industries like Fincha, Metehara and Wonji which controlled and managed by Sugar Development Agency (SDA). From this molasses can be produced as by-product in which ethanol can takes in largest part, but its consumption must attract the attention of the government policy makers in order to utilize as a bioethanol. Bioethanol or biofuel is ethanol based products that can route into liquid fuels for transport purposes (Teferra, 1986).

2.3. Feedstock for Bio-Ethanol Production

Bioethanol can be produced from any biological feedstock that have high amount of sugar or materials that can be renewed in to sugar such as starch and cellulose. Many different feedstock for production of bioethanol can be divided in to sugary, starchy and cellulosic feed stocks that may additional convert in to simple sugars that are prepared for fermentation (Izmirlioglu & Demirci, 2012). Corn, wheat, barley and other cereals are usual feed stock containing starch types in their kernels. Starch are relatively informal to convert into sugar and then by fermentation into ethanol (Maarel, 2007). There are also other feed stocks that can be used for production of bioethanol from biomass which comprises large amount of cellulose and hemi celluloses such as: agricultural wastes, forest residues, municipal solid wastes, crop residues and other lignocellulosic materials that can be converted to simple sugars. This kind of conversion is to a certain degree difficult than conversion takes place in starch (Rabelo et al., 2011). Lignocellulose material is the main component of the plant cell walls and is mainly composed of cellulosic material (40–60% of the total dry weight), hemicellulose material (10–20%), and lignin (10–25%). Since this lignocellulose biomass consists these compositions it can convert to bioethanol by some consecutive procedures (Chiaramonti et al., 2012). First the lignocellulose biomass is pretreated by steam or chemical and hydrolyzed by dilute acid or enzyme. Then the released sugars can be fermented using yeast as catalyst and separation will be applied for further purification of the bioethanol (Chiaramonti et al., 2012).

2.4. Compositions and Properties of Coffee Husk

Coffee husks are lignocellulosic biomass which are composed by the coffee berry outer skin, the pulp and the parchment, largely resulting from the coffee dry processing. Coffee husks are rich in total carbohydrates (62%), proteins (5.2%), fibers (15.8%) and minerals (5.7%)("Coffee husks as biofuel," 2007). Generally, the chemical composition of coffee husk is summarized in table 2.1 below:

Table 2.1 chemical composition of coffee husks

Table: The composition of coffee husk (% dry matter) according to (Franca and Oliveira, 2009)	
Total carbohydrate	58-85
Cellulose	53
Hemicellulose	10
Lignin	11
Protein	8-11
Minerals	3-7
Lipids	0.5-3

Coffee husks are agricultural wastes which are abundant, renewable and cost less energy sources in coffee growing counties (Ethiopia, USA, Vietnam, Brazils, and Uganda). These wastes are gathered every year in large quantities, causing environmental as well health problems (Sime et al., 2017). However, due to their chemical composition based on sugars and other compounds of interest, it could be developed for the production of a number of value added products, such as ethanol, food additives, organic acids, enzymes, and others. Therefore, in addition the environmental problems caused by their accumulation or removal in the environment, the non-use of these materials creates a loss of potentially valuable resources (Öztürk et al., 2015). Some important properties relating to the current conversion of coffee husk biomass to important fuels. Some of them were listed below:

Bulk density (BD): Bulk density states that the weight of the material per unit volume of the container when biomass particles are put into a container. It is an important physical property of a fuel which influences the treatment and flow characteristics as well as energy density of the fuel. For biomass, bulk density is usually expressed on an oven dry weight (MC=0%) basis

or as-is basis with corresponding symptom of moisture content (MC) (Engineering, 2015). A study on agricultural deposits of Uganda shows that coffee husk has the highest bulk density (225-275 kg/m3) compared to maize cobs, rice husk, ground nut shells and bagasse (Engineering, 2015).

Moisture content (MC): The MC of biomass is the amount of water in the material expressed as a percentage of the material's weight. It may be stated as a percentage of total wet mass (wet basis), percentage of dry mass (dry basis) and percentage of dry-and-ash free mass (dry-and-ash free basis) of the material. The basis on which the MC is measured must always be identified because it affects the value of biomass as fuel (Production of Bioethanol, 2014). MC considerably affects the net heating value of the fuel. The higher the MC the lower the heating value of the fuel because some of the energy is consumed in boiling the water. In energy applications, this water leaves the ignition products as steam and latent heat is not recovered (Engineering, 2015). A study on agricultural residue in Uganda displays that coffee husk has a MC of 14.073% (wb) (Engineering, 2015). A study in Brazil also shows that coffee husk has a MC of 13.1% (db). On the other hand, a study in Ethiopia shows that coffee husk (dry process) has a MC 12% (wb); whereas coffee husk (wet process) has lower MC (10% wb) (Production of Bioethanol, 2014).

Volatile matter content (VM): Volatile matter in biomass fuel constitutes all the liquid and tarry residues and low molecular weight gases which start to discharge after drying, leaving solid char in thermochemical processes. It considerably affects the fuel heating value as it is a combustible portion of the fuel. High volatile matter indicates high heating value. Biomass typically has VM content up to 80% however coal has low VM (<20%) or in the case of anthracite coal, a negligible one. A study in Brazil showed that coffee husk has VM of 73.2% (db) (Abraha, 2011). Although, a study in Tanzania indicated a considerably higher value of 83% (db). Another study in Uganda indicated still higher value of 89. 57% (db). A study in Ethiopia specified that coffee husk has VM of (65-72%. This suggests that coffee husk is easy to explode and suitable for large scale thermo-chemical conversion (Production of Bioethanol, 2014).

Fixed carbon content (FC): Biomass may be approximately divided into two parts, combustible and non-combustible. The combustible part constitutes of VM and FC, while the non-combustible portion constitutes moisture and ash. The heating value of a fuel is generally, the demonstration of carbon and hydrogen content of a fuel. Thus, FC is the most significant

constituent of a fuel. FC content is usually estimated as a "difference". That is to say, all the other constituents are subtracted from 100% and the remainder is assumed to be the FC content. A study in Tanzania demonstrations that coffee husk has a FC content of 14.3%. Conversely, a study in Brazil specified a considerably higher value of 23.1% (Production of Bioethanol, 2014).

Ash content (Ash): Ash refers to the inorganic component or mineral matter which remains as a deposit after complete combustion of the fuel. The total ash content as well as the chemical composition of the ash are vital in thermo-chemical conversion. Ash, being incombustible component, tends to poorer the heating value of a fuel. Moreover, this, low melting temperature ash (with high alkali metal content) can cause various problems including slag and blockage due to sintering and accumulation in high temperature operations. Normally, coffee husk has low ash content but the ash has low melting temperature which is attributed to the high contents of K_2O (36-38%). An ash content as low as 2% (db) described from a study in Tanzania, and a value of 3.7% (db) was informed from a study in Brazil. However, a small percentage higher value (6-9%wb) was reported from a study in Uganda (Abraha, 2011).

2.5. Biochemical Conversion of Coffee Husk to Bioethanol

According the green environment and renewable energy journals of Brazilian in 2010 suggests that bioethanol can be also produced from lignocellulose feed stocks like Agricultural and forest residues, loyal crops from production process wastes and so on. lignocellulosic biomass contains three main components which are called cellulose, hemicellulose, lignin, and a remaining smaller part (extractives and ash). Composition that lignocellulose biomass contains can extremely depends on its source. (Maurya et al., 2015). Coffee husks contains lignocellulosic biomass mean it is possible to extract bioethanol from coffee husk in which the lignocellulosic biomass converts to cellulose in the pretreatment process. There are two methodologies in which ethanol is transformed from cellulosic material that followed by distillation of fermented solution to extract pure ethanol (Kang et al., 2014). These are:

- Separate Hydrolysis and Fermentation (SHF)
- Simultaneous Saccharication and Fermentation (SSF)

2.6. Separate Hydrolysis and Fermentation (SHF) Methods

In this process the utilizing of lignocellulose biomass to bio ethanol can be accomplished by four major steps as shown in figure 2.2 below.

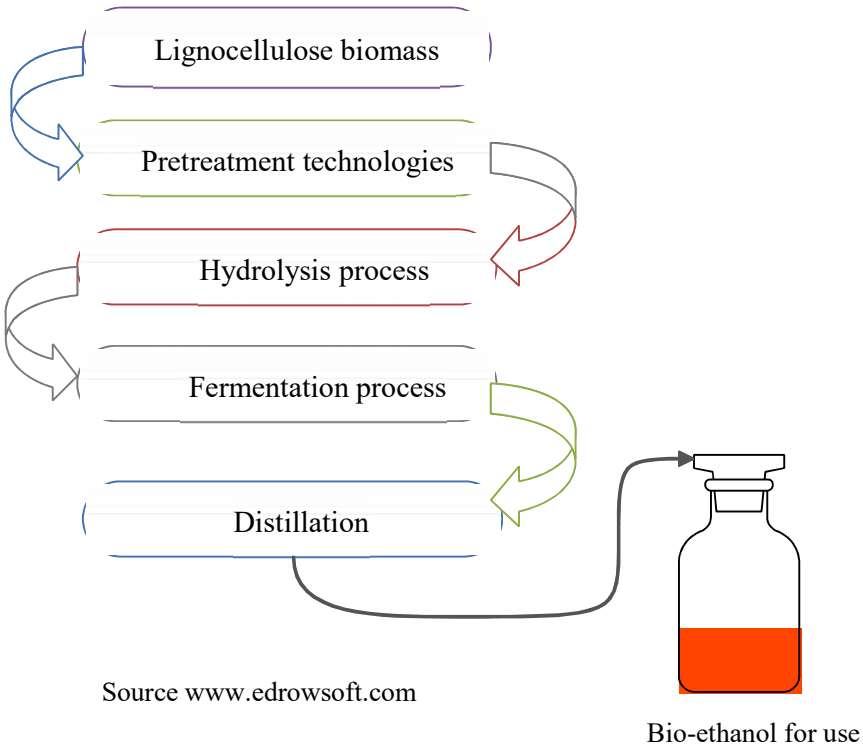

Figure 2.2: Annual bioethanol productions by main producers

1) Pre-treatment technologies

Pre-treatment is required to break down the crystalline structure of the lignocellulose material separating the cellulose away from the lignin in the cell walls for hydrolysis. A popular pretreatment should fulfill the requirements like;

- ✓ increase sugar formation,
- ✓ allow least degradation of carbohydrate,
- ✓ evade the production of hydrolysis and fermentation inhibitory byproducts and
- ✓ should be cost effective as well (Maurya et al., 2015)

There are 4 main types of pretreatment methods:

a) Physical pretreatment method: These contains such as Chipping, milling, grinding. Physical pretreatment used as to increases surface area and it requirements high power consumptions (Harmsen & Huijgen, 2010).

b) **Chemical (acidic)pretreatment methods:** Mineral acids such as H_2SO_4 and HCl have been used to pretreat the coffee husk (lignocellulose materials). Although concentrated mineral acids (hydrochloric acid, HCl, sulfuric acid, H_2SO_4 and nitric acid, HNO_3) are powerful representatives for cellulose pretreatment but they are toxic, corrosive and hazardous and require reactors that are resistant to corrosion (Harmsen & Huijgen, 2010). Furthermore, the recovery of concentrated acid is problematic enough to make the process economically feasible. Whereas, dilute acid pretreatment has been effectively developed for pretreatment of lignocellulosic materials. The dilute acid pretreatment can suggestively improve the cellulose hydrolysis; its cost is usually higher than some physio-chemical pretreatment processes such as steam explosion or Ammonia Fiber Explosion/Expansion (AFEX) (Wei et al., n.d.).

c) **Alkaline pretreatment:** Alkaline pretreatment is one of the serious method used to pretreat the plant biomass, conversely the effect of alkaline pretreatment method depends on the lignin content of the materials. Dilute NaOH treatment of lignocellulosic materials affected swelling of lignocellulosic materials, leading to an increase in internal surface area, a decrease in the degree of polymerization, and crystallinity, separation of structural linkages between lignin and carbohydrates (Rabelo et al., 2011).

d) **Biological pretreatment methods:** These can be work apply microorganisms like white and soft-rot fungi to the raw material for pretreatment applications (Fungus & Chrysosporium, 2012).

e) **Physiochemical pretreatment methods:** These includes physical and chemical pretreatment methods by using steam as pretreatment agent (steam explosion) and it can be upgraded using acid catalyst (Harmsen & Huijgen, 2010).

2) Hydrolysis process

When pre-treatment is complete, the cellulose formed undergoes hydrolysis. Hydrolysis is the transformation of cellulose to glucose also known as saccharification. Lignocellulose biomass is a combination of three basic components: lignin, cellulose and hemicellulose (National & Energy, 2005). Lignin cannot be converted to sugar but cellulose and slightly hemicellulose can break down to sugars, by means their elementary structure of these biomasses must be attacked by enzyme or acid. Once the structure of the biomass is disrupted, the cellulose can be converted to sugars enzymatically or by acid hydrolysis. Two methods of hydrolysis are acid hydrolysis and enzymatic hydrolysis (Sun & Cheng, 2002).

a) **Enzymatic hydrolysis:** Enzymes are naturally occurring in nature used for several chemical reactions and both bacteria and fungi can be estimated in this process. The enzymatic hydrolysis reaction is carried out by means of enzymes that act as catalysis to breakdown the glycoside bonds of the cellulose. The process of breaking the glucoside bonds of the cellulose to glucose is called hydrolysis because of water molecule must be supplied to purify each broken bond inactive. It has frequent advantages like less cost, insignificant conditions needed and improved yields (Mushimiyimana & Tallapragada, 2016). The degradation of cellulose can be accomplished by the cellulase-producing microorganisms: aerobic bacteria and fungi hide away soluble extracellular enzymes known as non-complexed cellulase system and anaerobic cellulolytic microorganisms produce complexed cellulase systems, called cellulosomes (Isaacs, 1984).

b) **Acid hydrolysis:** Acid hydrolysis is perhaps currently the most technologically matured method of sugar release from biomass. Acid hydrolysis can be of two types, dilute and concentrated acid hydrolysis. Depending on the concentration of the acid and the other parameters can be used as: dilute acid maybe used at high temperature and pressure while concentrated acids maybe used at very low temperature and pressure (Karim et al., 2014). For example in the case when sulfuric acid can be used as concentrated (25-80%) or dilute (3-8%), measured as the weight of acid in the weight of acidified aqueous solution that is present with the feedstock (Mushimiyimana & Tallapragada, 2016). The most advantages of using acid hydrolysis is: It is a faster reaction that requiring much less residence time in the reactor and its main disadvantages is; its lower conversion of cellulose to glucose due to process is more equilibrium driven.

3) Estimation of Reducing Sugars

Two methods are in common use for the estimation of sugars.

(i) Chemical, depending upon the reducing properties of certain sugars

(ii) Polari metric, depending upon the optical activity of the sugars concerned.

The second method is most accurate and fast method and is of substantial technical importance. The chemical method, although less accurate than the polar metric method, is of great value for the estimation of sugars in biological fluids (Benedict, 2015). Using chemical methods, the

reducing sugars like glucose and fructose may be estimated quantitatively be oxidizing agents like:

(i) Benedict's solution

(ii) Fehling's solution

(i) **Determination of Glucose by Benedict's Solution:** Glucose freely reduces Benedict's solution, which is an alkaline solution of cupric ions. It is prepared by dissolving copper sulphate, sodium carbonate, sodium citrate, potassium thiocyanate and potassium Ferro cyanide in appropriate proportions in distilled water. Three procedure are mandatory in this methods (Benedict, 2015).

a) **Preparation of Standard glucose solution:** Balance accurately known grams of glucose and transfer to a volumetric flask. Dissolve it in small quantity of distilled water and make up to the mark.

b) **Titration with standard glucose solution:** Fill the burette with the standard glucose solution. Now pipette out 25 cm^3 of Benedict's solution in a conical flask. Boil the contents of the conical flask and add regularly the glucose solution with continuous shaking till the blue color of the solution just disappears and white precipitate of CuCNS begins to appear. Keep the conical flask on burner during the addition of glucose solution Records the observation correctly (Manual & Chemistry, 2015).

c) **Titration with unknown solution:** Repeat the above process with unknown glucose solution. Records the observation correctly (Manual & Chemistry, 2015).

4) Media Preparation

Media can be prepared using different chemicals that could be used for the fermentation process of lignocellulosic biomass. For example, for fermenting of cellulosic materials to reducing sugar like glucose, according to the necessities it would be required that, Saccharomyces cerevisiae, Sugar (Dextrose), peptone, Urea, yeast extractor, make up water, and the pH is adjusted as 5. Then the media must have autoclaved at 121 ℃ for 15 min to destroy some microbes. After that, strain yeast (Saccharomyces cerevisiae) was added to the media in a conical flask. Then the flask was placed in shaking incubator for 24 hrs at around 30 ℃ and 200rpm (Breeding et al., 2015).

5) Fermentation process

Fermentation is the chemical conversion of organic substance into simpler compounds of different products by the action of enzymes. Initially the term fermentation was used to mean

the enzymatic breakdown of carbohydrates in the absence of air. Later in industrial practice, fermentation refers to any process by which raw materials are changed by the controlled action of carefully selected strains of organisms into definite products (Mushimiyimana & Tallapragada, 2016). The process of fermentation contains of microbes which consume sugar as food and in the process lead to the production of bioethanol and other varieties of products. The microorganisms used must compatible with the fermentation conditions i.e. pH, temperature, growth rate, tolerance to inhibitory compounds, output result, osmotic tolerance, specificity, yield, stability etc. (Sime et al., 2017). The fermentation can be done in batch, fed batch and continuous process. The selection of the method depends on properties of microbes and nature of lignocellulosic hydro lysate other than economic aspects (Dombek & Ingram, 1987). The glucose produced from the hydrolysis described above is fermented with yeast to produce bioethanol. Carbon-dioxide is also produced as by product when glucose is consumed. The simplified reaction equation is:

$$C_6H_{12}O_6 \longrightarrow 2C_2H_5OH + 2CO_2$$

6) Product separation using distillation

This is a separation of mixtures based on their relative volatilities (boiling points) of the individual components of the mixture. Moreover fermented products are unstable, so distillation is widely used equipment for the recovery of the bioethanol and other products from a number of impurities (Ohimain et al., 2012). Bioethanol is recovered in the distillation unit which is stabilized to be about 99.6% to lessen the bioethanol loss (Ohimain et al., 2012).

7) Fourier Transform Infrared Spectroscopy (FTIR) analysis

Fourier Transform Infrared Spectroscopy (FTIR) analysis is an analytical technique used to identify organic, polymeric, and in some case inorganic materials. The FTIR analysis method uses infrared light to scan test samples and observe chemical properties (Broberg & Pedersen, 2013). The FTIR instrument sends infrared radiation of about 10000 to 100 cm-1 through a sample, with some radiation absorbed and some passed through. The absorbed radiation is converted in to rotational or vibrational energy by the sample molecules. The resulting signal at the detector presents as a spectrum, typically from 4000cm^{-1} to 400cm^{-1}, representing molecular fingerprint of the sample. Each molecule or chemical structure will produce a unique spectral fingerprint, making FTIR analysis a great tool for chemical identification (Broberg & Pedersen, 2013).

2.7. Simultaneous Saccharification and Fermentation (SSF)

The simultaneous Saccharification and fermentation process combines hydrolysis of cellulose with simultaneous fermentation of its basic derived sugar (glucose) to ethanol in the same fermenter (Otulugbu, 2012). Simultaneous Saccharification and fermentation (SSF) is the best method for enzymatic transformation of cellulose to bioethanol. In SSF, enzymatic cellulose hydrolysis and glucose fermentation to bioethanol by yeast under goes simultaneously inside one vessel or fermenter (Otulugbu, 2012).

2.8. Coffee Husk in Ethiopia

Ethiopia is one of the prevalent coffee producer countries in the world. This coffee generates large amount of coffee husks during processing. Depending upon the method of coffee cherries processing, different residues are obtained. Coffee husks are the major solid residues from the handling and processing of coffee, since for every 2kg of coffee beans produced, approximately 1kg of husks are generated (Sime et al., 2017). In Ethiopia 192000metric tons of coffee is Husk cast adrift as byproduct per year. Coffee grounds are highly pollutant due to the presence of organic material that demands a great quantity of oxygen in order to degrade. Proposed alternative uses for coffee husks include employing this solid residue as a supplement for animal feed, direct use as fuel, and fermentation for the production of a diversity of products (enzymes, citric acid and flavoring substances), use as a substrate for growth of mushrooms and use as adsorbents (The Potential of Coffee Husk, 2014). However, considering the high amounts generated, there is still a need to find other alternative uses for this solid residue. Given that, such residue consists mainly of the pulp and hull of the coffee fruit, it presents a high concentration of carbohydrates and thus can be viewed as a potential raw material for bio-ethanol production. There is 134,400 metric ton of coffee husk disposed per year in Jimma area. So, this project is conducted in this area, in order to convert coffee husk wastes in to bio-ethanol. Thus, bio-ethanol is used for vehicles (car, lorry, bicycles, motors and etc.).

3. MATERIALS AND METHODS

3.1. Characterization of Raw material (Coffee husk)

Here the amount of moisture content, fixed carbon content, ash content and volatile matter content of samples coffee husks was determined.

3.1.1. Materials

The materials used to run the characterization of coffee husk were:

- coffee husk as raw material
- Digital balance**s (**Model-Sartorius and model EP214C) and
- Ovens- Loading model 100 -800.

3.1.2. Methods

The proximate analysis method was used to characterize the sample coffee husk. Here moisture content (MC), volatile matter content, the fixed carbon content, the ash content (the inorganic residue remaining after combustion of the sample) of the sample coffee husk was evaluated.

Determination Moisture Content (MC): The moisture content of the waste coffee husk was determined using an oven drier. The sample was weighed to the in-Petri dishes and then dried at 105 °C. It was then cooled and reweighted (Engineering, 2015). Then moisture was determined using the following eq.

$$\text{MC (\%)} = \frac{(W1 - W2)}{W1} \text{ X } 100 \quad (3.1)$$

Where:

W_1= Initial weight of sample and W_2= weight of sample after drying

Determination Volatile Matter Content (VM): Volatile matter of the coffee husk was determined by heating the sample in Furness in absence of oxygen at 950^{o}C for six minutes (Abraha, 2011). The volatile matter was computed as the difference between the initial weight and final weight of the sample to the ratio of the original weight of the sample as follows.

$$\text{VM (\%)} = \frac{(W1 - W2)}{W1} \; X \; 100 \quad (3.2)$$

Where: W_1= Original weight of sample

W_2= weight of sample after cooling

Ash content (Ash): Ash content of the coffee husk was determined by heating the coffee husk sample in a crucible at 750^{o}C for three hours in the Furness (Abraha, 2011). The ash content was calculated as the proportion of the weight of the ash in the coffee husk to the weight of coffee husk sample as follows.

$$AC\ (\%) = \frac{(W1 - W2)}{W1}\ X\ 100 \qquad 3.3)$$

Where:

W_1= Original weight of sample

W_2= weight of sample after cooling

Fixed carbon content (FC): The percentage of fixed carbon content of the coffee husk was computed by subtracting the sum of volatile matter (VM), ash content (AC), and MC (moisture content) from 100 (Abraha, 2011).

$$FC\ (\%) = 100 - (MC\% + VM\% + AC\ \%) \qquad (3.4)$$

3.2. Experimental Investigation for 'Separate Hydrolysis and Fermentation' Methods

In this sub title, the major experimental analysis of separate hydrolysis and fermentation method was discoursed in detail for utilization of coffee husk to bio ethanol.

3.2.1. Materials

The main materials used to investigate the experimental procedure for valorization of coffee husk to bioethanol were listed below.

Chemicals: Coffee husk as a raw material, Sodium Hydroxide (NaOH, min. assay 98% BDH Chemicals Ltd pool England cellulose), Sulphuric acid (H2SO4, (98%, France)), Dextrose sugar, Yeast extract, Urea, MgSO4.7H2O, Yeast (Saccharomyces cerevisiae strain).

Equipment's: Digital balance**s (**Model-Sartorius and model EP214C), Vacuum Filter (model-BN 3 STAATLICH, Berlin), Crusher, Sieves (mesh size of 1.0 mm, Sortmks-3332, PFEUFFR, Germany), Shaking Incubator, Vertical Autoclave, pH- Meter, Ovens.

3.2.2. Methodology

The methodology of this thesis was: Valorization of coffee husk to bioethanol using separate hydrolysis and fermentation (SHF) methods in the laboratory scales. The major experimental processes for valorization of coffee husk to bioethanol were summarized in the following procedures.

a) Coffee husk collection

The coffee husk was collected from the area of Jimma town from dry coffee processing in Jan 2018. Then, it was dried in oven at 105°C for 24 hours). Size reduction (grinding) and sieving was continued. The sample was characterized before pretreatment process was started. The sample was kept at room temperature until the next stage of the experiment.

Figure 3.1: Coffee plant, Coffee husk and Powder coffee husk

b) **Pre -treatment of coffee husk**

Chemical pretreatment method by using dilute sulfuric acid (H_2SO_4) was used to destroy hemicellulose, lignin shell, to protect cellulose and to decrease crystallinity of cellulose. The pretreatment was done by mechanical stirrer at 200 rpm for 24 hrs in the laboratory. The amount of sulfuric acid used in the pretreatment step was 1% (v/v) ratio with distill water that means for 1ml of H_2SO_4, it requires 99 ml of distill water.

The amount of solid sample (powder coffee husk) can be determined based on the literature that I used as 1:5, i.e. for 5ml of solution it requires 1gram of sample coffee husk. Then,

For 1gm = 5ml of solution then what for 100 ml of solution =? Y gm of sample, solving for Y gives as; Y = $\frac{1gm\ X\ 100ml}{5ml} = 20gram\ of\ sample$

Therefore, for 100ml of solution it requires 20gm of sample coffee husk powder in the pretreatment. Then for total amount of sample coffee husk which is 376gram can be calculated:

For 20gm = 100ml of solution then what for 376gm =? X ml of solution, solving for X gives as: X = $\frac{376gm\ X\ 100ml}{20gm}$ = 1880 ml of solution requires. Here the amount of sulfuric acid in the solution can be determined as; From the above, for 100ml of solution it requires 1.02ml of H_2SO_4 then, what for 1880ml =? Z,

Z = $\frac{1880ml\ so|n\ X\ 1ml\ H2SO4}{100ml\ so|n}$ = 18.8ml of H_2SO_4 was required

The remaining was distilling water i.e. = 1880ml-18.8ml = 1861.2ml of distill water. Therefore, for pretreatment of 376 gram of sample coffee husk powder I have used;

- ✓ 18.8ml of H_2SO_4

- ✓ 1861.2ml of distill water

By mixing together in 250 ml of flask with 200rpm of speed of stirrer for 24 hrs was set in the laboratory.

c) Filtration

The sample from pretreatment was filtered using vacuum filter. The solution from this was acidic and further adjusted its pH by adding NaOH solution and wash using distill water until the pH becomes 5-6. After that the solid part of the sample was dried in oven for 24hrs at 105 ℃. Then the sample was stored in plastic bag at room temperature.

d) Dilute Acid Hydrolysis

In this step the pretreated sample that means the cellulose part can be degraded or converted to glucose. The weight of the solid part (cellulose) of the sample after pretreatment was decreased to 201grams. This sample was hydrolyzed with three factors and three levels and its explains further in the experimental design procedure. Literature works on the dilute acid hydrolysis of different lignocellulosic materials have defined optimal process conditions for temperature, dilute sulfuric acid and reaction time as follows:

- Temperature90-200℃,
- Sulfuric acid concentration 0.5–8%, and
- Reaction time 15-2000 min (Izmirlioglu & Demirci, 2012).

So, for this experiment the dilute acid hydrolysis procedure was started by adding 1%, 3%, and 5% (v/v) diluted sulfuric to the distill water and coffee husk sample was added to each of the solution prepared. Then, the coffee husks were hydrolyzing in the autoclave at three levels of temperature (120, 130, and 140℃), time of (30, 50, and 70min). But in response surface CCD (central composite design) understands as five levels by adding two other center points for each experimental solution. So, the hydrolysis step requires three different solutions with:

- ❖ 1% H_2SO_4
- ❖ 3% H_2SO_4
- ❖ 5% H_2SO_4

Figure 3.2: Coffee husk solutions ready for hydrolysis

With constant amount of pretreated coffee husks 33.5gm for each solution. Same way the temperature and time also minimized in to three. Temperature (120,130 and 140℃) and time (30, 50 and 70mins). These three-parameters were applied to hydrolysis step of the experimentation with 30-minute time, 120 ℃ temperature and 1% of acid concentration at minimum levels and 70-minute time, 140℃ temperature and 5% acid concentration at maximum levels. The process of valorization of coffee husk to bio-ethanol was conducted in a completely randomized design using Design expert® 7.0 software.

Table 3.1: Experimental design formulated for hydrolysis stage

S. No	Factor 1 Acid con. (%)	Factor 2 Hydrolysis T° ℃	Factor 3 Hydrolysis time(min)	S. No	Factor 1 Acid con. (%)	Factor 2 Hydrolysis Temp. ℃	Factor 3 Hydrolysis time(min)
1	1	120	30	11	3	120	50
2	5	120	30	12	3	140	50
3	1	140	30	13	3	130	30
4	5	140	30	14	3	130	70
5	1	120	70	15	3	130	50
6	5	120	70	16	3	130	50
7	1	140	70	17	3	130	50
8	5	140	70	18	3	130	50
9	1	130	50	19	3	130	50
10	5	130	50	20	3	130	50

After hydrolysis the solid part was separate from the liquid by vacuum filtration as shown (to remove the non-fermentable lignin portion). Finally, the soluble component was mixed with the previously filtered solution from the pretreatment step for the next procedure.

e) pH Adjustment

Before addition of any micro-organism to the above prepared sample, pH of these sample was adjusted. This was done because of the micro-organism will die in hyper acidic or basic state. A pH of around 5 -6 was maintained. Pretreated and hydrolyzed sample was mixed, shaken substrate primarily checked for pH using a digital pH meter. Since, the mixed sample was more

acidic media, and then it must maintain the pH (5-6) by adding sodium hydroxide solution. Then the pH was maintained 5.5 and it was stored until the next procedure.

f) Media Preparation

Chemicals that was used for preparation 150 ml media for the fermentation process were: 2 gm of Sugar (Dextrose) 2 gm, peptone 0.5 gm, Urea gm, 1 gm yeast extractor, make up water = 150 ml, and the pH is adjusted as 5. The media was autoclaved at 121℃ for 15 min to destroy some microbes. Then 0.5 ml of strain yeast (Saccharomyces cerevisiae) which was taken from Ethiopian Institute of Biodiversity in Addis Ababa was added to the 150ml of media in a 250ml of flask. Then the flask was placed in shaking incubator for 24 hrs at 30 ℃ and 200rpm.

g) Cell Counting using champers method

The total number of cells used to formulate the above media was counted as: Primarily, six test tubs which contains 6ml of distilled water. One ml was pipetted out from the 250 ml growth media and taken in to the first test tube and also successive addition for the remaining test tubes. Then the test tubes were well mixed by vortex. Another media was prepared based on the proportion written on the nutrient agar. Six agar plates with 100ml volume were equipped to count the cells and pipetted out one ml from each of the six test tubes and 100ml from the nutrient agar to each of the agar plats and well distribute it over the agar plate. The agar was then incubating for 3 days at 30 ℃ temperature for the colony formation. The acceptable number of cells to foment cellulose is 300-3000 and 1^{st} 2^{nd} and 3^{rd} agar plates were have being above 3000 cells which is not accepted. 5^{th} and 6^{th} agar plats also not in the acceptable manure which counts less than 300 cells. 350 cells were gotten in the 4^{th} agar which is in the acceptable manner. Consequently, the total number of cells was calculated based on the following eq.:

Total number of cells in the fermentation = number of cells * dilution factor * amount of growth media (ml)which is added to the sample (3.5)

h) Fermentation

fermentation of the hydrolyzed sugar was continued to produce bio-ethanol. First the optimum (maximum) result was selected from hydrolysis step for fermentation process. It known that maximum glucose yield gives maximum ethanol yield. To know the amount of ethanol produced may not require to ferment all the experiments and also it is not feasible in terms of economic analysis. So, only experiment number 14 was selected for production of ethanol in this case which gives me maximum glucose yield 46.75% with hydrolyzing parameters of acid

concentration 3%, hydrolysis temperature 130℃ and hydrolysis time 70min. Then this sample (sample 14 that contains maximum glucose yield) and media were mixed in the 500ml flasks with the ratio of 10:1 (1%media with 10% sample) based on the recommended data in the literatures. The volume of the sample was 210ml and 21ml of media was added based on the proportion above. After well mixing the sample and the media, it was placed on shaking incubator at a temperature of 30 ℃ and at 200rpm for 72 hours to ferment the produced glucose to bioethanol in the biochemical engineering laboratory as shown in the figure.

Figure 3.3: Sample ready for fermentation and fermenter

General Fermentation Procedures:

Major processing steps in alcoholic fermentation are: Raw material (substrate) preparation, Yeast propagation (inoculums preparation), and Final fermentation.

- The sample was conditioned at temperature of 30℃ before fermentation step was started.
- The adapted media with the proportion of 1:10 to the soluble sample was Autoclave set at 30 ℃ and 200 rpm and then mixed the prepared sample with the media prepared into the autoclave using sterilized funnel.
- The parameters of fermentation i.e. fermentation time, yeast concentration (yeast proportion) and fermentation temperature were set to be at 72 hour, 10% (with the proportion of 1:10 that was the prepared media and sample respectively) and 30 ℃ respectively. And after 72 hours of fermentation, the samples were taken out and distilled.

i) Distillation

Distillation is a method used to separate two liquids based on their different boiling points (Mushimiyimana & Tallapragada, 2016). So, finally the product from fermentation was separated using simple distillation in this case rotary evaporator was used to separate the produced ethanol after fermentation at temperature of 78℃ for around 6hrs.

Figure 3.4: Rotary distillation

j) Determination of ethanol yield by colorimetric method

From the experimental work experiment number 14 gives maximum glucose yield (46.75%) and that value was selected for further analysis for the production of ethanol. This sample was ferment in the fermenter and separate the produced ethanol using rotary distillation. The ethanol produced from this sample was calculated by preparing standard ethanol solution in the following manner. Standard 1.6 mg/mL Ethanol concentration was used to prepare stock solution in water. One ml of ethanol was poured in volumetric flask containing one ml water to prevent loss due to volatility. To the standard stock solution containing 1.6 mg/mL, 25ml of chromic acid reagent was added in 50 mL of volumetric flask. The mixture was shaken gently for 1 min and allowed to place the tube in a water bath at 70℃ temperatures for 15 min, resulted in formation of green colored reaction product. Take out the tubes and immediately 24 ml of distilled water was added to it to stop the reaction. Finally measure the absorbance at 600nm. The same procedure was taken to prepared the sample solution as the standard one. But the concentration (mass density) of the sample was determined by density meter which was red 1.78 mg/ml. then ethanol yield was calculated as:

$$\text{Percentage of ethanol in sample (\%)} = \left(\frac{Cs}{Cu}\right)\left(\frac{As}{Au}\right) \times 100 \qquad (3.7)$$

Where, Cs = Concentration of standard, Cu = Concentration of sample, As = Absorbance of standard, Au = Absorbance of sample.

3.3. Measurement of Reducing Sugars

In this case the reducing sugars are determined and checked by Benedict's solution.

3.3.1. Materials

The mail materials used in this procedure were listed below.

Standard glucose, Benedict's solution, Balance, Test tubs, Waterbus, UV- spectroscopy

3.3.2. Methods

The reducing sugars (glucose) was analyzed or identified by using benedicts solution method. In this method of testing reducing sugars (glucose) is readily reduces benedict's solution. Consequently, one can understand the reducing sugar could be glucose solution.

Determination of Glucose by Benedict's Solution: Glucose readily reduces Benedict's solution, which is an alkaline solution of cupric ions. It is prepared by dissolving copper sulphate, sodium carbonate, sodium citrate, potassium thiocyanate and potassium ferrocyanide in proper proportions in distilled water. Sodium citrate prevents the precipitation of cupric hydroxide by forming a complex, while potassium thiocyanate is used to precipitate copper ions as copper thiocyanate (Benedict, n.d.).

Figure 3.5: Preparation of standard glucose solutions

Preparation of Standard glucose solution: glucose was prepared and weighed accurately as 0.05, 0.075, 0.1, 0.125, 0.15 grams and 6 other test tubs was prepared with 5ml of distill water for each test tubes. Then dissolve these glucose samples in 5ml distill water for each and one was remained free since the glucose samples are 5 in numbers. Then shake the sample until the glucose is completely dissolve in the distil water. In other case 6 test tubs was prepared with 5ml of benedict's solutions for each. Then transfer one ml from the different glucose solutions to the benedict solution. This sample was insert in waterbus at a temperature of 90℃ for five

minutes. Based on the amount of glucose concentration the sample changes its color from blue to red colors as shown below.

Determination of the concentration of unknown samples: First standard glucose solution was filtered and measured their absorbance's which was used to determine the concentration of unknown reduced sugars (glucose) in the sample hydrolysates. Using slope and intercept from the standard glucose curve the unknown glucose concentrations was calculated. The standard glucose curve was determined by mini tab software.

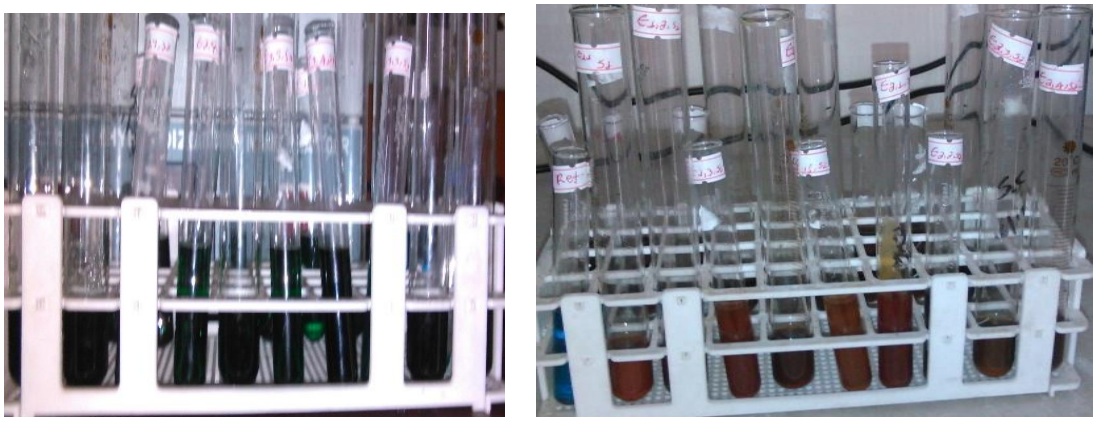

Figure 3.6: determination of samples concentration

$$Y = m\,x + b \tag{3.8}$$

Where, y = absorbance, x = glucose concentration, m = slop = -0.03921, b = intercept = 0.02766

Then 5ml of benedict solution in 20 test tubs same as the standard one was prepared. Then 1ml from each of the 20 samples was added to the test tubs with benedict solution. It was then Boiled in waterbus with 90℃ temperatures for 5min and each of their absorbance was measured. The concentration of unknown sample was then calculated by using their absorbance and slop and intercept from standard curve using the following equation.

$$\text{Concentration of unknown sample} = \frac{(\text{absorbance of unknown sample}) - (\text{y-intercept})}{\text{slop}} \tag{3.9}$$

Then yield can also be calculated as:

$$\text{Yield} = \frac{\text{gram of glucose produced}}{\text{raw material used}} \text{x} 100\% = \frac{\text{C,ini-C,final}}{\text{C,final}}\ x\ 100\% \tag{3.10}$$

After calculating the concentration and yield of each unknown samples, the result with maximum yield was selected for further process instead of using all in the next steps that consume more time and due to in availability of gas chromatography to measure the amount of

ethanol and also the fermenter was busy to run all the experiments instead I was select the maximum result from hydrolysis step and that result was fermented to produce bioethanol. Experiment 14 was selected in which maximum yield was registered in this experiment.

3.4. Fourier Transform Infrared Spectroscopy (FTIR) Analysis

Fourier Transform Infrared Spectroscopy (FTIR) analysis is an analytical technique used to identify organic, polymeric, and in some case inorganic materials. The FTIR analysis method uses infrared light to scan test samples and observe chemical properties (Bromberg & Pedersen, 2013). The FTIR instrument sends infrared radiation of about 10000 to 100 cm-1 through a sample, with some radiation absorbed and some passed through. The absorbed radiation is converted in to rotational or vibrational energy by the sample molecules. The resulting signal at the detector presents as a spectrum, typically from 4000cm-1 to 400cm-1, representing molecular fingerprint of the sample. Each molecule or chemical structure will produce a unique spectral fingerprint, making FTIR analysis a great tool for chemical identification (Bromberg & Pedersen, 2013). Fourier Transform Infrared Spectroscopy (FTIR) analysis is an analytical technique used to identify organic, polymeric, and in some case inorganic materials. The FTIR analysis method uses infrared light to scan test samples and observe chemical properties (Bromberg & Pedersen, 2013). The FTIR instrument sends infrared radiation of about 10000 to 100 cm-1 through a sample, with some radiation absorbed and some passed through. The absorbed radiation is converted in to rotational or vibrational energy by the sample molecules. The resulting signal at the detector presents as a spectrum, typically from 4000cm-1 to 400cm-1, representing molecular fingerprint of the sample. Each molecule or chemical structure will produce a unique spectral fingerprint, making FTIR analysis a great tool for chemical identification (Bromberg & Pedersen, 2013). FTIR analysis was used to, identify and characterize the ethanol produced.

3.5. Analyzing of The Data Using Design Expert Software

Design expert® 7.0 software experimental method was used to determine the effect of three operating variables of the acid hydrolysis in bio ethanol produced from coffee husk. Response surface methodology (RSM) was used to understand the effect of the factors for the valorization of coffee husk to bioethanol for the different hydrolysis variables on the glucose yield. The central composite design CCD was applied to study process variables. The experimental runs were carried out according to a CCD design for the three identified design independent variables, namely, hydrolysis temperature, hydrolysis time and acid concentration.

4. RESULTS AND DISCUSSION

This work was consisting of four major parts: pretreatment to remove lignin, reduce cellulose crystalline, sterilize the coffee husk and increase the porosity of the materials, dilute sulfuric acid hydrolysis to degrade cellulose to glucose, fermentation of glucose to produce bio-ethanol and distillation to separate pure ethanol. The experimental outcomes of those particular results were measured in the hydrolysis of cellulose to know the yield of sugar concentration. There were 20 experiments conducting by varying hydrolysis time, hydrolysis temperature and diluted sulfuric acid concentration. The amount of product obtained for each sample in the hydrolysis was measured and recorded, to select the optimum value for further process like fermentation, and distillation to obtain final product bioethanol and finally chemical composition of this product (bioethanol) were analyzing using FTIR.

4.1. Results for Raw Material (Coffee Husk) Characterization

The Moisture Content (MC) Determination: The moisture content of the waste coffee husk was determined using an oven drier. The sample was weighed to the nearest 10 g in Petri dishes and then dried at 105 ℃. It was then cooled and reweighted which is maintained as 8.8 g. Then moisture was determined using the following eq.

$$\text{MC (\%)} = \frac{(W1 - W2)}{W1} \text{X } 100$$

Where: W_1= Initial weight of sample

W_2= weight of sample after drying

$$\text{MC (\%)} = \frac{10g - 8.8g}{10g} \text{X } 100 = 12\%$$

The Volatile Matter Content (VM) Determination: Volatile matter of the coffee husk was determined by heating the sample in Furness in absence of oxygen at 950^{o}C for six minutes (Abraha, 2011). The volatile matter was computed as the difference between the initial weight and final weight of the sample to the ratio of the original weight of the sample as follows.

$$\text{VM (\%)} = \frac{(W1 - W2)}{W1} \ X \ 100$$

Where: W_1= Original weight of sample

W_2= weight of sample after cooling

VM (%) $= \frac{8.8g - 1.6g}{10g}$ X 100 = 72%

Ash content (Ash): Ash content of the coffee husk was determined by heating the coffee husk sample in a crucible at 750^{o}C for three hours in the Furness (Abraha, 2011). The ash content was calculated as the proportion of the weight of the ash in the coffee husk to the weight of coffee husk sample as follows.

AC (%) $= \frac{(W1 - W2)}{W1} X\ 100$

Where:

W_1= Original weight of sample

W_2= weight of sample after cooling

AC (%) $= \frac{1.97g - 1.6g}{10g}$ X 100 = 3.7%

Fixed carbon content (FC): The percentage of fixed carbon content of the coffee husk was computed by subtracting the sum of volatile matter (VM), ash content (AC), and MC (moisture content) from 100.FC (%) = 100 – (MC% + VM% + AC %)

FC (%) = 100 – (72+12+3.7) = 12.3%

4.2. Results for Reducing Sugar Measuring (Characterizing)

Here the reducing sugars can be known using benedict's solution in part three. If samples can have reducing sugars like glucose in this case the benedict solution changes its color from blue to red.

Preparation of Standard glucose solution: Preparation of standard solution is important to determining the slop and intercept of the standard one which are important in determining of the concentration of the unknown samples. The preparetion step is identified in part three aready.

Data recorded from prepared glucose powder plus benedict's solution were:

Absorbance (540 nm)	0	0.187	0.356	0.552	0.602	0.834
Glucose concentration (mg/ml)	0	10	15	20	25	30

Fitted Line: Absorbance versus glucose concentration

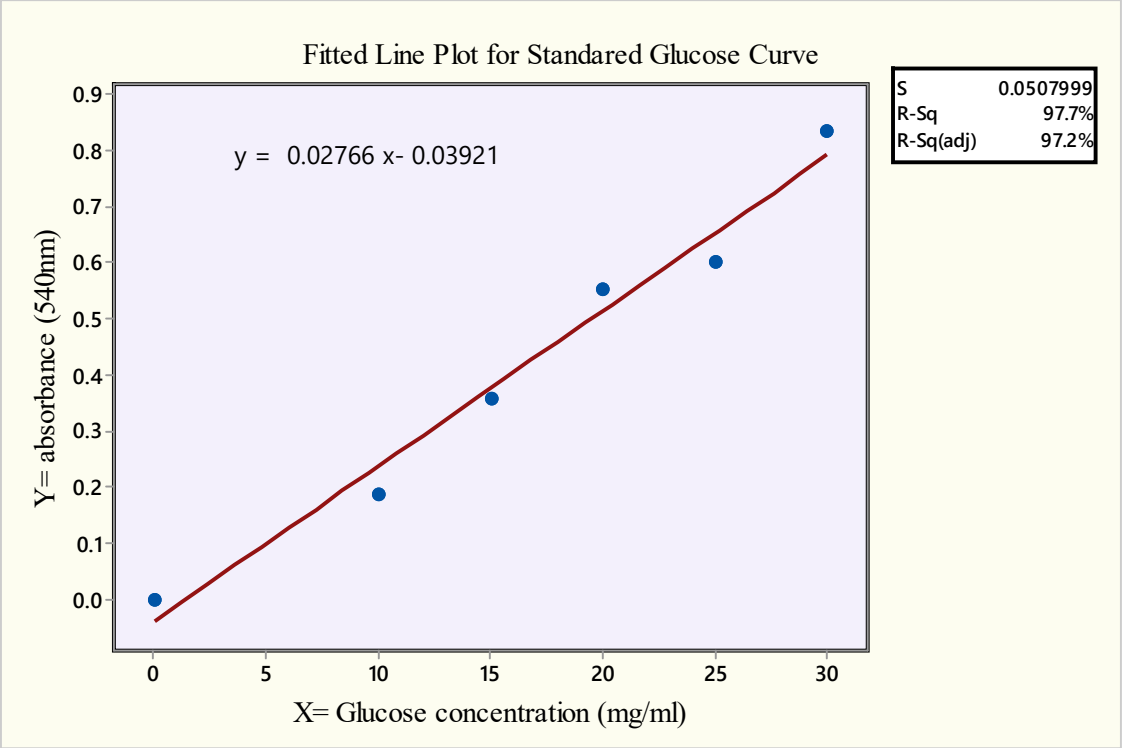

Figure 4.1: Calibration curve of glucose standard

The concentrations of unknown sugar samples were determined from a standard curve of glucose solution prepared that gives the following equation.

$$y = 0.02766\,x - 0.03921$$

Determination of glucose concentration of unknown samples: her the method is written in part three and the equation used to determine the unknown concentration of the samples is:

$$\text{Concentration of unknown sample (C)} = \frac{(\text{absorbance of unknown sample})-(\text{y-intercept})}{\text{slop}}$$

$$C_1 = \frac{(\text{absorbance 1})+ 0.03921}{0.02766} = \frac{0.183+ 0.03921}{0.02766} = 8.03\text{mg/ml}$$

$$C_2 = \frac{(\text{absorbance 2})+ 0.03921}{0.02766} = \frac{0.258 + 0.03921}{0.02766} = 10.7451\text{mg/ml},$$ likewise it continues until 20^{th} concentration.

$C_{20} = \frac{(\text{absorbance } 20) + 0.03921}{0.02766} \quad \frac{0.709 + 0.03921}{0.02766} = 27.0502\text{mg/ml}$, and yield can be calculated as: $\text{Yield} = \frac{\text{gram of glucose produced}}{\text{raw material used}} \text{ x } 100\%$

$y_1 = \frac{8.03\text{mg/ml}}{\text{raw material used}} \text{ x } 100\%$, but the amount of raw material used can be calculated as:

Raw material used ($C_{initial}$) = gram of sample used/ ml solution = 6.7gm/0.2913ml = 23.039mg/ml

Then, $y_1 = \frac{8.03\text{mg/ml}}{23.039\text{mg/ml}} \text{ x } 100\% = 34.8538\%$

$y_2 = \frac{10.7451\text{mg/ml}}{23.039\text{mg/ml}} \text{ x } 100\% = 36.1922\%$, likewise it continues until 20^{th} step.

$y_{20} = \frac{27.0502\text{mg/ml}}{23.039\text{mg/ml}} \text{ x } 100\% = 45.2804\%$

4.3. Yield Analysis of the Parameters Using Design Expert Software

The experimental data from hydrolysis step that were used for the analysis and design of the experiment are tabulated in table 4.1 below.

Table 4.1: Measured Absorbance's and glucose yield of unknown samples of the 20 experiments

S. No	Factor 1 Acid con. (%)	Factor 2 Hydrolysis temp. (℃)	Factor 3 Hydrolysis time(min)	Response 1 Absorbance (540nm)	Response 2 Glucose conc.(mg/ml)	Response 3 Glucose Yield (percent)
1	1.00	120.00	30.00	0.183	8.03362	34.8538
2	5.00	120.00	30.00	0.258	10.7451	36.1922
3	1.00	140.00	30.00	0.185	8.10593	36.9695
4	5.00	140.00	30.00	0.256	10.6728	38.0765
5	1.00	120.00	70.00	0.207	8.9013	35.2421
6	5.00	120.00	70.00	0.296	12.1189	38.3903
7	1.00	140.00	70.00	0.225	9.55206	36.2833
8	5.00	140.00	70.00	0.314	12.7697	39.4315
9	1.00	130.00	50.00	0.283	11.6489	39.6383
10	5.00	130.00	50.00	0.394	15.662	40.0591
11	3.00	120.00	50.00	0.613	23.5795	41.7273
12	3.00	140.00	50.00	0.668	25.568	43.9087
13	3.00	130.00	30.00	0.478	18.6988	41.9182
14	3.00	130.00	70.00	0.769	29.2194	46.7511
15	3.00	130.00	50.00	0.715	27.2672	45.2672
16	3.00	130.00	50.00	0.707	26.978	44.1647
17	3.00	130.00	50.00	0.697	26.6164	45.5863
18	3.00	130.00	50.00	0.71	27.0864	45.3383
19	3.00	130.00	50.00	0.688	26.291	44.0656
20	3.00	130.00	50.00	0.709	27.0502	45.2804

Hydrolysis of coffee husk using sulfuric acid the produced glucose concentration increases with increasing time and temperature as shown in the table above. Based on this, the maximum yield of glucose concentration was noted for 3% of acid concentration, at a temperature of 130℃ obtained 46.7511%. The experiment that produces high amount of glucose concentration was selected for further bioethanol production procedure, in this case experiment 14 is selected which is optimum yield of glucose attained. So as the glucose yield varies with the different parameters, this indicated us the Ethanol yield also varies at different acid concentration, time and temperature. This indicates the optimum glucose yield gives optimum ethanol yield. The Experimental results, can be analyzed using Design expert® 7.0.0 software. From table above, the maximum glucose yields 46.7511% was obtained at an experiment number 14 at 130℃, of temperature, 3 % acid concentration, and at 70-minute time. While the minimum yield 34.8538% was obtained at experiment number 1, at a temperature of 120℃, 1% acid concentration, and 30 minutes of hydrolysis time. The decrease and increase of the yield was depending on the level of factors. This difference may be due to the different method of hydrolysis. There resulting data, from the above table, were analyzed using Design expert® 7.0.0 software to determine the effect of temperature, acid concentration, and time. The dependent variable used as a response parameter was the initially for glucose yield but further ethanol yield. All experiments were carried out in a randomized order to minimize the effect of unexpected variability in the observed response due to extraneous factors. Table 4.2 below shows the generalized design of the experiments.

Table 4.2: Design summery

Design Summary			
Study Type	Response Surface	Experiments	20
Initial Design	Central Composite	Blocks	No Blocks
Design Model	Quadratic		

Response	Name	Units	Obs	Minimum	Maximum	Trans	Model
Y_1	Absorbance	mg/ml	20	0.18	0.77	None	No model chosen
Y_2	Glucose concentration	mg/ml	20	8.03	29.22	None	Quadratic
Y_3	Yield	%	20	34.8538	46.75	None	Quadratic
Factor	Name	Units	Type	Low Actual	High Actual	Low Coded	High Coded
A	Acid concentration	%	Numeric	1.00	5.00	-1.000	1.000
B	Hydrolysis temperature	℃	Numeric	120.00	140.00	-1.000	1.000
C	Hydrolysis time	minute	Numeric	30.00	70.00	-1.000	1.000

In order to determine whether or not the quadratic model is significantly affect by the parameters listed in the design, it was crucial to perform analysis of variance (ANOVA). The probability values (P-values) were used to perform as a device to check the significance of each coefficient, which also showed the interaction strength of each parameter. The smaller the p-values are, the bigger the significance of the corresponding coefficient.

Table 4.3: Analysis of variance (ANOVA)

ANOVA for Response Surface Quadratic Model Analysis of variance table [Partial sum of squares] Response: Glucose Yield						
Source	Sum of squares	DF	Mean Square	F- Value	Prob > F	
Model	281.70	9	31.30	31.10	< 0.0001	Significant
A	8.40	1	8.40	8.34	0.0162	
B	6.83	1	6.83	6.78	0.0263	
C	6.54	1	6.54	6.50	0.0289	
AB	6.693E-003	1	6.693E-003	6.650E-003	0.9366	
AC	1.85	1	1.85	1.84	0.2046	
BC	0.46	1	0.46	0.46	0.5145	
A^2	73.33	1	73.33	72.85	< 0.0001	
B^2	13.24	1	13.24	13.16	0.0046	
C^2	1.26	1	1.26	1.26	0.2887	
Residual	10.07	10	1.01			
Lack of Fit	7.90	5	1.58	3.65	0.0908	Not significant
Pure Error	2.16	5	0.43			
Cor Total	291.76	19				

An ANOVA study for the quadratic model was used to evaluate the impact and significance of terms individually and interactions in the regression equation. In this case ANOVA was carried out to determine the statistical significance of the quadratic response surface model, and it can be seen from Table 4.3 that the p-value of the model was less than 0.0001, which indicates the model was statistically significant. The analysis of variance F- Value is a test for comparing model variance with residual (error) variance. If the variances are close to each other, the ratio will be close to one and it is less likely that any factors have a significant effect on the response. It is calculated by model mean square divided by residual mean square 31.3/1.01= 31.1. Here the model F- Value of 31.1implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. The values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B, C, A^2, B^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. The "Lack of

Fit F-value" of 0.0908 implies that, Lack of Fit is not significant relative to the pure error. The lack of fit was found to be small with a value of 9.08%, and this indicating that the model was adequate to fit the experimental data in other case, the model adequately described the relationship between the dependent variable (i.e., glucose yield) and the independent variables (i.e., acid concentration, hydrolysis temperature and hydrolysis time) Non-significant lack of fit is good. Beucause, we want the model to fit.

The coefficient of determination (R^2) was found to be 0.9655, which indicates that 96.55% of the experimental data were relevant and only 3.55% of the total variations was not explained by the model. In general, a high value of R^2 indicates that there is good fit between the predicted data and experimental data. The adjusted coefficient of determination ($AdjR^2$) was found to be very high with a value of 0.9345, which indicates that that the model accounted for 93.45% of the variability in the data. The coefficient of variation (C.V.) was found to be 2.45%, this low value of CV indicating that the deviations between the predicted data and experimental data were small, that means the experiments were precise and reliable. Adequate precision measures the signal to noise ratio. A ratio of adequate precision greater than 4 is desirable. In this study, the adequate precision value was 14.809, as shown in table 4.4 which indicated an adequate signal.

Table 4.4: Model adequacy measures

Std. Dev.	1.00	R-Squared	0.9655
Mean	40.96	Adj R-Squared	0.9345
C.V.	2.45	Pred R-Squared	0.7801
PRESS	64.17	Adeq Precision	14.809

The “pre-R- squared” of 0.9655 is as close to the “Adj R- square” of the 0.9345 in less than 0.03 difference as one might expect. The difference between Adj R-Squared and Pred R-Squared is 0.1544 (i.e. they are reasonably close to each other). This indicated a close fit of the model to the actual response data. “Adeq precition” measures the signal to disturbance ratio due to random error. A ratio greater than 4 is desirable. Here the ratio of 14.809 indicates an adequate signal. Therefore, the developed model is suitable and can be used to navigate the designed space and predict the response. The regression coefficients and the corresponding 95% CI (Confidence Interval) High and Low were presented in table 4.5 below. If zero was in the range High and Low 95% Confidence Interval, the factors have no effect. From the 95%

CI High and Low values of each model term, it could be concluded that the regression coefficients of acid concentration and the interaction terms of time and acid concentration have highly significant effect in ethanol production.

Table 4.5: High and low 95% confidence interval

Factor	Coefficient Estimate	DF	Standard Error	95% CI Low	95% CI High
Intercept	44.98	1	0.34	44.21	45.74
A-Acid concentration	0.92	1	0.32	0.21	1.62
B-Hydrolysis temperature	0.83	1	0.32	0.12	1.53
C-Hydrolysis time	0.81	1	0.32	0.10	1.52
AB	-0.029	1	0.35	-0.82	0.76
AC	0.48	1	0.35	-0.31	1.27
BC	-0.24	1	0.35	-1.03	0.55
A^2	-5.16	1	0.60	-6.51	-3.82
B^2	-2.19	1	0.60	-3.54	-0.85
C^2	-0.68	1	0.60	-2.03	0.67

Using the designed experimental data, the quadratic polynomial model for glucose yield and further for bio-ethanol yield from coffee husk by separate acidic hydrolysis was retreated and shown as below:

Final Equation in Terms of Coded Factors:

$$\text{Glucose yield} = +44.98 + 0.92 * A + 0.83 * B + 0.81 * C - 0.029 * A * B + 0.48 * A * C - 0.24 * B * C - 5.16 * A^2 - 2.19 * B^2 - 0.68 * C^2 \quad (4.1)$$

Final Equation in Terms of Actual Factors:

The actual versus predicted values using model in the above equation (in terms of actual factors) are tabulated in table blow.

Glucose yield = -362.43160 +7.79006 * Acid concentration +5.85250 *Hydrolysis temperature +0.32959 * Hydrolysis time -1.44625E-003 * Acid concentration * Hydrolysis temperature +0.012034* Acid concentration * Hydrolysis time -1.19850E-003 *Hydrolysis temperature * Hydrolysis time -1.29094 * Acid concentration ^{2} -0.021945* Hydrolysis temperature^{2}-1.69452E-003*Hydrolysis time^{2} (4.2)

Table 4.6: Actual versus model Predicted values of glucose yield

Diagnostics Case Statistics							
Standard Order	Actual Value	Predicted Value	Residual	Leverage	Student Residual	Cook's Distance	Outlier T
1	34.85	34.60	0.25	0.793	0.555	0.118	0.535
2	36.19	35.53	0.66	0.793	1.455	0.812	1.555
3	36.97	36.79	0.18	0.793	0.392	0.059	0.375
4	38.08	37.60	0.47	0.793	1.039	0.414	1.044
5	35.24	35.73	-0.49	0.793	-1.080	0.447	-1.090
6	38.39	38.59	-0.20	0.793	-0.433	0.072	-0.415
7	36.28	36.97	-0.68	0.793	-1.496	0.859	-1.611
8	39.43	39.70	-0.27	0.793	-0.596	0.136	-0.576
9	39.64	38.90	0.74	0.491	1.038	0.104	1.043
10	40.06	40.73	-0.67	0.491	-0.934	0.084	-0.928
11	41.73	41.95	-0.23	0.491	-0.317	0.010	-0.302
12	43.91	43.61	0.30	0.491	0.421	0.017	0.403
13	41.92	43.49	-1.57	0.491	-2.194	0.464	-2.890
14	46.75	45.11	1.64	0.491	2.298	0.509	3.173
15	45.27	44.98	0.29	0.118	0.310	0.001	0.295
16	44.16	44.98	-0.81	0.118	-0.860	0.010	-0.848
17	45.59	44.98	0.61	0.118	0.649	0.006	0.629
18	45.34	44.98	0.36	0.118	0.385	0.002	0.368
19	44.07	44.98	-0.91	0.118	-0.966	0.012	-0.962
20	45.28	44.98	0.31	0.118	0.324	0.001	0.309
* Case(s) with |Outlier T| > 3.50							

4.4. Diagnostic Case Plots

4.4.1. Normal probability plot

Normal probability plot of the raw data used to check the assumption of normality the experiment when using design expert software. In the analysis of variance, it is usually more effective (strait line) to do this with the residuals which is shown below.

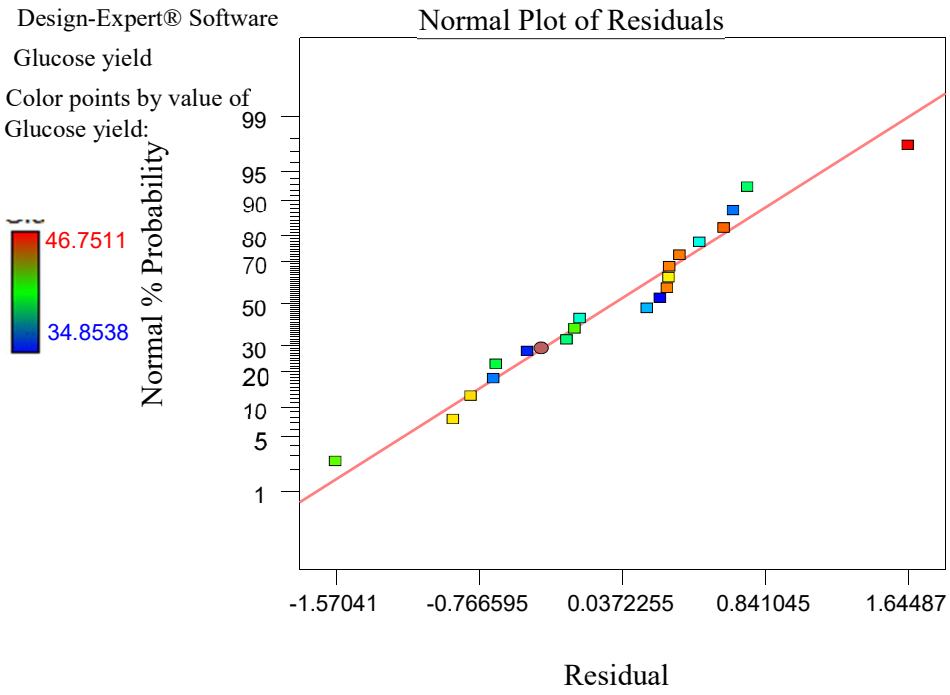

Figure 4.2: Normal Probability Plot of Residuals

The normal probability plot, (Fig.4.2 above), indicates the residuals following a normal distribution, in which case the points follow a straight line. This shows that the quadratic polynomial model satisfies the assumption of ANOVA.

4.4.2. Residual vs Predicted plot

For correct model and satisfied assumptions, the residual value must be structure less; in particular, they should be unrelated to any other variable including the predicted response. A simple check is to plot the residuals versus the fitted (predicted) values. A plot of the residuals versus the rising predicted response values tests the assumption of constant variance. The plot shows random scatter or structure less which satisfied the assumption of the constant variance as shown in figure 4.3.

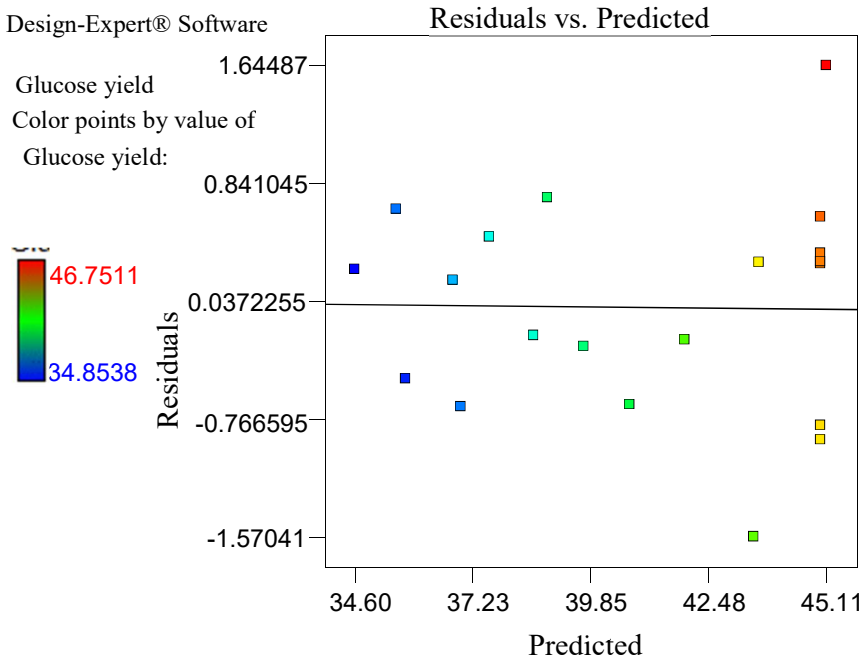

Figure 4.3: Plot of residuals versus model predicted values

4.5. Model Graphs for Individual Effects of Experimental Parameters (Variables) On Glucose Yield

Optimum glucose production can give optimum ethanol production. So, parameters that can affect glucose production indirect they can also affect ethanol production. Let's see the effect of the parameters on glucose yield one by one.

4.5.1. The effect of acid concentration on the glucose yield

The resulting plot of acid concentration versus the glucose yield, when temperature and hydrolysis time were actual factors, is shown in Figure 4.4 below. As shown from the plot increasing acid concentration from 1% up to 3%, glucose yield increased and glucose yield decreases when the acid concentration increases from 3% to 5%, the reason that the glucose yield was decreased due to degradation of pentose's, hexoses, and the lignin present and consequently ethanol yield also decrease. These products can include furfural, acetic acid, and formic acid. So acid concentration highly affects to the yield rather than the other parameters. In this case, the optimum acid concentration was found to be 3% and the glucose yield at this acid concentration was 46.75%.

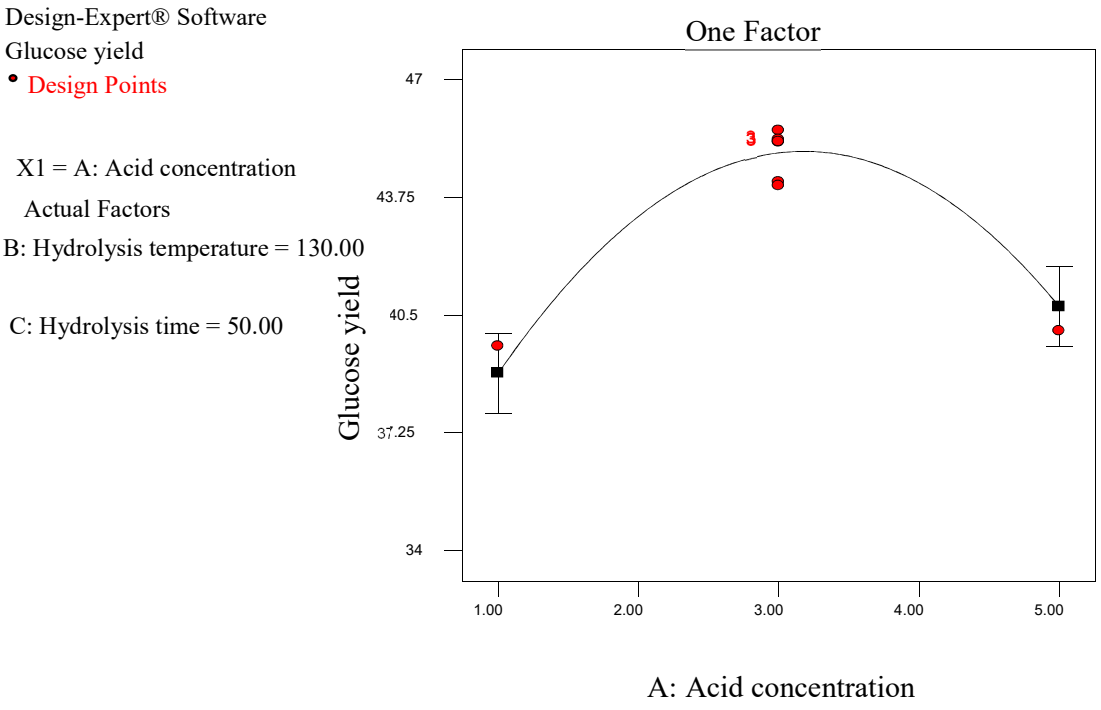

Figure 4.4: The effect of acid concentration on glucose yield

4.5.2. The effect of hydrolysis temperature on the glucose yield

The resulting plot of temperature versus the glucose yield, when Acid concentration and hydrolysis time were actual factors, is shown in Figure 4.5 below. From the plot as temperature increases from 120℃ to 130℃, glucose yield also increased and beyond 130℃, decreases. The yield was decreased may be due to further conversion of other by product. Therefore, the optimum temperature was found to be 130℃ and the yield at this temperature was 46.75%.

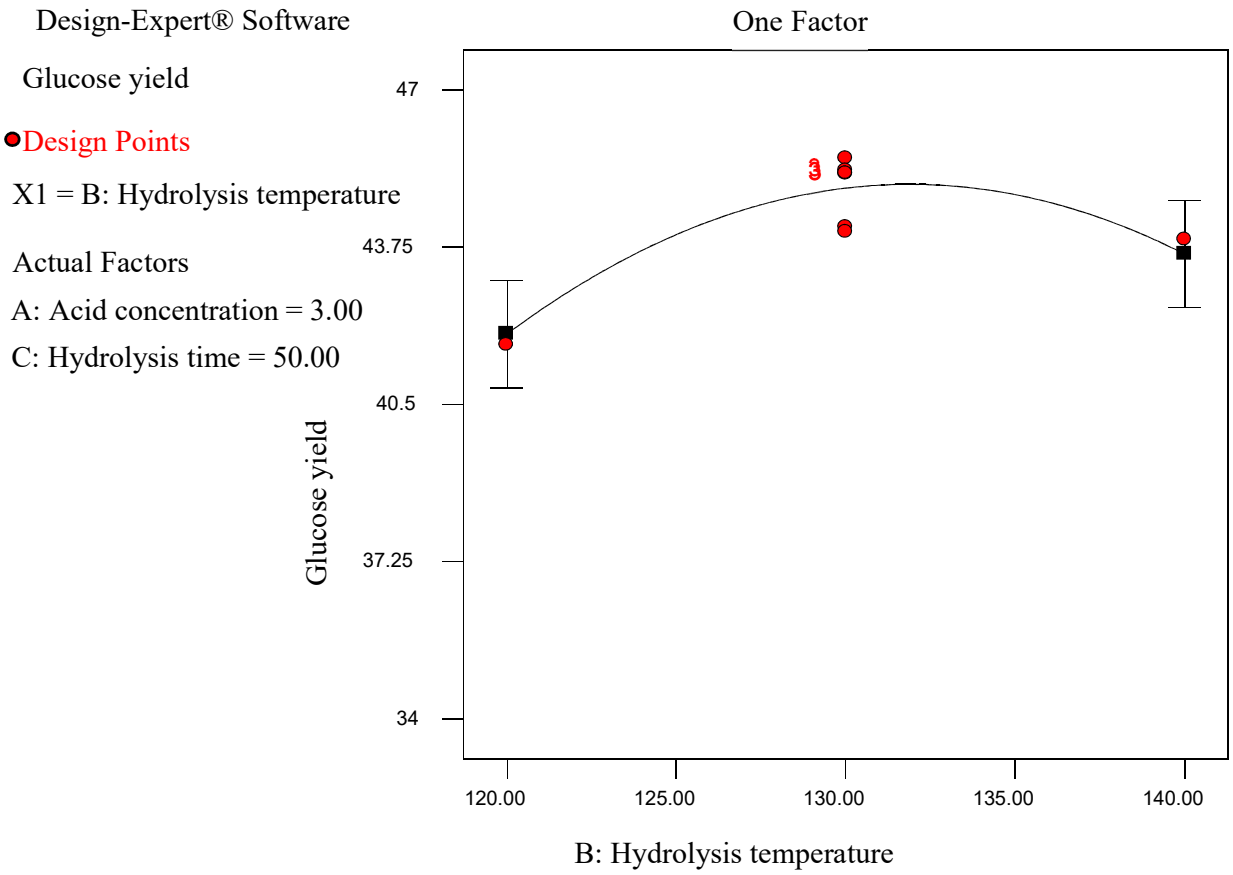

Figure 4.5: The effect of hydrolysis temperature on glucose yield

4.5.3. The effect of hydrolysis time on the glucose yield

The resulting plot of time versus the glucose yield, when Acid concentration and hydrolysis temperature were actual factors, is shown in Figure 4.6 below. As shown from the plot as increasing the hydrolysis time, glucose yield also increased and reached maximum yield (46.75%).

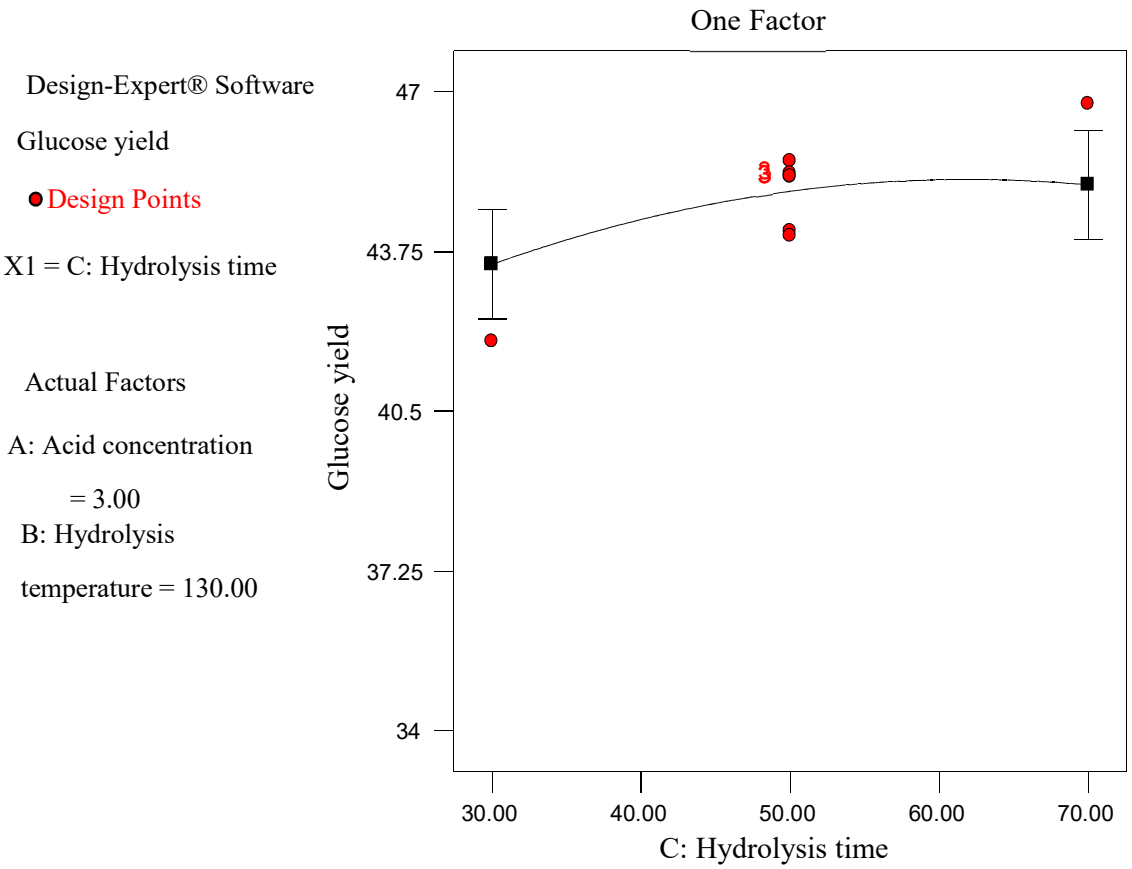

Figure 4.6: The effect of hydrolysis time on glucose yield

4.6. Model Graphs for Interaction Effects of Experimental Parameters (Variables) on Glucose Yield

4.6.1. The effects of hydrolysis temperature and acid concentration on glucose yield

The effects of hydrolysis temperature and acid concentration on glucose yield were shown in the following different figures by holding the hydrolysis time at middle. For the interaction figures, black and red line indicates low and high level of parameters respectively.

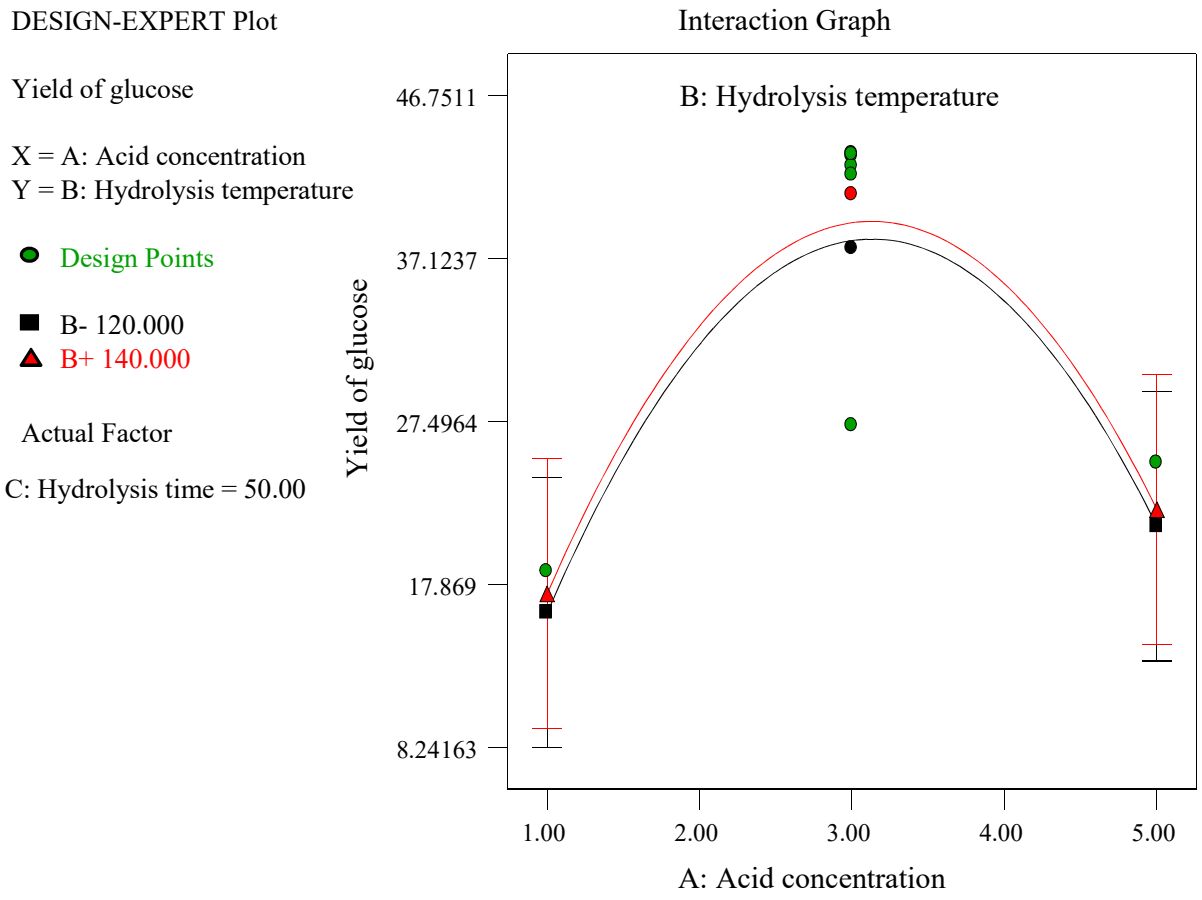

Figure 4.7: Interaction effects of acid concentration and temperature (fixed) on the yield of glucose when the time was at the center point

The interaction effects of acid concentration and hydrolysis temperature on the yield of glucose when, time was selected at the center point, are shown in figure 4.7 above. The interaction graph of acid concentration and hydrolysis temperature at lower and higher time the graph has uniform shape that means the interaction graph is almost parallel it can't cross one over the other. Therefor their effect is the same at lower and higher hydrolysis time that means the interaction graph has insignificance effect on glucose yield. But generally, at lower acid

concentration and temperature, glucose yield decreases and at the center high glucose yield was given. At lower level the cellulose might not hydrolysis to simple glucose and at higher acid concentration and temperature the cellulose might convert to other molecules rather than glucose.

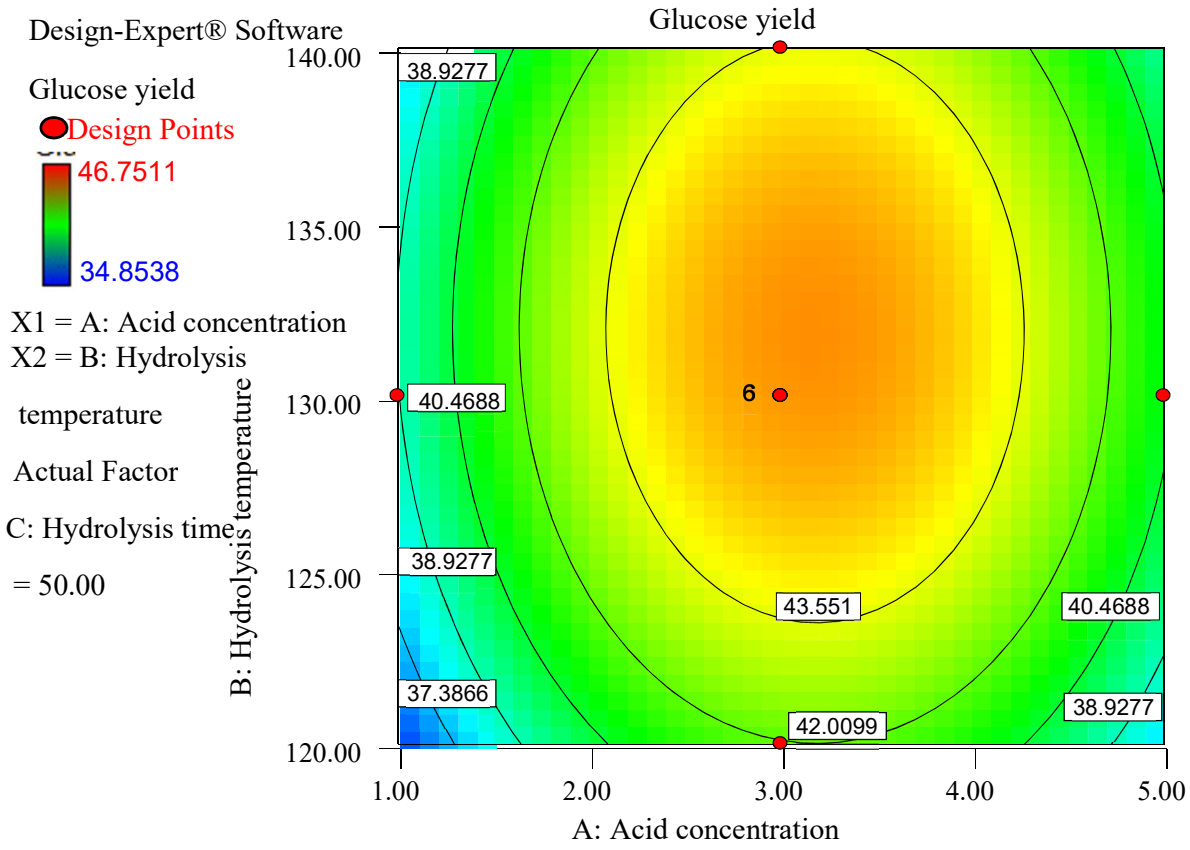

Figure 4.8: Contour plots of the effects of acid concentration and temperature on glucose yield

The effects of processing variables on glucose yield were analyzed using contour plots as well. Figure 4.8 showed the effects of two independent variables on the response while the other one variable was held constant at the middle range. So, the contour plot graph in the above shows predicted response of glucose yield as a function of hydrolysis temperature and acid concentration. As hydrolysis temperature and acid concentration increases towards the center, the yield was registered higher value as shown from the graph above (yellow red color). Further increasing the value of the parameters (hydrolysis temperature and acid concentration) the yield starts to decrease as shown from the figure (blue green color).

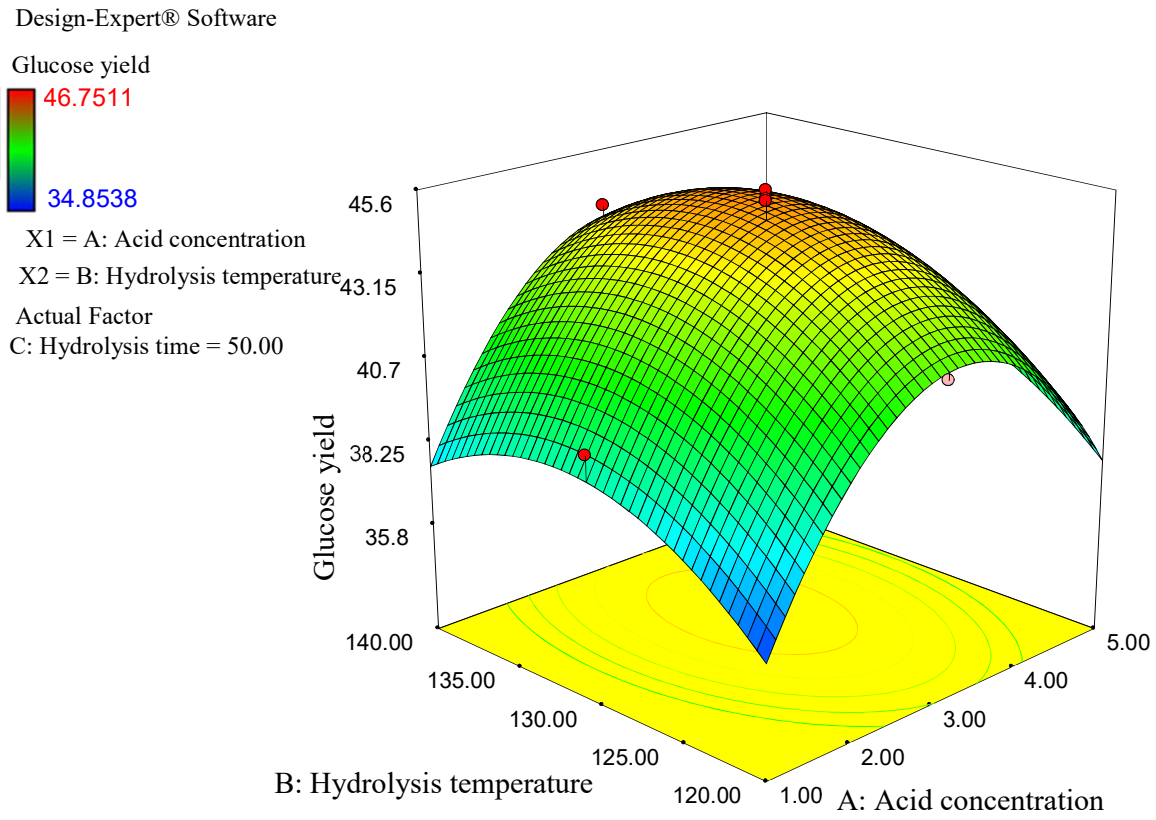

Figure 4.9: Response surface plots (3D) of the effects of acid concentration and temperature on glucose yield

The best way to show the effects of parameters for glucose yield is to generate response surface plots. The response surface plot figure 4.9 obtained from hydrolysis temperature and acid concentration was conical shape. This response surface shows that, at the minimum value of temperature and acid concentration the yield was minimum around 34.85% (blue green color) at the corners (blue color) and at the center the yield becomes maximum 46.75% (yellow red color).

4.6.2. The effects of acid concentration and hydrolysis time on glucose yield

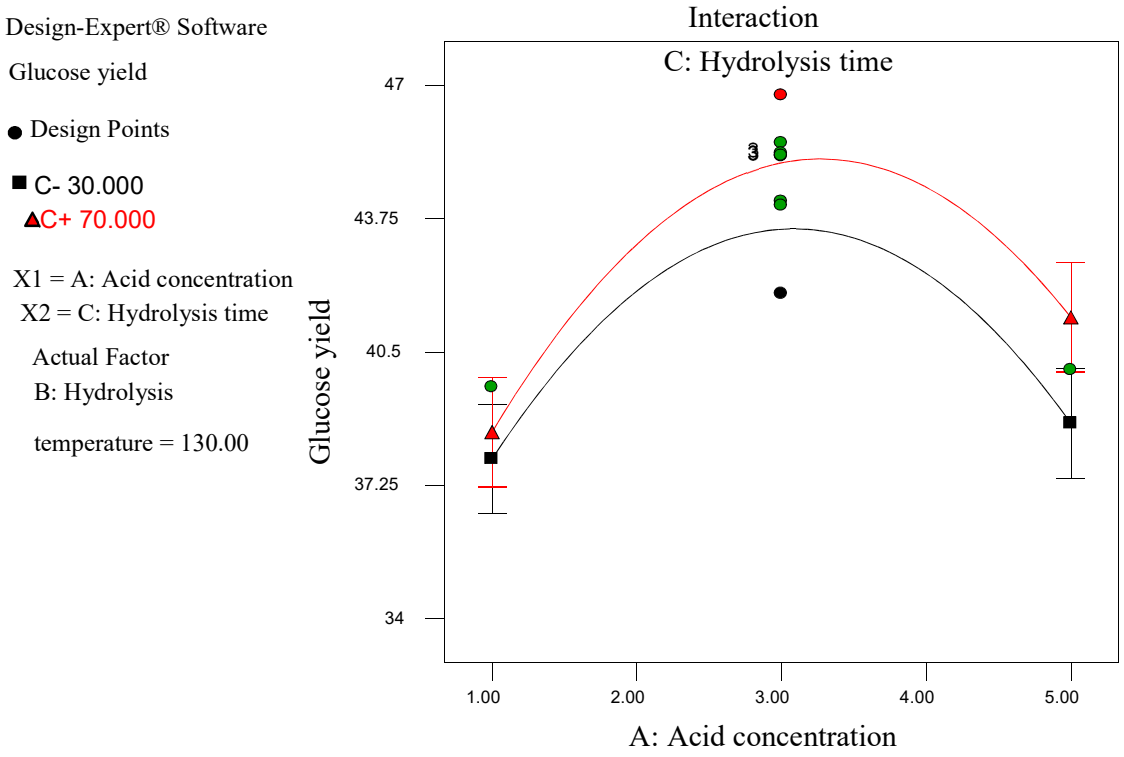

Figure 4.10: Interaction effect of hydrolysis time and acid concentration

The interaction graph between acid concentration and hydrolysis time was not parallel as shown from figure 4.10 above. This implies that the interaction graph of acid concentration and hydrolysis time can affect for the yield of glucose when temperature was changed from minimum to maximum values. Generally, as the graph indicates when the levels of acid concentration increases hydrolysis resulted in higher yield of glucose. However, after some increments of acid concentration, the yield of glucose became decreases since the possible formation of other molecules instead of glucose formation due to high acid concentration. Similarly, at low and high time, the yield of glucose decrease.

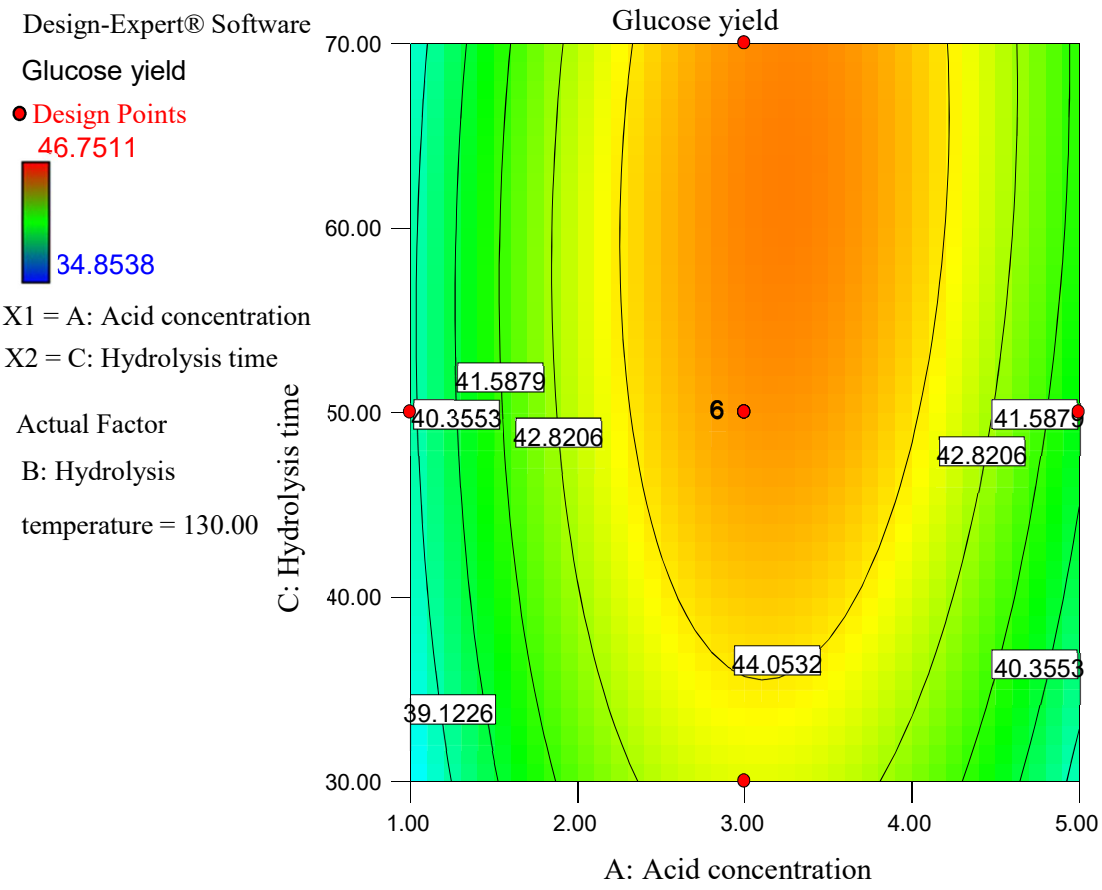

Figure 4.11: Contour plot of the effects of hydrolysis time and acid concentration on glucose yield

It can also possible to analyze the effects of processing variables on glucose yield using contour plots which shows predicted response of glucose yield as a function of hydrolysis time and acid concentration as shown in figure 4.11 above. This contour plot shows higher glucose yield at the center (yellow red color) when both hydrolysis time and acid concentration increase towards the center. But at lower and higher-level acid concentration and hydrolysis time, the value of glucose yield becomes lower as shown from the graph (blue green color).

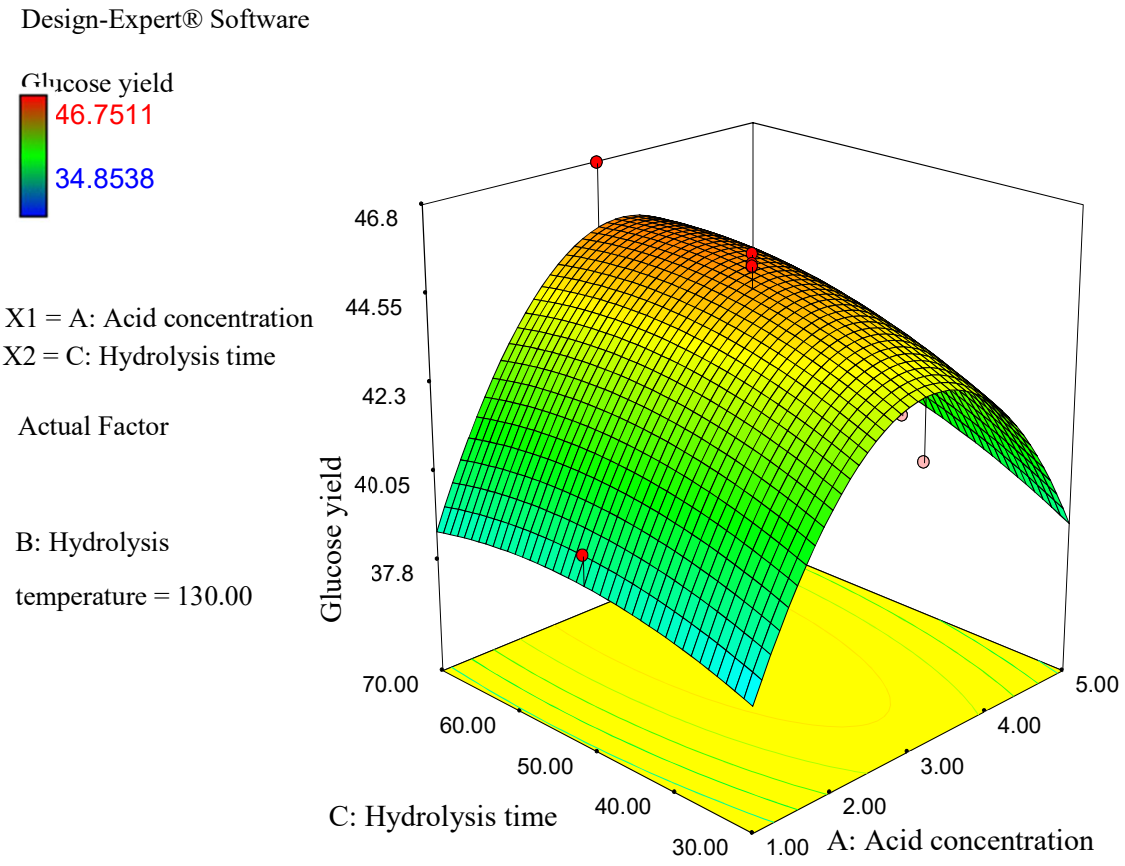

Figure 4.12: The effect of hydrolysis time and acid concentration in response surface (3D) plot

The response surface plot is the best method to analyze the effect of parameters on yield by showing 3D form as shown in the figure 4.12 above. In the above graph at the corners that means at minimum and maximum values of hydrolysis time and acid concentration shows minimum value of glucose yield (blue green color) and at the center point (middle point of parameters) the graph shows maximum yield of glucose (yellow red color). In general, it is simple to understand the effect of process parameters by using response surface plot on our yield.

4.6.3. The effects of hydrolysis temperature and time on glucose yield

For the interaction effects of hydrolysis time and temperature on the yield of glucose when,

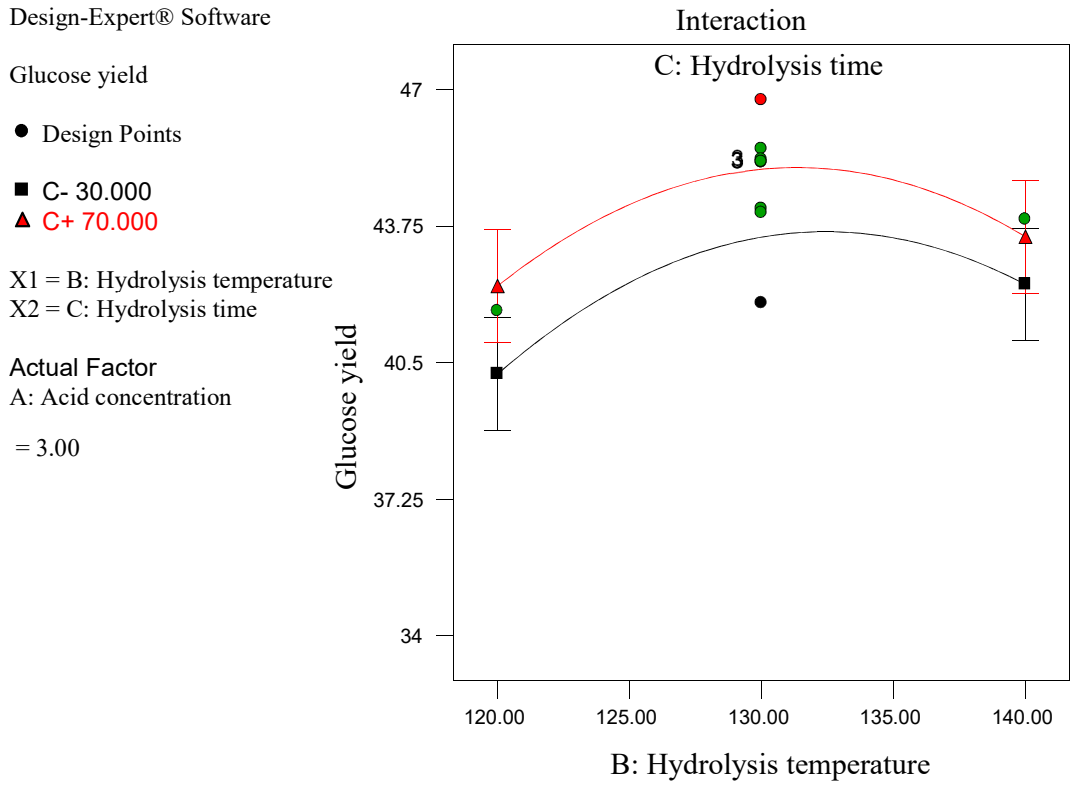

Figure 4.13: The effects of temperature and time (fixed) on the yield of glucose, when acid concentration was at the center

acid concentration was at the center point, were shown in figure 4.13. Since the plot of the two parameters is not parallel the interaction graph has some effect on the yield at different values of acid concentration. But in general case, as hydrolysis temperature increases yield of glucose also increases. However, as you seen from the graph after some increments of temperature, the yield of glucose became slightly decreases since the possible formation of other molecules instead of glucose formation due to high temperature. Similarly, at low and high time, the yield of glucose decreases.

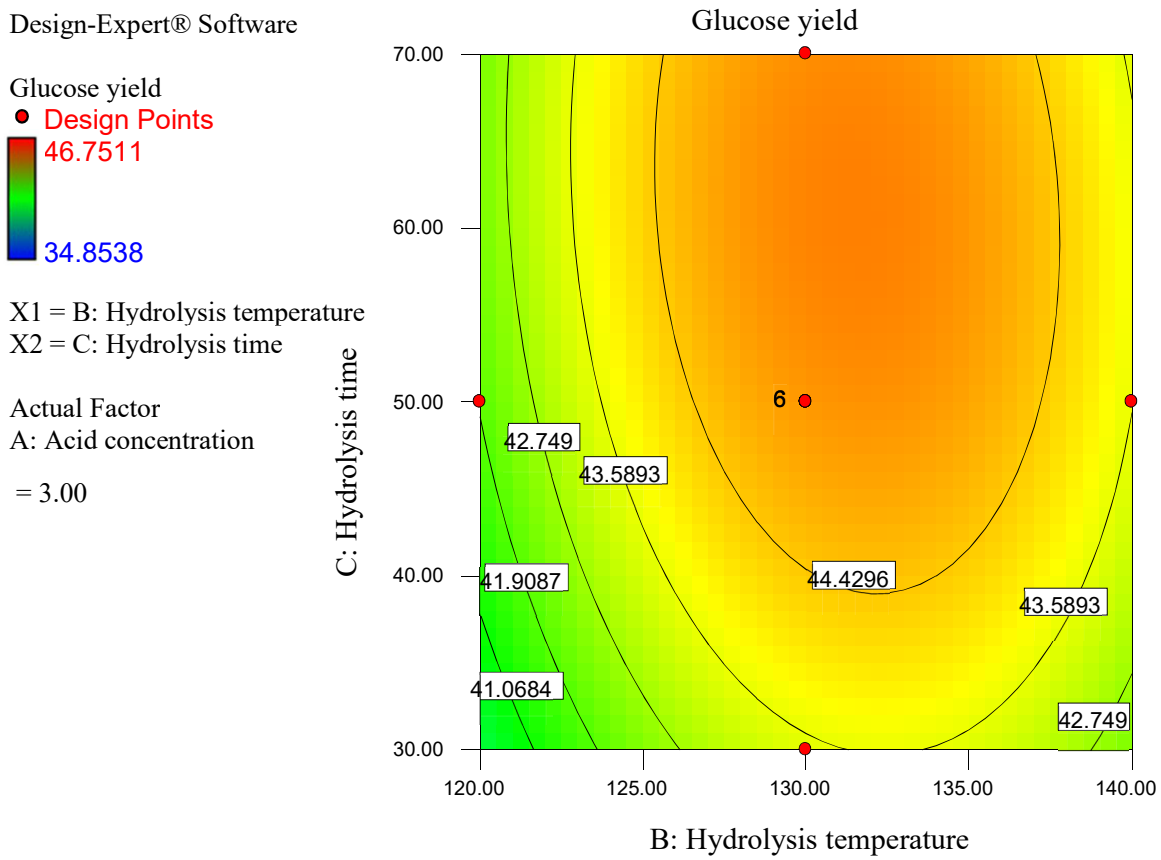

Figure 4.14: Contour plots of the effects hydrolysis time and temperature on glucose yield

From the contour plot graph showing predicted response of glucose yield as a function of hydrolysis time and hydrolysis temperature was shown in figure 4.14. most probably the contour plot shows minimum value at lower values slightly at higher value of hydrolysis temperature and time (green color) and maximum value at middle of hydrolysis temperature and time (yellow red color). At lower and higher values of hydrolysis time with lower value of hydrolysis temperature gives minimum value of the yield around 41-42% as shown from the figure (green color) and the vice versa gives somewhat higher than this around 42-43% as shown from the figure (green yellow color).

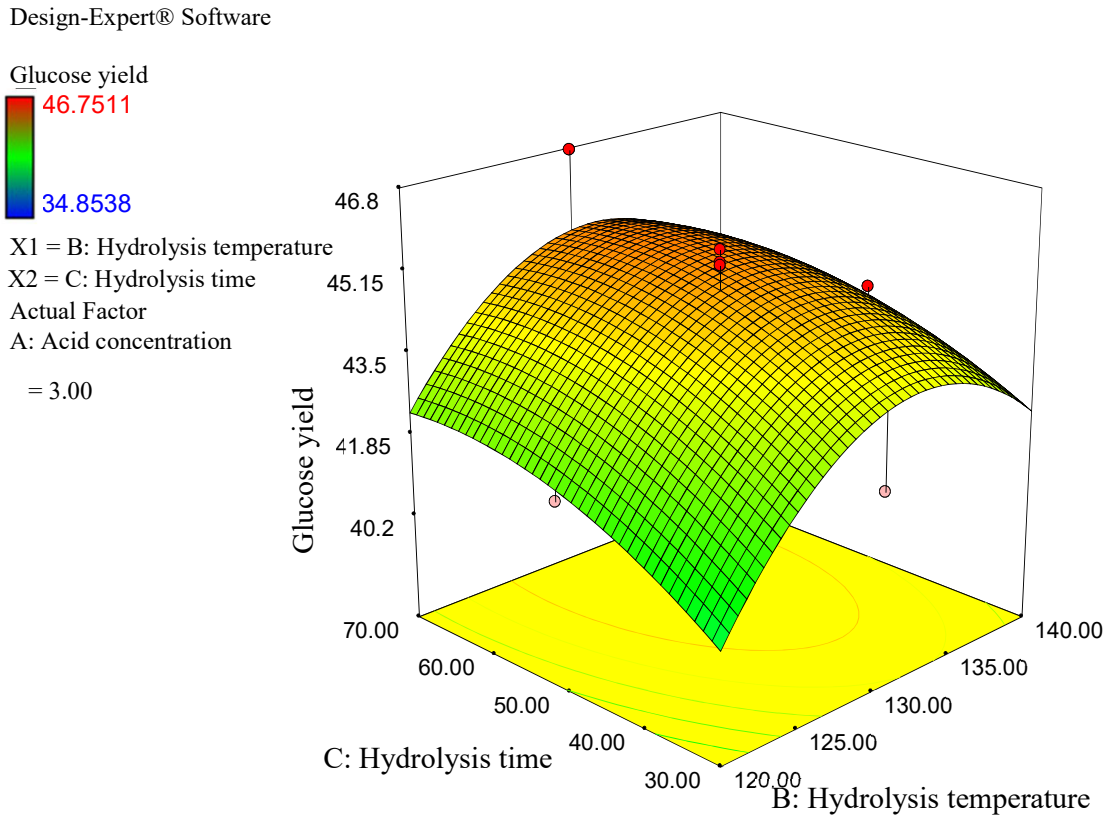

Figure 4.15: Response surface plots of the effects of hydrolysis time and temperature on glucose yield

The response surface plot obtained from hydrolysis time and hydrolysis temperature when acid concentration was held at the center was shown in the figure 4.15 above. This figure shows a maximum value at the center points (yellow red color) and its value decrease as it moves toward the corner (green color). This shows hydrolysis time and temperature can highly affect to glucose yield and also it can suggest that based on the model developed there were well-defined optimum operating conditions.

4.7. Process Optimization

The yield of glucose can be optimized or maximized by manipulating the process parameters such as acid concentrations, hydrolysis temperature and hydrolysis time in the process optimization. In order to optimize the response, the function of desirability was applied using Design Expert software version 7.0. In this study, numerical optimization was chosen which presents a comprehensive and up-to-date description of the most effective methods in process optimization. To do so, the upper and lower limit of each variable (H_2SO_4 concentration, hydrolysis temperature and hydrolysis time) and its response as predicted by the model were provided based on the contour and surface plot obtained previously. The ultimate goal of this optimization was to obtain the maximum response by minimizing the model variables. The table below shows constraints of each variable and the desired response.

Table 4.6: Constraints of each variable for the optimization of the glucose yield

Constraints			
Type of variable	Goal	Lower Limit	Upper Limit
H_2SO_4 Acid concentration	minimize	1	5
Hydrolysis tempreture	minimize	120	140
Hydrolysis time	minimize	30	70
Glucose yield	maximize	34.8538	46.7511

Table 4.7: Optimum conditions for maximization of the yield (glucose yield)

Solutions found						
Number	Acid concentration	Hydrolysis tempreture	Hydrolysis time	Glucose Yield	Desirability	Selecting optimum result
1	2.22	123.78	30.00	41.0215	0.735	Selected
2	2.23	123.76	30.00	41.0195	0.735	-
3	2.23	123.77	30.00	41.0293	0.735	-
4	2.23	123.78	30.00	41.0339	0.735	
5	2.23	123.80	30.00	41.0341	0.735	-
6	2.22	123.79	30.00	41.0271	0.735	-
7	2.23	123.79	30.00	41.0411	0.735	-
8	2.22	123.77	30.00	41.0116	0.735	-

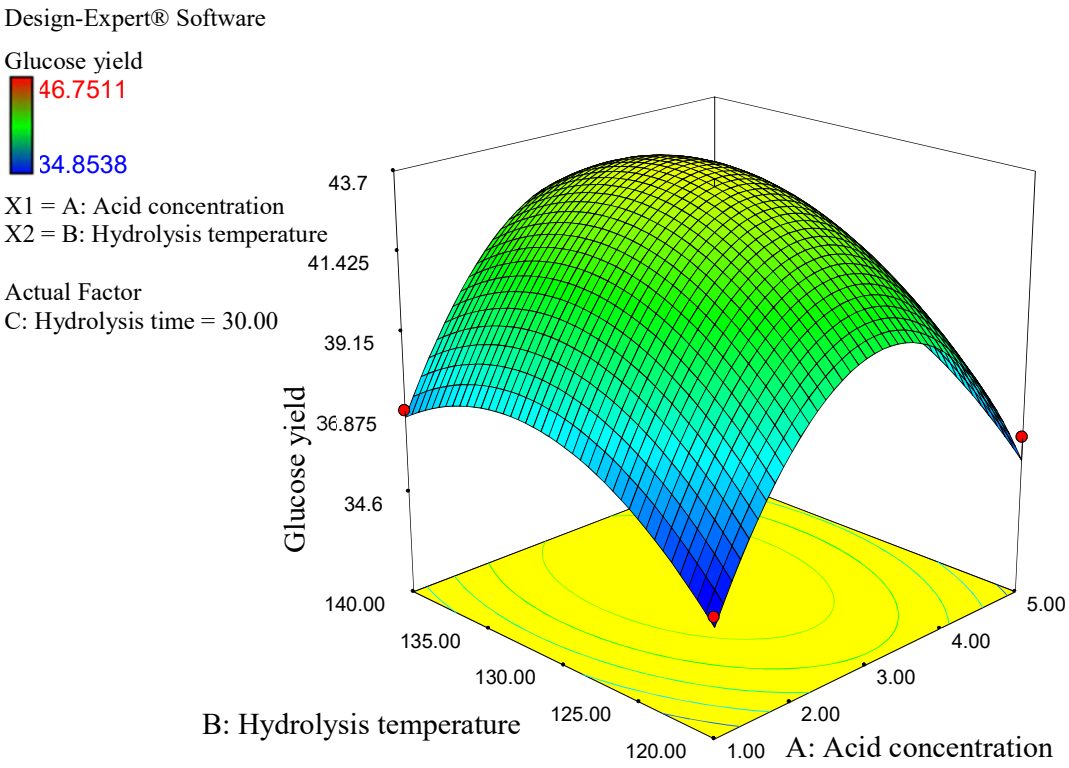

Figure 4.16: Response surface plots for the optimization process of the effects hydrolysis temperature and acid concentration on glucose yield

The optimum response surface figure 4.16, obtained from hydrolysis temperature and acid concentration was conical shape. Hence from the result, there were well defined optimums operating conditions. At optimum hydrolysis temperature and at optimum level of acid concentration and the graph shows an optimum positive effect on the yield of glucose. Therefore, optimization of the process was gives an optimum value parameter like acid concentration 2.22%, hydrolysis temperature 123.78℃ and hydrolysis time 30.0min with optimum yield of glucose 41.0215%.

4.8. Validation of The Model

According to the central composite design result using Design-Expert® 7.0. software, an experiment with acid concentration, hydrolysis temperature and hydrolysis time were conducted in order to study the effects of experimental parameters of the design. As you see from figure 4.11 and 4.12 above, glucose yield of average predicted value was 44.05%. numerical optimization was carried out to maximize the yield of glucose, using the response optimizer in Design expert®7. The optimal values of test variables were calculated as 30 min, 123.75 °C and 2.22% v/v acid concentration. The analyses show that the average yields of glucose were 41.02%. This is in good agreement with the predicted one. As a result, the model was considered to be accurate and reliable for predicting the yield of glucose from coffee husk using dilute acid hydrolysis.

4.9. Cell Counting Result

The number of cells was counted using chambers method based on equation 3.5.

Total number of cells in the fermentation = number of cells * dilution factor * amount of growth media (ml)which is added to the sample

Total number of cells in the fermentation = $350*1x10^4/ml*21ml = 73.5x10^6$ cells

4.10. Determination of Bio-Ethanol Yield

From the experimental work experiment number 14 gives maximum glucose yield (46.75%) and that yield was selected for further analysis for the production of ethanol. This sample was ferment in the fermenter and separate the produced ethanol using rotary distillation at 78°C temperature and 3hrs time. The yield of ethanol produced was then calculated by using equation 3.5 as follows.

Standard ethanol concentration (mass density) C_S = 1.6 mg/mL (given)

Sample ethanol concentration (mass density) C_u = 1.78 mg/ml (measured)

Absorbance of standard A_S = 0.471 (measured)

Absorbance of sample A_u = 0.829 (measured) then yield would be calculated as:

Percentage of ethanol in sample (%) = $\frac{Cs}{Cu}) (\frac{As}{Au})$ x 100

$$= \frac{1.6\ \text{mg/ml}}{1.78\ \text{mg/ml}}\left(\frac{0.4\ \ 71}{0.829}\right) \text{x } 100$$

$$= 51.03\%$$

4.11. Fourier Transform Infrared Spectroscopy (FTIR) for Bioethanol Characterization

Alcohols are characterized using FTIR absorptions linked with the O-H, C-O and the C-H stretching vibrations. When run as a liquid film the region 3500-3200 cm^{-1} with a very powerful and broad band indicated the O-H stretch of alcohols, while the region 1260-1050 cm^{-1} confirms the C–O stretch. The groups at around 2880 and 2930 cm^{-1} were assigned as the symmetric stretching modes of the $–CH_2$ and $–CH_3$ groups, respectively (Zabed et al., 2014). This shows that the product obtained from coffee husk was ethanol based on the graph shown below.

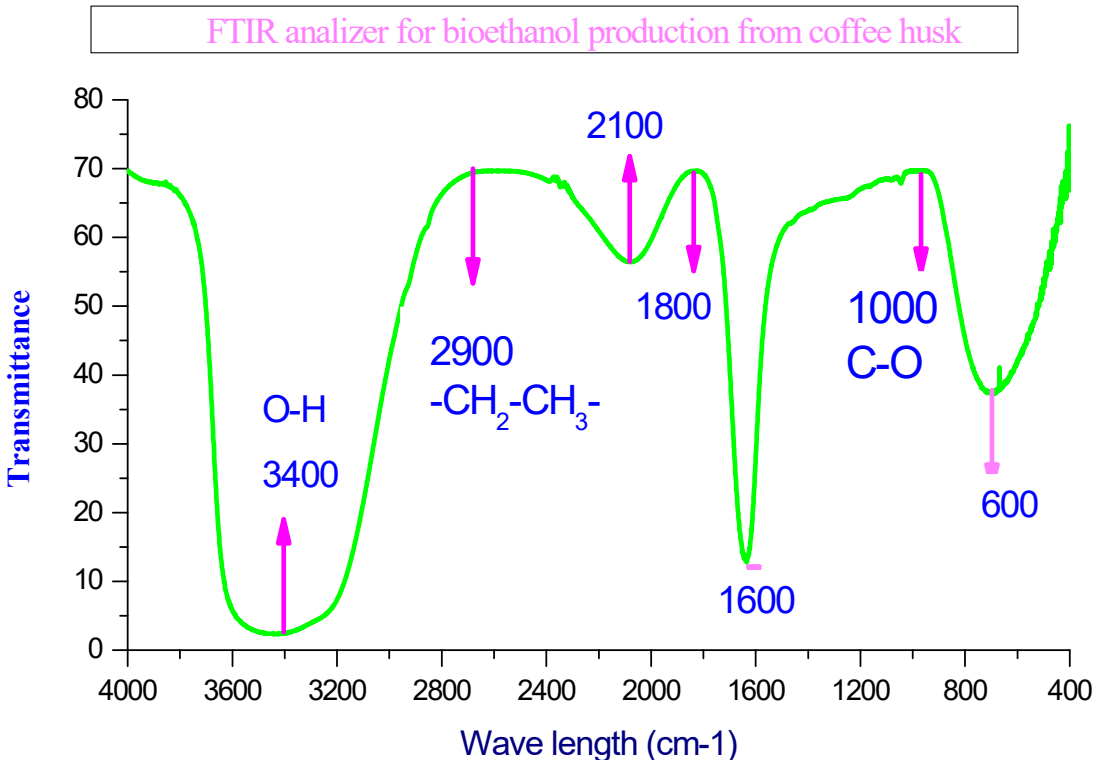

Figure 4.17: FTIR analyzer for bioethanol produced

5. CONCLUSION AND RECOMMENDATION

5.1. Conclusion

Coffee generates large amount of coffee by-products/coffee husk during its dry processing methods. Its great importance in terms of renewable energy production and environmental pollution protection to convert these residues to value added products. Consequently, in this work, coffee husk residue was valorizing to produce bioethanol by the method of separate hydrolysis and fermentation (SHF) in the laboratory scales. Here coffee husk residue was characterized before the experimental work was began and the results were found to be more or less similar to the values obtained from literature. Based on the experimental analysis for testing the composition of coffee husks by using benedict solution shows a presence of reducing sugars(glucose). This indicates that valorization of coffee husk to bio-ethanol is a best option in determining the limitation of renewable source of energy and also in minimizing the environmental pollutions. To understand the effect of parameters 20 experiments were conducted with a fixed size less than one mm in the laboratory. Response surface methodology based on the Central Composite experimental design(CCD) was then used to analysis and to optimize the experimental operating parameters of the hydrolysis processes. Quadratic regression models were developed in this study and used to predict the reducing sugar yield from hydrolysis step. The process variables were varied three time with each experiment using response surface CCD design methods. The one which was hydrolyzed with 3% dilute H_2SO_4, 130℃ hydrolysis temperature and 70min hydrolysis time gives maximum glucose yield. From 33.5g of coffee husk powder 46.75% of yield (glucose) obtained from experiment number 14 and this experiment was selected for further analysis (fermentation and distillation) for the bioethanol production and gives 51.03% ethanol yield. Based on central composite design ANOVA was carried out to determine the statistical significance of the quadratic response surface model, in which the p-value of the model was less than 0.0001, which indicates the model was statistically significant and the model parameters (acid concentration, hydrolysis temperature and time were found to have a significant effect on the hydrolysis yield. The coefficient of determination (R^2) was found to be 0.9655, which indicates that 96.55% of the experimental data were relevant and only 3.55% of the total variations was not explained by the model. The effects of the process parameters were conducted by model graphs for individual effects and interaction effects using counter and response surface plot of experimental parameters on glucose yield. Numerical optimization was carried out to maximize the yield of glucose, using the response optimizer in Design expert®7.0 in process

optimization. The bioethanol produced from coffee husk was characterized and measured by FTIR analyzer and according to that instrument the result contains O-H, C-O, -CH_2, and CH_3 functional groups which indicate the presence of ethanol in the product.

5.2. Recommendation

Based on the current study of this work or investigation the following recommendations are forwarded:

- The development of processes for valorization of coffee husks to bio-ethanol is feasible in terms of energy sector and also an environmental friendly process for waste management. So, this research should be integrated to large scale in our country which can solve the shortage of energy and environmental pollution as well and there should be an economic feasibility analysis on a large-scale basis of the overall conversion of coffee husk residue to bio-ethanol for the purpose of commercialization.
- It is also possible to produce other very important products from coffee husk rather than bioethanol.
- Further investigation should be done to characterize the reducing sugar produced during the hydrolysis step.
- More work should be undertaken to optimize the glucose yield in the process optimization rather than using simply numerical optimization by soft war.
- In addition to characterizing the product by FTIR analyzer it is better to add another characterization method to be have enough knowledge about the composition and properties of the new product.

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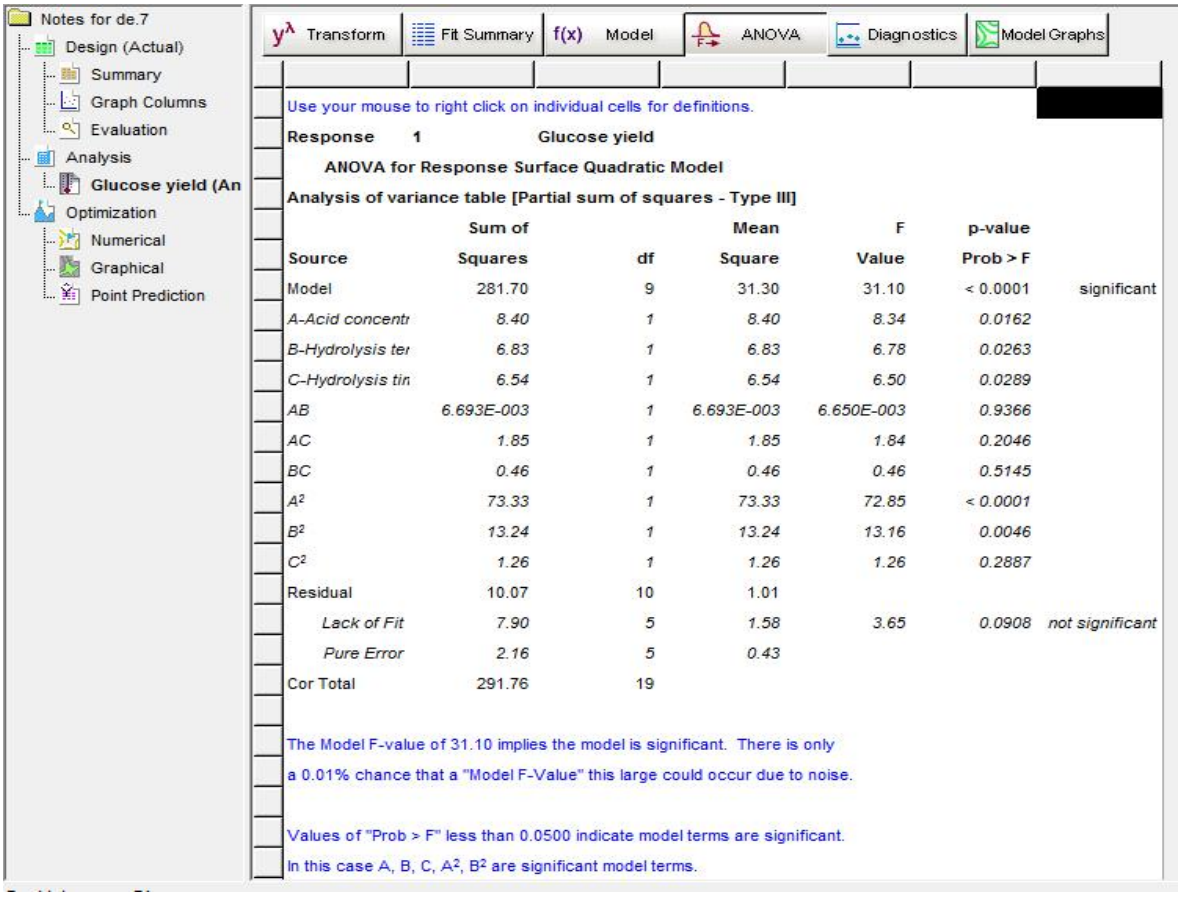
APPENDIX

Appendix A: Properties of Ethanol

Physical Properties	Descriptions
Molecular formula	CH_3CH_2OH
Molar mass	40.06844 g/mol
Appearance	Colorless clear liquid
Density	0.789 g/cm^3
Melting point	- 114.3 ℃
Boiling point	78.4℃
Viscosity	1.200 mpa.s(cp) at 20℃
Dipole moment	5.64 fc.fm (1.69 D) (gas)
Flash point	286.15K(13 ℃)

Appendix B: ANOVA in the design expert software for response surface quadratic model



Appendix C: Laboratory work pictures

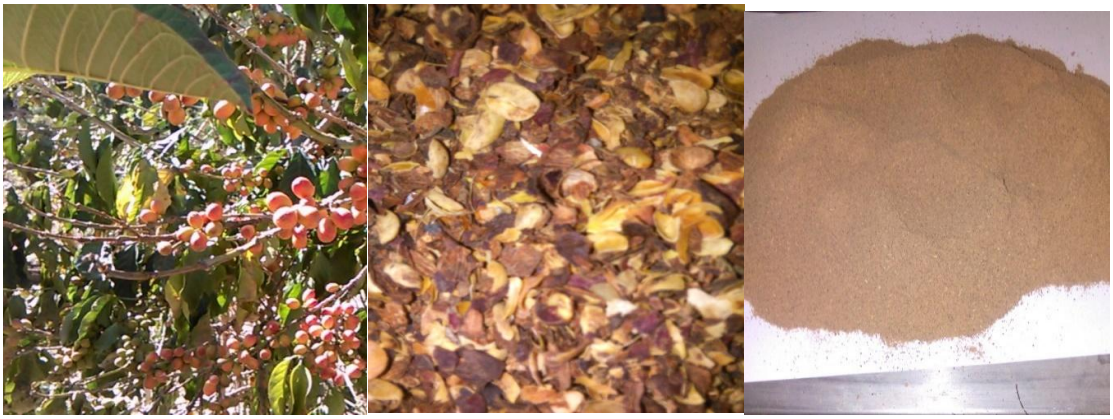


Figure 1: Coffee plant, dry coffee husk and coffee husk powder

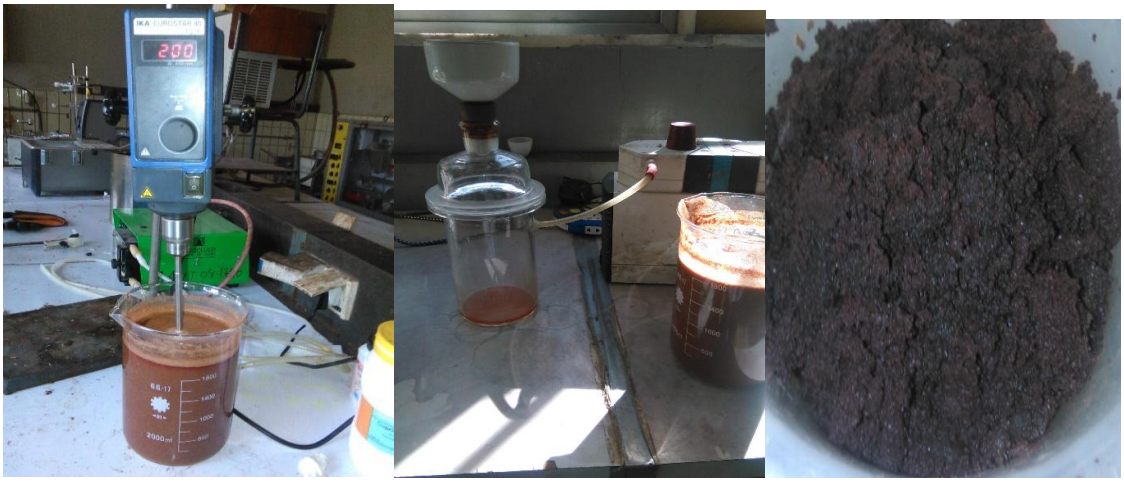


Figure 2: Pretreating coffee husk by stirrer, filter and coffee husk waste (solid part)

Figure 3: Sample hydrolysate, waterbus and samples ready for measuring absorbance

Figure 4: Prepared of standard glucose solution and standard solution after boiled by waterbus

Figure 5: UV spectroscopy, sample and media prepared for fermentation

Figure 6: Fermenter, rotary distillation and final produced ethanol