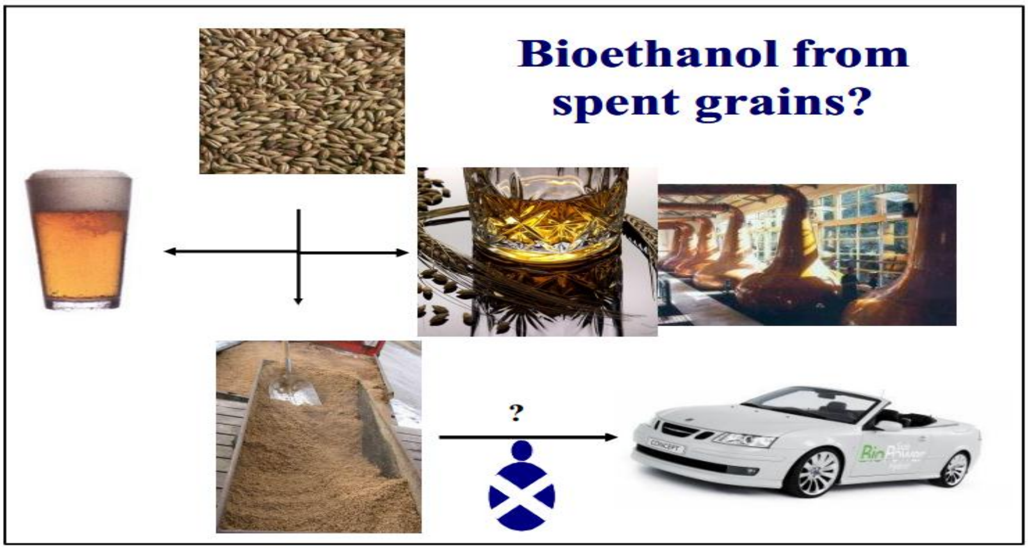
ADDIS ABABA UNIVERSITY
ADDIS ABABA INSTITUTE OF TECHNOLOGY (AAIT)
SCHOOL OF CHEMICAL AND BIO ENGINEERING
PROCESS ENGINEERING STREAM

BIOETHANOL PRODUCTION FROM BARLEY- SPENT GRAIN (BREWERIES SPENT GRAIN)



By: Amare Fissha

JUNE 2016

ADDIS ABABA, ETHIOPIA

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A thesis submitted to the Research and Graduate School of Addis Ababa University, Addis Ababa Institute of Technology, School of Chemical and Bio engineering in partial fulfillment of the requirements for the attainment of the Degree of Master of Science in Chemical Engineering under Process Engineering Stream.

By: Amare Fissha

Advisor: Dr.S.Anuradha Jabasingh (Associate Professor)

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DECLARATION

I declare that this thesis for the M.Sc. Degree at Addis Ababa University, hereby submitted by me, is my original work and has not previously been submitted for the degree at this or any other university, and that all resources of materials used in this thesis have been duly acknowledged.

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This thesis has been submitted for examination with my approval as University Advisor.
Name: Dr. S. Anuradha Jabasingh
Signature: _________
Date: _________

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Table of Contents

Contents **Page**

List of Tables

List of Figures

Figure No. **Description** **Page**

List of Acronyms

AFER	Ammonia Fiber Explosion
ANOVA	Analysis of variance
BSG	Barley spent grain
CCD	Central Composite Design
CI	Confidence Interval
Cp	specific heat capacity
E	Purchased Equipment cost
DC	Direct cost
DF	Degree of Freedom
FCI	Fixed capital investment
FTIR	Fourier Transform Infrared Spectroscopy
IC	Indirect cost
MPa	Mega Pascal
MTBE	Methyl Tertiary Butyl Ether
pH	potential of hydrogen
rpm	revolution per minute
R^2	Regression Coefficient
RSM	Response Surface Methodology
TCI	Total Capital Investment
TDC	Total direct cost
TPC	Total production cost
TRS	Total Reducing Sugar
USA	United State America
WC	Working Cost

Abstract

The objective of this study was production of bioethanol from Barley spent grain by using two-stage diluted acid hydrolysis. The production process was carried out in four main stage such as pretreatment, hydrolysis (first and second stage), fermentation and distillations. The first stage diluted acid hydrolysis was used as chemical pretreatment stage and the process variables were fixed at the best optimum condition (liquid/solid ratio of 8 g/g, 100 mg H_2SO_4/g of dry matter, 17 min reaction time and 121^oC temperature). Before the first stage hydrolysis process was carried out, the wet Barley spent grain was dried using oven at 70^oC temperature for 24 hours. For the second stage hydrolysis, 2^3 full factorial central composite design (CCD) was applied to investigate the effect of temperature (130-150^oC), reaction time (20-30min) and acid concentration (1.5-2%) using Design expert® 6 software. RSM was applied to investigate the interaction effect of hydrolysis process variables and to find the optimum yield of ethanol from BSG. After hydrolysis process sugar content of the hydrolyzate was quantified using quantitative benedict reagent solution. Fermentation of the hydrolyzate was performed using 5g/L Saccharomyces cerevisiae at 30^oC temperature, pH 5.0 and 72h fermentation time for all samples. After fermentation the specific gravity of the produced alcohol was measured by pycnometer (specific gravity bottle) and alcohol concentration was obtained from the relationship between the specific gravity and the proportion of ethanol in alcohol solution at 20^oC (Perry Chemical Engineering Handbook). Significance of the process variables were analyzed using analysis of variance (ANOVA) and second order polynomial function was fitted to the experimental results. Thus, the influence of all experimental variables, factors and interaction effects on the response was investigated. Hydrolysis temperature, time and interaction between temperature and acid concentration have significance effect on the yield of ethanol. As the result of RSM optimization, the best yield of TRS and bioethanol were found at 144.29^oC hydrolysis temperature, 26.26 min reaction time and 1.68% w/w acid concentration. Under these condition 47.60%w/w and 8.33mL per 50 g dry barley spent grain of TRS and bio-ethanol was obtained respectively.

***Keywords**: Bioethanol, Barley spent grains, diluted-acid hydrolysis, and Saccharomyces cerevisiae*

1 INTRODUCTION

1.1 Background of the study

In the 20th century, the world economy has been dominated by technologies that depend on fossil energy, such as petroleum, coal, or natural gas to produce fuels, chemicals, materials and power. The continued use of fossil fuels to meet the majority of the world's energy demand is threatened by increasing concentration of CO2 in the atmosphere and concerns over global warming (Demirbas et al., 2004). The combustion of fossil fuel is responsible for 73 % of the CO2 emission (Sun and Cheng, 2002).

Interest in alternative transportation fuels are growing due to oil supply insecurity and its impending peak, and the imperative to lower GHG emissions from fossil fuel use in order to stave off adverse global climatic changes (Farrell et al., 2006). The main contributors to air pollution are vehicular emissions of GHG and particulate matter. Renewable energies are essential contributors to the energy supply portfolio as they contribute to world energy supply security, reducing dependency of fossil fuel resources, and providing opportunities for reducing emissions of GHG (IEA, 2006). Energy security and climate change imperatives require large-scale substitution of petroleum-based fuels as wellas improved vehicle efficiency.

Bioethanol as fuel has been known over hundred years. In 1860 Nicholas August Otto from Germany employed ethanol as fuel in his internal combustion engine. From the beginning of last century up to 1960, mixed ethanol with gasoline was used widely for transportation in many European countries such as Germany, France, Italy, Sweden, England as well as Brazil and USA. In 1960's the interest to use of ethanol decreased due to the low price of oil (in comparison with the price ethanol). The new interest in Bioethanol once more started in many countries (Brazil in 1979, USA in 1980 and Europe in 1990) owing to technological developments, market factors and some other factors such as national energy security concern and governmental motivations. Nowadays a huge amount of ethanol is produced around the world. For instance, in 2008, USA was the first fuel ethanol producer with 9000 million gallons. In that year Brazil with 6,472.2 and the European Union with 733.6 million gallons was the second and third producer of fuel ethanol in the world respectively. The main raw materials in those countries were sugar or starch (corn, wheat, sugar cane and sugar beets)

Brewery spent grain (BSG) is a by-product of the brewing process, consisting of the solid residue remaining after mashing and lautering. It consists primarily of grain husks and other residual compounds not converted to fermentable sugars by the mashing process. BSG is the most abundant brewing by-product, corresponding to around 85% of total by-products (consisting of 85% malt and 15% wheat) generated and is mainly used as low-value cattle food or discarded as waste. The chemical composition of BSG varies according to barley variety, harvesting time, malting and mashing conditions, and the quality and type of adjuncts added in the brewing process (Huige 1994; Santos et al., 2003). BSG contains mainly hemicellulose in the form of arabinoxylans from the barley grain and cellulose. BSG has the potential to serve as a low-cost feedstock for the production of ethanol since hemicellulose and cellulose content corresponds to 60% w/w of dry BSG (Dehnavi 2009).

1.2 Statement of the Problem

The issue of energy security resulting from the depletion of world petroleum reserves, increase of petroleum price and environmental concerns has stimulated governments and researchers to look for alternative renewable energy sources that are technically feasible, economically competitive and environmentally acceptable. Among alternative energy sources, biofuel derived from biomass, have been gaining increasing attention recently as a replacement for fossil fuels, hence bioethanol is an important biofuel which can be produced from different types of biomass and used for different purpose.

Now a days, the numbers of brewery industries are increasing in Ethiopia and they generate a high amount of barley spent grain. This by-product is mainly used as a low value cattle feed or simply deposited as a waste into land fill. Beside this, Barley spent grain remains from animal feed also creates bad odour on the environment which inturn affects health of people who live around the area, so that Conversion of such waste into biofuel helps to reduce environmental pollution and energy problem of developing countrys like Ethiopia.

The commercial production of fuel ethanol in the world relies mainly on the fermentation of sugar and starch, but production of ethanol from such “first generation” feedstock is often viewed as competing with food production and increasing prices of food. So that productions of bioethanol or biofuel from agricultural wastes such as BSG negotiate the debate of ‘food versus energy’ controversy. Therefore this study was intended to solve such type of problem and to convert this low valuable by product into highly valuable product.

1.3 Objectives of the study

1.3.1 General objective

The general objective of the study was to develop the production process of bioethanol from barley–spent grain (brewer’s spent grain).

1.3.2 Specific objective

The specific objectives of the study were:-

- To quantify the sugar content of barley spent grain after diluted acid hydrolysis
- Evaluate the effect of process variables (reaction time, H_2SO_4 concentration and temperature) in the second hydrolysis stage using response surface methodology (RSM).
- Optimize operating process variables in hydrolysis process (time, acid concentration and temperature) by using RSM to give optimum yield of TRS and ethanol.
- To perform material and energy balance on bioethanol production
- Sizing of equipments for bioethanol production from BSG
- To perform preliminary engineering economic analysis of bioethanol production from Barley spent grain.

1.4 Significance of the study

- This study was performed preliminary analysis of barley spent grain as an input for ethanol production.
- Revealed for researchers to see this very cheap and highly available by-product for other investigation.
- This study also highly contributes in the substitute fossil fuel by biofuel.
- Bioethanol production from BSG is considered a 2nd generation biofuel process since it has no direct conflict with human food, as the case of 1st generation biofuels produced from agricultural crops, such as corn, sugarcane and soybean oil.

2 LITERATURE REVIEW

2.1 Bioethanol

The use of ethanol as an automobile fuel is not a new invention. Already in 1908, Ford's model T could be adjusted to run on either gasoline or alcohol (DiPardo 2000). However, after World War II the interest in using ethanol as a fuel declined because cheap gasoline made from petroleum was available. In 1970's, the interest in fuel ethanol was renewed due to the oil crisis (DiPardo 2000). More recently, ethanol has become used as an additive in gasoline. MTBE (methyl tertiary butyl ether) is used as a gasoline additive to increase the oxygen content and the octane number. During the last few years, the use of MTBE has been banned in several states of USA due to the risk of contamination of water. Many companies have replaced MTBE with ethanol to give the gasoline similar clean burning and octane boosting properties as MTBE-blended gasoline (F.O. Licht's World ethanol and biofuels report 2006; Sun and Cheng, 2002). Today, there are several flexi fuel automobile models (vehicles that can run on mixtures of ethanol and gasoline containing up to 85% ethanol) available from various manufacturers (BAFF, 2006).

About 99% of the fuel ethanol is produced from cultivated crops (BAFF, 2006). Brazil has for a long time been the leading ethanol producer of the world. However, during the last few years USA has increased its production and in 2012 both countries have an annual production of about 16 million m^3 (F.O. Licht's World ethanol and biofuels report, 2006). The Brazilian ethanol is mainly produced from sugarcane. Brazil is the world leader in the use of ethanol as an automobile fuel. In Brazil, the ordinary gasoline, which is used in about 7, 000, 000 cars, contains about 24% ethanol. In addition, 4 million automobiles drive on a blend of 95% ethanol and 5% water (BAFF, 2006). In USA, ethanol is mainly produced from corn.

In Sweden, about 55,000 m^3 of fuel ethanol is produced per year from wheat and about 18,000 m^3 from spent sulfite liquor (Agroetanol AB, 2006; Jordbruksverket, 2006). In Sweden, the ordinary gasoline typically contains 5% ethanol and the number of flexi fuel automobiles is increasing (Jordbruksverket, 2006). The Swedish ethanol production does not cover the demand and therefore Sweden is a net importer of ethanol. However, initiatives have been taken to increase the future national ethanol production. In 2004 ethanol from lignocellulose pilot plant was inaugurated in the city of Örnsköldsvik (Sweden). Agro-ethanol plans to expand its production of ethanol from grain with 150, 000 m^3 in 2008.

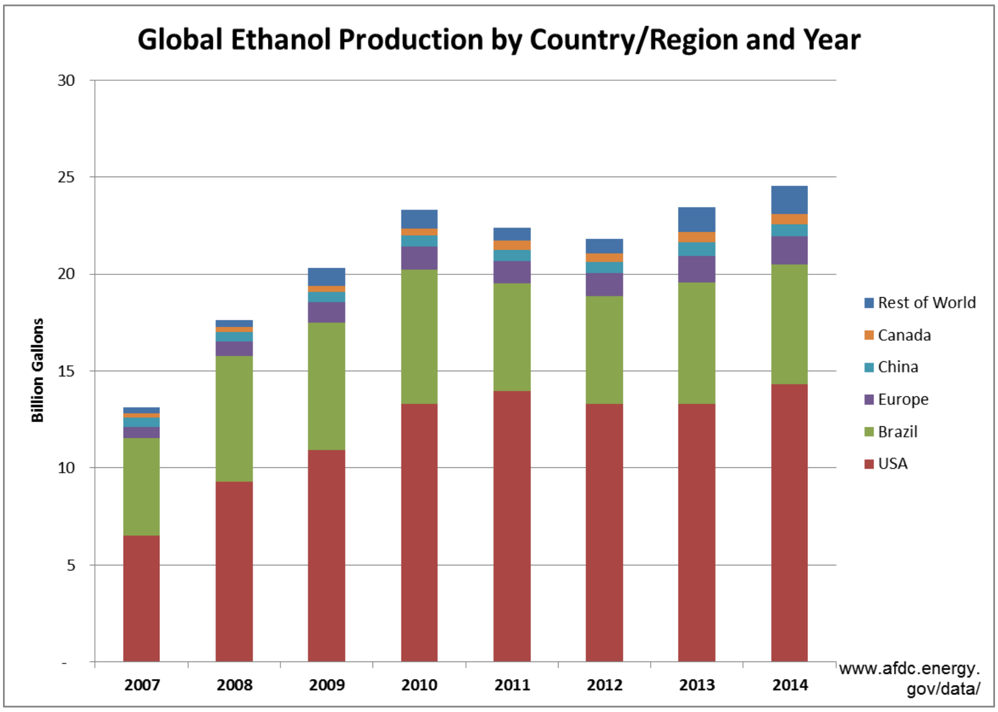
2.1.1 World fuel ethanol production

The commercial production of fuel ethanol relies mainly on the fermentation of sugar and starch, while lignocellulosic ethanol entered the market only recently (Balat et al., 2011). USA and Brazil have been the leading countries in the production of ethanol from corn starch and sugarcane respectively, and the amount of ethanol produced by these two countries together in 2013 was 19,567 billion liters, accounting for 84% of the world's production in that year.

Table 2.1. World fuel ethanol production by country or region ((Million Gallons))

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

Data Source: F.O. Licht, cited in Renewable Fuels Association, Ethanol Industry Outlook 2008-2014 reports. Available at www.ethanolrfa.org/pages/annual-industry-outlook



Data Source: F.O. Licht, cited in Renewable Fuels Association, Ethanol Industry Outlook 2008-2014 reports. Available at www.ethanolrfa.org/pages/annual-industry-outlook

Figure 2.1**.** Global ethanol productions by country/region and year

2.1.2 Ethanol production in Ethiopia

Ethanol production in Ethiopia has been started since 1998/99 in Fincha sugar factory with the capacity of 1,907 m^3 per year. In 2010/11 Ethanol production started in Metahra sugar factory with the capacity of 6,373 m^3 per year. Currently, both factories produce 19,805m^3 ethanol per year (Ethiopia Sugar Corporation). The main raw material for this product is molasses which is a by-product of sugar production from the crystallization process.

The worldwide recent awareness for the use of ethanol to replace petroleum and generation of power along with sugar mill plants should have led to setting up of number of ethanol plants and co-generations. Ethiopia has several sugar real estates such as Fincha, Metehara and Wonji Shoa are among the industries which are run and administered by Sugar Development Agency. Among molasses derived products ethanol takes the largest part, but its utilization must attract the attention of the government policy makers in order to utilize as a bioethanol. Bioethanol or biofuel is ethanol based products that can process into liquid fuels for transport purposes (ESDA, 2015).

Table 2.2. Ethanol produced in Ethiopia since 1998/99

Year	Ethanol produced (liters)		
	Fincha sugar factory	Metahra Sugar Factory	Total
1998/99	1,907,000	_	1,907,000
1999/00	720,000	_	720,000
2000/01	1,790,571	_	1,790,571
2001/02	209,444	_	209,444
2002/03	894,624	_	894,624
2003/04	911,431	_	911,431
2004/05	1,636,047	_	1,636,047
2005/06	6,847,816	_	6,847,816
2006/07	6,066,860	_	6,066,860
2007/08	5,330,337	_	5,330,337
2008/09	5,878,516	_	5,878,516
2009/10	7,116,585	_	7,116,585
2010/11	7,127,895	6,373,775	13,501,670
2011/12	6,794,000	7,658,000	14,452,000
2012/13	7,620,500	7,063,000	14,683,500
2013/14	11,678,000	7,767,000	19,445,000
2014/15	10,999,000	8,806,000	19,805,000

Data source: Ethiopia Sugar Corporation (by interview from sugar corporation management Addis Ababa)

2.2 Feedstocks for Bioethanol Production

Ethanol can be produced synthetically from petroleum or by the microbial fermentation of sugars. The three main groups of raw materials for the production of ethanol by fermentation are sugar, starch, and lignocellulose (Lin and Tanaka, 2006).

Sugar-containing raw materials include sugarcane, sugar beets, fruits and sweet sorghum. The advantage with the sugar-based raw materials is that they can be converted into ethanol directly without hydrolysis. A disadvantage is that many of these raw materials are considered to be a human food resource and will therefore be too expensive to use for ethanol production (Badger, 2002).

Starch-based materials that are commonly used for ethanol production include corn, potatoes, cassava, and various cereal grains. Starch is a biopolymer and defined as a homopolymer consisting only one monomer, D-glucose (Pongsawatmanit et al., 2007). During bioethanol production from starch, it is necessary to break down the chains of this carbohydrate for obtaining glucose syrup, which can be converted into bioethanol by yeasts. This type of feedstock is the most utilized for bioethanol production in North America and Europe (Sanchez, 2007). Starch can be converted to fermentable sugar by a method called the hydrolysis technique. Hydrolysis is a reaction of starch with water, typically performed by cooking the starch at high and low temperatures which is normally used to break down the starch into fermentable sugar. Dextrin oligosaccharides are generated by adding α-amylase and glucoamylase to obtain glucose (Wheals et al., 1999). A disadvantage of using starch-based materials for ethanol production is high hydrolysis cost due to high energy costs of the cooking step and the high costs of the amylolytic enzymes (Lin and Tanaka, 2006).

Lignocellulose-based feedstocks that can be considered for ethanol production are wood residues, agricultural residues, and the spent sulfite liquor from pulp and paper mills. The advantage of using lignocellulose as raw material for ethanol production is due to its abundance and relatively cheap (Wheals et al., 1999).

In contrast to sugar-containing crops, the utilization of lignocellulose as a substrate for ethanol production is difficult or relatively recalcitrant to hydrolysis (Gray et al., 2006). Because of its complex structure, this resists degradation. The basic structure of all lignocellulosic biomass consists of cellulose $(C_6H_{10}O_5)_x$, hemicelluloses $(C_5H_8O_4)_m$, and lignin $[C_9H_{10}O_3.(OCH_3)_{0.9-1.7}]_n$. Production of ethanol from lignocellulosic waste materials, such as sawdust from the forestry industry and BSG from Beer industries have a benefits from the fact that the energy input for the overall process can be kept low. Energy-related expenses for planting, fertilization and harvesting can be avoided if waste materials are used. If lignocellulosic waste materials are used, there will not be any competition for the limited agricultural land available, which instead might be needed for food production (Sun and Cheng, 2002).

2.2.1 Lignocellulosic Material as Sources of Ethanol

Lignocelluloses biomass is an organic residue which consists of mainly cellulose, lignin and hemicelluloses, whose basic units are sugars that can be fermented into ethanol or other chemical. These structural materials are produced by plants to form the cell walls, leaves, stems, stalks, and woody portions of the plant. The carbohydrate polymers (cellulose and hemicelluloses) are tightly bound to the lignin. Up to 80% of the lignocelluloses are polysaccharide (Kaparaju et.al., 2009).Lignocelluloses plant structures also contain a variety of plant-specific chemicals in the matrix, called extractives (resins, phenolic, and other chemicals), and minerals (calcium, magnesium, potassium, and others).

Lignocellulosic biomass comprising forestry, agricultural and agro-industrial wastes are abundant, renewable and inexpensive energy sources. Such wastes include a variety of materials such as sawdust, poplar trees, sugarcane bagasse, waste paper, brewery spent grains (BSG), switch grass, straws, stems, stalks, leaves, husks, shells and peels from cereals like rice, wheat, corn, sorghum and barley. Lignocellulose wastes are accumulated every year in large quantities, causing environmental problems. However, due to their chemical composition based on sugars and other compounds of interest, they could be utilized for the production of a number of value added products, such as ethanol, food additives, organic acids, enzymes, and others. Therefore, besides the environmental problems caused by their accumulation in the nature, the non-use of these materials constitutes a loss of potentially valuable sources (Mussatto and Teixeira, 2010).

The major constituents of lignocellulose are cellulose, hemicellulose, and lignin, polymers that are closely associated with each other constituting the cellular complex of the vegetal biomass. Basically, cellulose forms a skeleton which is surrounded by hemicellulose and lignin (Figure 2.2).

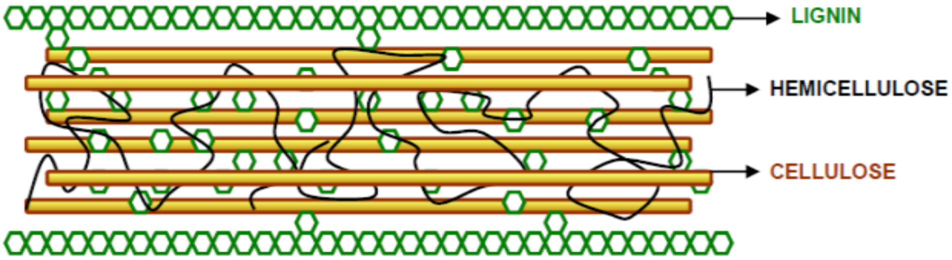
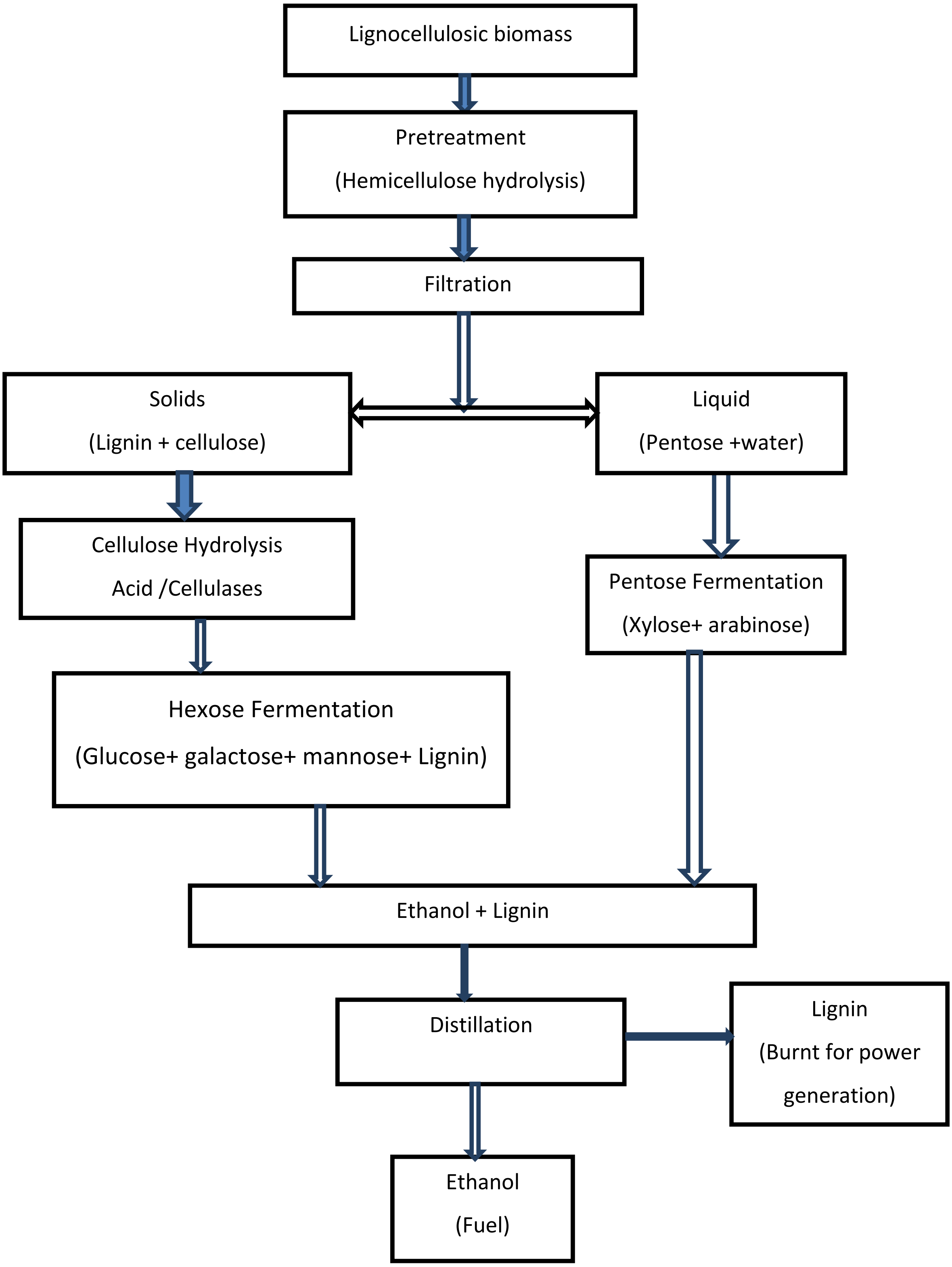


Figure 2.2. Representation of lignocellulose structure, showing cellulose, hemicellulose and lignin fractions (Mussatto and Teixeira, 2010



Data sources: United States Departments of Energy and Renewable Fuel

Figure 2.3. Overview of the process for ethanol production from lignocellulosic biomass

2.2.2 Composition of Lignocellulosic material

Cellulose

Cellulose is a polysaccharide of hundreds or thousands of molecules of glucose with the formula $(C_6H_{10}O_5)n$. Cellulose molecules consist of long chains of glucose molecules like starch molecules with a different structural configuration. The structure of cellulose plus the encapsulation of cellulose by lignin in lignocellulosic materials makes cellulosic materials more difficult to hydrolyze than starch polymers (Harinen, 2004).

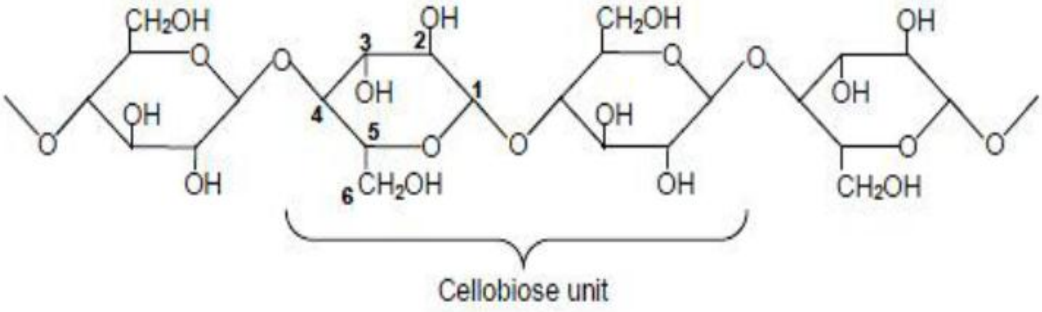


Figure 2.4. Schematic illustration of the cellulose chain

Hemicelluloses

It is a complex polysaccharide made from a variety of five- and six-carbon sugars. While cellulose is crystalline, strong, and resistant to hydrolysis, hemicellulose has a random, amorphous structure with little strength, as such it is easily hydrolyzed by dilute acid or hemicellulase enzymes. It consists of several matrix polysaccharides (hetero-polymers), such as arabinoxylans, present a long with cellulose in almost all plant cell wall.

Hemicelluloses differ in three ways from cellulose; by presence of shorter chain, branching of the main molecule and composition of several sugar units (Idi and Mohamad .,2011).Hemicelluloses include long chains of sugar molecules consisting of: (1) five-carbon sugars (D-xylose, L-arabinose), (2) six-carbon sugars (D-galactose, D-glucose, D-mannose) (3) uronic acids

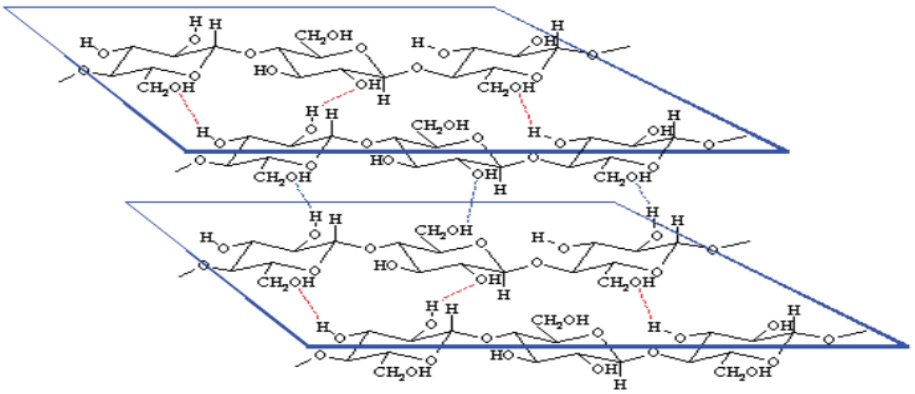


Figure 2.5. Structure of hemicellulose (Idi and Mohamad., 2011)

Table 2.3. Differences between cellulose and hemicellulose

Cellulose	Hemicellulose
Consists of glucose units	Consists of various units pentose and hexoses
High degree of polymerization	Low degree of polymerization
Forms fibrous arrangement	Do not form fibrous arrangement
Presents crystalline and amorphous regions	Present only amorphous regions
Slowly attacked by diluted inorganic acid in hot conditions	Rapidly attacked by diluted inorganic acid in hot conditions
Insoluble in alkalis	Soluble in alkalis

Lignin

Lignin is a highly complex, three-dimensional polymer of different phenyl propane units, which are bound together by ether and carbon-carbon bonds. It is one of the most abundant and important polymeric organic substance in the plant. Lignin is unusual because of its heterogeneity and lack of a defined primary structure. Few lignin structures have been known, but generally their structures remain unknown. Although there are great numbers of microorganisms, which are able to utilize hemicelluloses and cellulose, relatively few strains have the ability to decompose the lignin effectively (Laser et al., 2002).

Extractives and ash

Any numbers of different compounds (resins, phenolic, and other chemicals) in biomass that are not an integral part of the cellular structure are called extractives. These compounds can be extracted from biomass by means of polar and non-polar solvents including hot or cold water, ether, benzene, methanol, or other solvents that do not degrade the biomass structure.

Table 2.4. Main component of lignocellulose wastes (Mussatto and Teixeira, 2010).

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

2.2.2.1 Barley spent grain

Barley spent grains are Lignocellulosic materials, which are the main by-product of the brewing industry corresponding to around 85% of the total by-products. This byproduct of beer brewing consist of the residue of malt and grain which remains after the mashing and lautering; it is primarily a mixture of grain and grain husks from which a majority of its sugars were extracted. They are a rich source of lignocellulose, which may be converted to fermentable sugars. In addition, some investigation on the enzymatic hydrolysis of BSG has been carried out in order to release the ferulic and p-coumaric acids with an esterase and a xylanase. Although a huge amount of this byproduct is produced every year, only a few investigations have been carried out to consider it as a low-cost raw material for energy production (Mussatto et al., 2006; Santos et al., 2003).

The chemical composition of BSG varies according to the barley variety, harvesting time, malting and mashing conditions, and the quality and type of adjuncts added in the brewing process (Santos et al., 2003). Thus the composition of BSG as described in the literature is variable containing mainly hemicellulose in the form of arabinoxylans from the barley grain and cellulose (Mussatto et al., 2006; Santos et al., 2003) and has the potential to serve as a low-cost feedstock for the production of ethanol.

Table 2.5. Chemical composition of Barley spent grain (Dehnavi 2009)

Component	**% of dry material**
Hemicellulose	32.5
Cellulose	27.6
Lignin	13.4
Extractives	12.9
Ash	3.4
Water	10.2

Use of Barley Spent Grain (BSG)

Lignocellulosic substrates, being cheap and readily available, have recently gained considerable interest because of their possible use in secondary fermentation processes. However, the utilization of brewery's spent grain is limited especially in developing countries and new ways of making use of this residue would be beneficial for the process economy. Generally BSG is used:

- Animal nutrition and feed formulations
- Production of construction bricks
- Metal adsorption and immobilization
- Growth medium for microorganisms and enzyme production
- Lactic acid production
- Bioethanol production
- Hydroxycinnamic acids (ferulic and p -coumaric) extraction
- Xylitol production

2.2.2.2 Availability of Barley spent grain in Ethiopia

Currently, there is a rapid growth of brewery industries in Ethiopia. There are about eleven breweries in full function and production. There are also some breweries being expanded and expected to be completed for the next few years. In Ethiopia around 263,736 kg barley spent grain is generated daily. The spent grain from Ethiopian breweries, now a days is only used for animal feed. The individual production capacity of the Brewery industries is presented in Table 2.6 below.

Table 2.6. Capacity of spent grain generated from Ethiopian brewery industries

No	Name of Brewery in Ethiopia	Capacity of spent grain generated Kg per day
1	Dashen(Gonder)	31,000
2	Dashen (debrebrhane)	21,830
3	Saint George Hawassa	32,980
4	Saint George Addis Ababa	33,592
5	Saint George Kombelcha	32,640
6	Meta	21,930
7	Bedele	21,930
8	Harar	22,950
9	Waliya	31,980
10	Habesha	21,780
11	Raya	20,500
	Total	**263,736**

Data sources: Direct phone interview with the industry production managers

2.3 Bioethanol production process

The major unit processes which involves in the production of bioethanol from Lignocellulosic material are pretreatment, hydrolysis, fermentation, and fractionation.

2.3.1 Pretreatment

The goal of the pretreatment process is to remove lignin and hemicellulose, reduce the crystallinity of cellulose, and increase the porosity of the lignocellulosic materials. Pretreatment must meet the following requirements: (1) improve the formation of sugars or the ability to subsequently form sugar by hydrolysis, (2) avoid the degradation or loss of carbohydrate,(3) avoid the formation of byproducts that are inhibitory to the subsequent hydrolysis and fermentation processes, and (4) be cost-effective.

Pretreatment methods can be roughly divided into different categories: physical (milling and grinding), physicochemical (steam pretreatment/auto-hydrolysis), chemical (alkali, dilute acid, and organic solvents), biological or a combination of these. The above pretreatment technologies have been promising for cost-effective pretreatment of lignocellulosic biomass for biological conversion to fuels and chemicals.

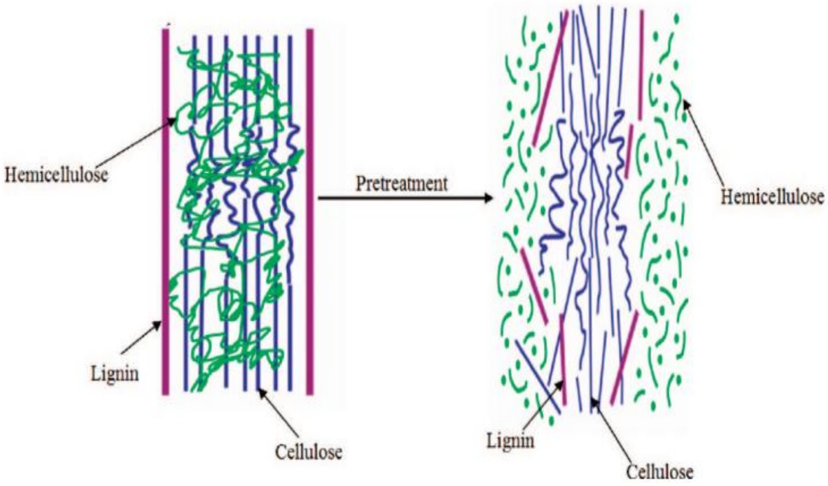


Figure 2.6. The role of pretreatment in the conversion of biomass to fuel (Hsu et al., 1980)

2.3.1.1 Physical pretreatment

Mechanical pulverization

Lignocellulosic biomass can be pulverized by chipping, grinding, shearing, or milling. The goal of mechanical pulverization is to reduce the particle sizes of the biomass and to increased surface area to volume ratio leads to improved cellulose hydrolysis. Some of the crystalline structure of cellulose is also destroyed using these methods, though the amount varies according to the type of biomass and power applied in milling or grinding. A vibration ball mill is the most effective mechanical tool for breaking the crystalline structure of cellulose (Millet et al., 1976). Mechanical pulverization methods generally are high cost and do not remove the lignin or hemicellulose**.**

2.3.1.2 Physicochemical pretreatment

Steam Explosion

Steam explosion is the most commonly used method for the pretreatment of lignocellulosic materials (Kumar et al., 2009). In this method, biomass is treated with high-pressure saturated steam, and then the pressure is suddenly reduced, which makes the materials undergo an explosive decompression. Steam explosion is typically initiated at a temperature of 160-260°C (corresponding pressure, 0.69-4.83 MPa) for several seconds to a few minutes before the material is exposed to atmospheric pressure (Kumar et al., 2009).The biomass/steam mixture is held for a period of time to promote hemicellulose hydrolysis, and the process is terminated by an explosive decompression. The process causes hemicellulose degradation and lignin transformation due to high temperature, thus increasing the potential of cellulose hydrolysis. Hemicellulose is thought to be hydrolyzed by acetic and other acids released during steam-explosion pretreatment. Grous et al. reported that 90% efficiency of enzymatic hydrolysis was achieved in 24 h for poplar chips pretreated by steam explosion, compared to only 15% hydrolysis of untreated chips. Removal of hemicelluloses from the micro-fibrils is believed to expose the cellulose surface and increase enzyme accessibility to the cellulose micro-fibrils. Lignin is removed only to a limited extent during the pretreatment but is redistributed on the fiber surfaces as a result of melting and depolymerisation/ repolymerization reaction (Kumar et al., 2009).

The removal and redistribution of hemicellulose and lignin increase the volume of the pretreated sample. Rapid flashing to atmospheric pressure and turbulent flow of the material cause fragmentation of the material, thereby increasing the accessible surface area depending on the severity of the pretreatment, some degradation of the cellulose to glucose water can be acting as an acid at high temperatures.

Addition of H_2SO_4 (or SO_2) or CO_2 [typically 0.3-3% (w/w)] in steam explosion can decrease time and temperature, effectively improve hydrolysis, decrease the production of inhibitory compounds, and lead to complete removal of hemicellulose (Kumar et al., 2009).

Ammonia fiber explosion (AFEX)

Ammonia fiber explosion is a physicochemical pretreatment process in which lignocellulosic biomass is exposed to liquid ammonia at high temperature and pressure for a period of time, and then the pressure is suddenly reduced. The AFEX process is very similar to steam explosion. In a typical AFEX process, the dosage of liquid ammonia is 1-2 kg of ammonia/kg of dry biomass, the temperature is 90 °C, and the residence time is 30 min. AFEX pretreatment can significantly improve the fermentation rate of various herbaceous crops and grasses. The AFEX technology has been used for the pretreatment of many Lignocellulosic materials including alfalfa, wheat straw, and wheat chaff. (Kumar et al., 2009). During pretreatment only a small amount of the solid material is solubilized; that is, almost no hemicellulose or lignin is removed. The hemicellulose is degraded to oligomeric sugars and de-acetylated, which is most likely the reason that the hemicellulose is not soluble. The structure of the material is changed, resulting in increased water holding capacity and higher digestibility (Kumar et al., 2009).

2.3.1.3 Chemical pretreatment

Chemical pretreatment involves the depolymerisation of lignocellulosic materials with chemical agents such as acid, inorganic solvents, alkali and peroxidases.

Dilute-acid pretreatment

Acid pretreatment firstly developed in Germany in 1898. In this method concentrated or dilute mineral acids like sulfuric acid are used in order to break down hemicelluloses into monomeric sugars and simultaneously removing part of the lignin. Dilute acid hydrolysis is widely used due to the high reaction time that can be achieved with hemicellulose which significantly improve the availability of the cellulose fraction for hydrolysis (Sanchez et al., 2008). The reaction is carried out between 121-220^{o}C under pressure; however temperature (~121^{o}C) are optimal to reduce the formation of inhibitors .The reaction time dependent on the temperature used. This method needs a small amount of water since a small amount of energy is required to get an optimum temperature. Some advantages of this method are: (1) High yield of hemicelluloses sugar, (2) Remove of lignin and hemicelluloses in this method increases exposing of cellulose to enzyme, (3) Remove of heavy metals in the raw materials. Some disadvantages of this method are: (1) Neutralization of acids is necessary, (2) Degradation of hemicelluloses sugar, (3) production of inhibitors like acetic acid and furfural, (4) High cost of reactor due to high pressure and temperature and resistance to low pH (Sanchez, Pilcher et al., 2004).

Organosolv process

Organosolv pretreatment is a method that offers potential to meet our pretreatment goals. One of the main advantages of this technique is recovery of lignin as valuable by-product and cellulose remained as a solid. In this technique, lignocellulosic materials are treated with a mixture of an aqueous- solvent and catalyst. Water and catalyst hydrolyze lignin and carbohydrates bonds and solvents provide an Organosolv condition for dissolving lignin.

Many kinds of solvents can be employed for this matter including various alcohols, glycerol, ethylene glycol, dioxane, ethylene glycol, tri-ethylene glycol, phenol and so forth. Two well-known catalysts are aluminum sulfate and aluminum chloride. For woody material, if the temperature is high enough (more than 180^{o}C), the acids released from the wood can act as catalyst and contribute to the release lignin parts. A result of Organosolv pretreatment is a high xylose yield due to the influence of organic solvents on hydrolysis kinetics.

After leaving the hydrolyzates from the reactor, the organic solvent fraction is recovered by evaporation in the liquid phase, and also the lignin fraction is precipitated and can be recovered by filtration of centrifugation. For this reason, this method has a positive effect on the process economy (Holtzapple and Humphrey 1984; Bjerre et al 1995; Zhao and Cheng et al., 2009). In acetosolv method, under mild operational conditions, both extensive delignification and hemicellulose removal are reached in a single reaction step, and the pulp shows superior characteristics (limited kappa number, good viscosity, low content of residual pentosans) for the manufacture of dissolving pulps. As hemicelluloses are hydrolyzed, acetyl groups are at once split off, leading to the formation of acetic acid, therefore, facilitating the recovery of solvent, an important factor in the economic analysis of Organosolv-based processes. In addition, by adding water, the dissolved lignin fragments (which are free from sulfur-containing groups) can be easily precipitated from the pulping liquors, enabling a useful separation of the compounds derived from hemicelluloses (Vila et al., 2003).

Alkali pretreatment

Alkali pretreatment is conducted under milder conditions at lower temperature and pressure compared with acid pretreatment. However, alkali pretreatment is much more time consuming and the reaction time greatly depends on the operation temperature selected (Mosier et al., 2005; Wyman et al., 2005). The major effect of alkali pretreatment is the saponification of intermolecular ester bonds which cross link lignin and carbohydrates, thus increasing porosity and internal surface area of the biomass matrix as well as decreasing the degree of crystallinity of cellulose (Sun and Cheng 2002).

Lignin can also be disrupted and removed from the biomass matrix, resulting in improved susceptibility of the remaining polysaccharides to enzyme attack during hydrolysis. One limitation related to alkali pretreatment is the formation of unrecoverable salts within the biomass feedstock. Bases such as sodium hydroxide, potassium hydroxide, and ammonia can be used for biomass pretreatment. Base solutions cause swelling of biomass, which subsequently leads to decrease in the degree of polymerization, decrease in crystallinity, disruption of the lignin structure, and separation of structural linkages between lignin and carbohydrates. Among the bases investigated, ammonia has the highest potential for use in commercial processes since it can be recovered and recycled due to its high volatility. Thus, it reduces chemical cost and waste treatment cost (Mosier et al., 2005)

Ozonolysis pretreatment

Ozone treatment is one way of reducing the lignin content of lignocellulosic wastes. These results in an increase of the digestibility of the treated material, and unlike other chemical treatments, it does not produce toxic residues. Ozone can be used to degrade lignin and hemicellulose in many lignocellulosic materials such as wheat straw, bagasse, green hay, peanut, pine, and cotton straw and poplar sawdust. The degradation is mainly limited to lignin. Hemicellulose is slightly affected, but cellulose is not (Parveen Kumar et al., 2009). Ozonolysis pretreatment has the following advantages: (1) it effectively removes lignin; (2) it does not produce toxic residues for the downstream processes; and (3) the reactions are carried out at room temperature and pressure. However, a large amount of ozone is required, making the process expensive (Parveen Kumar et al., 2009).

2.3.1.4 Biological treatment

Biological pretreatment employs wood degrading microorganisms, including white, brown, and soft rot fungi, and bacteria to modify the chemical composition and/or structure of the lignocellulosic biomass so that the modified biomass is more amenable to enzyme digestion. Most biological pretreatment so far has focused on the degradation of lignin in lingo-cellulosic biomass. However, degradation of lignin usually accompanies the loss of cellulose and hemi-cellulose. In order to reduce and eliminate the sugar loss during biological pretreatment, the microbial strains should have low cellulase activity. White rot fungi are the most widely studied for biological pretreatment since they can degrade lignin more effectively and more specifically. Biological pretreatment appears to be a promising technique and has very clear advantages, including no chemical requirement, low energy input, mild environmental conditions, and an environmentally friendly working manner. However, biological pretreatment is very slow (taking from weeks to a year) and requires careful control of growth conditions and a large amount of space to carry out. In addition, most lignolytic microorganisms solubilize or consume not only lignin but also hemicellulose and cellulose (Mosier et al., 2005).

2.3.2 Hydrolysis

Hydrolysis is a process where carbohydrate polymers are converted to simple fermentable sugars. This is facilitated through the pretreatment process, which changes the structure of the biomass (larger pores and higher surface area), thus allow the enzymes or chemical to enter the fiber (Alfani et al., 2000). Hydrolysis is essential before fermentation to release the fermentable sugars. In the process, cellulose is cleaved to glucose, while hemicellulose results in several pentoses and hexoses (Taherzadeh and Karimi, 2007).

$$\text{Cellulose} \xrightarrow{\text{Hydrolysis}} \text{Glucose} \tag{2.1}$$

$$\text{Hemicellulose} \xrightarrow{\text{Hydolysis}} \text{Pentose and Hexoses} \tag{2.2}$$

The hydrolysis step can be performed in different ways, either acidic (dilute and concentrated) or enzymatic hydrolysis (Galbe and Zacchi, 2002).

2.3.2.1 Diluted acid hydrolysis

Chemical hydrolysis means primarily the use of acids; diluted or concentrated. Due to environmental and corrosion problems, dilute-acid hydrolysis has been prioritized instead of concentrated acid (Balat, 2011). In chemical hydrolysis, the pretreatment and the hydrolysis can be combined. Hydrolysis by dilute-acid occurs under high temperature and pressure with a short residence time, resulting in degradation of hemicellulose and cellulose. However, the glucose yield is low, glucose decomposition occurs and there will be a formation of high amount of undesirable by-products. The harsh conditions (acid together with high temperature and pressure) lead to high utility costs and the process will also require downstream neutralization (Su et al., 2006).

The main drawbacks of dilute acid hydrolysis, especially those performed in batch processes, are sugar degradation during hydrolysis, low overall sugar yield and the formation of several by-products which inhibit the fermenting microorganism (Balat, 2011).The high hydrolysis temperature also causes corrosion problems even at low acid concentration and accelerates the sugar degradation. One way to reduce the sugar degradation is to perform a two-stage dilute acid hydrolysis. Here, mainly hemicellulose is hydrolyzed at relatively mild conditions to recover the 5-carbon sugars while the second stage is conducted under harsh conditions to recover the 6-carbon sugars (Balat, 2011). A two-stage dilute acid hydrolysis is generally preferred over a one stage hydrolysis, since e.g. sugar degradation is reduced and less inhibitor is produced.

2.3.2.2 Concentrated Acid Hydrolysis

Hydrolysis of cellulosic materials by concentrated sulfuric or hydrochloric acids is a relatively old process. The concentrated acid process uses relatively mild temperatures, and the only pressures involved are those created by pumping materials from vessel to vessel. Reaction times are typically much longer than for dilute acid (Demirbas, 2005). This method generally uses concentrated sulfuric acid followed by a dilution with water to dissolve and hydrolyze or convert the substrate into sugar. This process provides a complete and rapid conversion of cellulose to glucose and hemicelluloses to 5-carbon sugars with little degradation. The critical factors needed to make this process economically viable are to optimize sugar recovery and cost effectively recovers the acid for recycling. The solid residue from the first stage is dewatered and soaked in a 30 to 40% concentration of sulfuric acid for one to four hours as a pre-cellulose hydrolysis step. The solution is again dewatered and dried, increasing the acid concentration to about 70%. After reacting in another vessel for 1 to 4 h at low temperatures, the contents are separated to recover the sugar and acid. The sugar/acid solution from the second stage is recycled to the first stage to provide the acid for the first stage hydrolysis.

The primary advantage of the concentrated acid process is the potential for high sugar recovery efficiency. The acid and sugar are separated via ion exchange and then acid is re-concentrated via multiple effect evaporators. The low temperatures and pressures employed allow the use of relatively low cost materials such as fiberglass tanks and piping. The low temperatures and pressures also minimize the degradation of sugars (Demirbas, 2005). Unfortunately, it is a relatively slow process and cost effective acid recovery systems have been difficult to develop. Without acid recovery, large quantities of lime must be used to neutralize the acid in the sugar solution. This neutralization forms large quantities of calcium sulfate, which requires disposal and creates additional expense (Demirbas, 2005).

2.3.2.3 Enzymatic hydrolysis

Enzymatic hydrolysis can occur under milder conditions (typically 40-50^{0}C and pH 4.5-5), which give rise to two advantages of the process; low utility cost since there is low corrosion problems and low toxicity of the hydrolyzates (Taherzadeh and Karimi, 2007). In addition, it is also an environmental friendly process (Balat, 2011). However, enzymatic hydrolysis has also disadvantages compared to the dilute-acid hydrolysis; longer hydrolysis time, enzymes are more expensive than acid and end product inhibition can occur (Taherzadeh and Karimi, 2007). Although, many experts consider enzymatic hydrolysis as the most cost-effective process in the long run (Hamelinck et al., 2005) and it is thought to be the key process to achieve an economically viable ethanol production (Horn and Eijsink, 2010).

The degradation of cellulose to glucose in enzymatic hydrolysis is catalyzed by specific cellulolytic enzymes; cellulases. This is a group of enzymes with specificity to hydrolyse glycosidic bonds (Howard et al., 2003). Cellulases are naturally produced by microorganisms, mainly bacteria and fungi, which are capable of degrading cellulosic material.

2.3.3 Fermentation

Fermenting microorganisms are used for the conversion of monomeric sugars to ethanol. Different organisms such as bacteria, yeast and fungi can be used for the conversion, however the most frequently used organism in industrial processes are the robust yeast *Saccharomyces cerevisiae* (baker’s yeast). Under anaerobic condition S.cerevisiae produces ethanol from hexoses as the overall shows below in equation (2.3) and (2.4). The drawback of *Saccharomyces cerevisiae* is not produce ethanol from xylose but *Pichia stipitis yeast* is produce ethanol from both glucose and xylose sugar, however this type of yeast also has many drawbacks such as low ethanol ,temperature and inhibitor resistance (Galbe and Zacchi 2002).

$$C_6H_{12}O_6 \xrightarrow{\text{yeast}} 2C_2H_5OH + 2CO_2 \qquad (2.3)$$

$$3C_5H_{10}O_5 \xrightarrow{\text{yeast}} 5C_2H_5OH + 5CO_2 \qquad (2.4)$$

In theory, the conversion of glucose or xylose to ethanol is 0.51 g EtOH/g glucose. However, the fermenting efficiency of the yeast is generally assumed to be 90% and therefore result in a maximum conversion of 0.46 g EtOH/g glucose (Öhgren et al., 2007). When the glucose yield is high, *S. cerevisiae* has the ability to produce ethanol also under aerobic conditions (Brandberg, 2005). One drawback is that it cannot ferment pentoses, which are an interest when using lignocellulosic biomass. Studies have therefore been performed to genetically modify S. cerevisiae to become both a pentose and glucose fermenting yeast. Other microorganisms have the ability to ferment pentoses and another way to ferment lignocellulosic material is therefore to use different yeasts and to separate the two processes; glucose fermentation and pentose fermentation (Galbe and Zacchi, 2002).

The efficiency of fermenting process depends on several factors; choice of microorganism, raw material, pretreatment method, hydrolysis method and environmental factors such as pH, temperature, substrate and ethanol concentration. Common conditions for fermentation with S.cerevisiae are normally pH 5.0 and a temperature of maximum $37^{0}C$ (Alfani et al., 2000). The performance of the process is affected by different inhibitors generated from the upstream process steps. The hydrolyzates contain, together with fermentable sugars, inhibitors which restrict the fermenting microorganisms and thus, decrease the ethanol yield. Recirculation of the process water increases these compounds further (Olsson and Hahn-Hägerdal, 1996). The mixture of inhibitors inhibits the growth and ethanol production of the microorganism. Different bacteria have varying tolerance against these inhibitors, thus S. cerevisiae has proven to be the most robust one (Almeida et al., 2007). In addition, ethanol, the product itself, has an inhibiting effect on the fermenting microorganism, thus limits the conversion of glucose to ethanol (Olsson and Hahn-Hägerdal, 1996).

2.3.4 Distillation

To separate the ethanol from the other broth (water and cell mass) a distillation step is required after fermentation. Ethanol is recovered in a distillation column while the water is condensed and remains with the solid parts. The ethanol vapor is then concentrated in a rectifying column (Hamelinck et al, 2005). The residual, consisting of water and solids (stillage) are rich in organic material and can further be used for example as biogas substrate.

2.4 Parameters affecting hydrolysis of lignocellulosic materials

2.4.1 Properties of the substrate

The properties of the substrate can affect the hydrolysis. These properties are: neutralizing capacity, proportion of easily hydrolysable hemicellulose and cellulose, amount and rate of hydrolysis of the difficult to hydrolyze materials, the length of the macromolecules, degree of polymerization of cellulose, configuration of the cellulose chain, and association of cellulose with other protective polymeric structures within the plant cell wall such as lignin, pectin, hemicellulose, protein, mineral elements, etc. Particle size is also one of the effective parameters.

2.4.2 The acidity of the system

Another parameter affecting the hydrolysis is the acidity of system. The acidity is dependent on the type and concentration of the acid used, amount of acid solution, amount of acid released from the biomass during hydrolysis, liquid to solid ratio, the neutralizing capacity of the lignocellulose, and movement of the solution during heating. When dilute acid hydrolysis is applied in a continuous process such as a screw fed co-current reactor, it requires a relatively short residence time. Therefore, the penetration of acid catalyst in the biomass, as well as dispersion in the reactor, can significantly affect the overall reaction, and consequently the reactor performance. Diffusivity of sulfuric acid is dependent on the nature of the lignocellulosic materials (Demirbas, 2005).

2.4.3 Rate of decomposition of hydrolysis products during hydrolysis

The rate of decomposition of the products during the hydrolysis process depends on temperature, acidity, reaction time and the concentration of sugars. Under hydrolysis conditions that produce a solution containing in excess of 10 percent glucose, reversion phenomena are suggested to be very important. The reversion phenomena result in much of the glucose being present not as free glucose but as dimers, oligomers, and anhydrosugars which are unavailable to the microorganism used in fermentation (Harris et al., 1984). It was recently reported that metals and /or metal ions can also catalyze glucose decomposition during acid hydrolysis of Lignocellulosic materials.

2.5 Factors Affecting Fermentation

Microorganisms for ethanol fermentation can best be described in terms of their performance parameters and other requirements such as compatibility with existing products, processes and equipment. The performance parameters of fermentation are temperature, pH, alcohol tolerance, growth rate, productivity, osmotic tolerance, specificity, yield, genetic stability, and inhibitor tolerance (Demirbas, 2005).

2.5.1 Effect of sugar concentration

The concentration of sugar can affect the microbial ethanol fermentation in various ways. Use of concentrated sugar substrate is one of the ways to obtain high ethanol yield during fermentation. The amount of ethanol produced is proportional to the amount of sugar added; thus, high sugar concentrations are desired. However, too high sugar concentrations can inhibit metabolism due to increased osmotic pressure. Very low levels of sugar may limit the rate of ethanol production (Jones et al., 1981).

Hence, each fermentation process will have an optimal glucose or equivalent sugar concentration (Sofer and Zaborsky, 1981). A sugar concentration of 10-18% is usually satisfactory, although other concentrations are used (Dunn, 1959).

2.5.2 Effect of temperature

Temperature has an important factor on the growth rate of the microorganisms and the rate of ethanol production. Wine and beer fermentations are generally conducted below 20°C, whereas higher temperatures (30-38°C) are being examined for industrial alcohol production by yeast cultures (Sofer and Zaborsky, 1981). Too high temperature kills yeast, and low temperature slows down yeast activity and growth. Thus, specific range of temperature is required (Onuki, 2005).

All the recombinant strains are mesophilic organisms and have best function between 30 to 38 °C. Operating at greater temperatures is desirable for the following reasons:

- High fermentation temperature increases growth rate and productivity exponentially
- Plant capital cost is less due to higher productivity per unit volume of ferment or vessel and cooling equipment investment is lowered.
- Operating costs are less since less energy is required to maintain desired fermentation temperature and recover the ethanol.
- Contamination risk is less as fewer organisms exist at high temperatures.

The enzyme hydrolysis process for saccharification able to operate up to 55 °C may be combined with fermentation, further reducing capital and glucose inhibition (Hettenhaus, 1998).

2.5.3 Effect of pH

A very important factor for cellular growth is external pH. Most alcoholic yeast fermentations are conducted below pH 4.5, although this may not be the optimal pH for growth or ethanol production. Yeast cultures can grow over a wide range from 3 to 8 with an optimum for growth generally in the slight acidic range. Shifts in pH can also affect the final ratio of organic waste products produced by yeast cultures. Thus, the optimal pH for a fermentation process must support a balance among ethanol production, cellular growth, and physicochemical effect on waste product pathways. Low pH values in yeast fermentation help to inhibit growth of contaminating bacterial cultures. Bacterial cultures generally have a pH optimum around 7-7.5, with less tolerance than yeast to acid conditions (Sofer and Zaborsky, 1981).

2.5.4 Ethanol concentration

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

2.5.5 Osmotic tolerance

The semi-permeable membrane surrounding the cell must be able to withstand wide osmotic pressure changes in extracellular fluids that impact the relative osmotic pressure difference. If not, the cells may be severely damaged or even killed. The cells may burst in a hypotonic solution, when the solution becomes more dilute than the intracellular fluid. If hypertonic, the cells will shrink from the osmotic pressure difference. Osmotic pressure limits can be one of the factors that restrict maximum substrate concentration (Sofer and Zaborsky, 1981).

2.5.6 Inhibitor tolerance

McMillan, (1994) grouped the fermentation inhibitors into three classes:

1) Compounds originating in the biomass by hydrolysis. These include organic acids such as acetic, glucuronic and galacturonic acids from the hemicellulose, and phenolic compounds from the lignin. The inhibitoriest of these for both yeast and bacteria is acetic acid and solubilized lignin.
2) Compounds formed by degradation of the products resulting from pretreatment and hydrolysis of the biomass. Furfural from xylose and hydroxymethylfurfural from glucose lead this group. It is completed by an assortment of aldehydes, acids and alcohols from lignin, sugar and protein degradation.
3) Compounds from other sources. Metal ions resulting from equipment corrosion, sulfites, sulfur dioxide and lactic acid introduced with other streams containing nutrients, cleaning solutions and backset.

3 MATERIAL AND METHODS

The experimental work was done in laboratory of Addis Ababa institute of Technology ,School of Chemical and Bio-Engineering and college of natural science of Addis Ababa University school of physics and chemistry department, Addis Ababa Ethiopia.

3.1 Material and Equipments

3.1.1 Quantify the sugar content of barley spent grain

Materials used during quantifying sugar conten of Barley spent grain were:1000ml Graduated cylinder , Oven, Sieves (Retsch, AS200 digital), test tubes, Electronics weighting balance , Electric heater ,Distilled water, quantitative Benedict reagent solution, Digital spectrophotometer (MAX ELECTRONICS, model no 802), Autoclave (ADOLF WOLF, La-MCS-204) etc.

3.1.2 Evaluate the effect of hydrolysis process variables (time, acid concentration and temperature) in the yield of ethanol

The effect of hydrolysis process vbariables were evaluated using analysis of variance (ANOVA) and the material and equipment were, 98% Sulfuric acid (H_2SO_4), Sodium hydroxide (NaOH), Dextrose sugar, yeast extracts, Urea and Mg SO_4.7 H_2O, Yeast (*Saccharomyces cerevisiae*), digital pH meter (Janway, model 3510), thermometer, pycnometer vessel, Fourier transform infrared spectroscopy (prinks Elmer spectrum, 65 FT-lR), simple distillation, Shaker incubutor (EDISON, Nj.USA-C4) etc.

3.2 Experimental methods

3.2.1 Quantify the sugar content of barley spent grain

3.2.1.1 Raw material preparation

Barley Spent grain (BSG) which remains after the mashing and lautering process, was obtained from BGI brewery industry, Addis Ababa, Ethiopia. This sample was packed in polyethylene bags and transported to Addis Ababa institute technology (AAiT), School of chemical and bio engineering laboratory. The Samples used for this study were prepared in biochemical engineering laboratory. 3kg of BSG was washed in order to remove unwanted matter and dried at 70°C for 24h until 10% moisture content remain. Then the dried sample was sieved and milled the over size in to appropriate particle size which is less than 2mm. The milled sample was sterilized at 121°C for 15 min and stored at less than 4°C refrigerator.

3.2.1.2 Moisture content determination

A clean aluminum dish was dried in an oven at 130°C for 1h and placed in desiccators to cool, and the weight of dish (W_1) was determined. About 100 g of sample was weighed in dry aluminum dish (W_2) and dried at 70°C until constant weight was obtained, then the final dreid sample was weighed (W_3) again. The moisture content was determined using Eqn. [3.1]

$$\text{Moisture content\%} = \frac{(W2-W3)}{(W2-W1)} * 100 \qquad \text{......} [3.1]$$

3.2.1.3 First stage diluted acid hydrolysis (pretreatment)

The process parameters were fixed at best process conditions e.g. (liquid/solid ratio of 8 g/g, 100 mg H_2SO_4/g of dry matter, 17 min reaction time and 121°C) for extraction of hemicellulosic sugars (Mussatto and Roberto, 2005).The pretreated feedstock was mixed with 1.25% (w/w) or 100 mg H_2SO_4/g of dry matter sulfuric acid solutions in 500-mL closed universal flasks with a liquid-to-solid ratio of 8 (w/w). The mixtures were allowed to stand for 10 min at room temperature in order to equilibrate the acid concentrations between the bulk phase and the biomass. Hydrolysis was performed in an autoclave at 121°C for 17 min. The flasks were placed inside the autoclave at 100°C, and the heating time to reach 121°C.

3.2.1.4 Second stage diluted acid hydrolysis

The main purpose of this process was to degraded cellulose in to its monomer in the optimal condition of temperature, acid concentration and reaction time. The procedure for this stage was similar to first stage hydrolysis but energy required to degrade cellulose is higher than hemicellulose so it needs higher temperature and concentration of acid. The solution separated from the first stage hydrolysis was washed and dried before introduced in to the second stage hydrolysis. In second stage hydrolysis, the process conditions were optimized: Temperature (130°C and 150°C), reaction time (20 min and 30min) and acid concentration (120 and 160 mg of H_2SO_4 per gram of dry mass) using full factorial central composite design (CCD).

Figure 3.1 autoclave

3.2.1.5 Filtration

The lignin and degraded cellulose which is called monomeric sugar was separated by using vacuum filtration unit. Then the sugar solution or filtrate was neutralize and introduced into fermentation. The lignin which obtained from this filtration process was dried and weight before use for another purpose.

Figure 3.2.Vacuum filtration unit

3.2.1.6 Sugar content determination

The concentration of total reducing sugar (TRS) content of hydrolyzate which obtained from hydrolysis was determined using digital spectrophotometer (MAX ELECTRONICS, model no 802) by measuring absorbance vs. sugar concentration at 540nm wave length. Quantitative benedict solution and standard glucose solution was used for assays to plot the calibration curve. Benedict’s solution is designed to detect the presence of reducing sugars. In hot alkaline solutions, reducing sugars reduce the blue copper (II) ions to brick red copper (I) oxide precipitate. As the reaction proceeds, the color of the reaction mixture changes progressively from blue to green, yellow, orange and red. When the conditions are carefully controlled, the colour developed and the amount of precipitate formed depends upon the amount of reducing sugars present. Hence, in most conditions, a sufficiently good estimation of the concentration of glucose-equivalent reducing sugars present in a sample can be obtained.

Calibration plot for glucose standard

- Standard glucose dilution series solution was prepared at different concentration of 10, 8, 6, 4, 2, 1 and 0%.
- Pipette 0.5 mL from each of the dilution series into labeled test tubes, each containing 5 mL of Benedict's solution. Mix by shaking
- All the labeled test tubes were heated at 90^{o}C water bath for 5 minutes.
- The test tubes were removed from the water bath and filtered using filter paper to remove red precipitate formed when reducing sugar in the samples reacted with Benedict reagent.
- After filtered the precipitate, % absorbance was measured using spectrophotometer at 540nm.
- Calibration curve was plotted to show the % of absorbance of blue light by the standard glucose solution.

Determination of the total reducing sugar in the hydrolyzate

- Pipette 0.5 mL of each of the samples into labeled test tubes, each containing 5 mL of Benedict's solution. Mix by shaking
- All the labeled test tubes were heated to 90^{o}C in water bath for 5 minutes.
- The test tubes were removed from the water bath and filtered using filter paper to remove red precipitate formed when reducing sugar in the samples reacted with Benedict reagent.
- After filtered the precipitate, % absorbance was measured using spectrophotometer at 540nm.
- The concentration of sugar in each samples were read from the calibration curve of the standard glucose solution.

$$\text{CTRSUS} = \frac{(\text{absorbance of unknown sample}) - (\text{y} - \text{intercept})}{\text{Slope}} \text{..........................} \quad [3.2]$$

Where

CTRSUS = Concentration of total reducing sugar of unknown sample

3.2.2 Evaluate the effect of hydrolysis process variables (time, acid concentration and temperature) in the yield of ethanol

3.2.2.1 Fermentation

The fermentation process was carried out in shaker incubator, at 30 °C, with stirring at 175 rpm, for a 72h. All assays were performed with 10% (v/v) of inoculum. The prepared hydrolyzates were adjusted to pH of 5.0 which is optimum for Saccharomyces cerevisiae using 2M sodium hydroxide solution.

Media Preparation

The culture medium was prepared in 250 mL test tube by composed of (g/l), Yeast extract (10); Dextrose (20); Urea (5); $Mg\ SO_4.7\ H_2\ O$ (5); Peptone (20).

Procedures in Media Preparation

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

Figure 3.3. Cultured media

The Procedure for Fermentation

- The hydrolyzates sample was conditioned at temperature of 30^oC. This temperature is favorable for the fermentation by which *Saccharomyces cerevisiae*.

- The pH of the sample was adjusted using 2M NaOH to make solution pH from (4.5-5.0) to establish a favorable condition for *S. cerevisiae*.
- The hydrolyzate sample with 10% inoculum was placed into shaker incubator at 30°C and 175 rpm for 3 days.
- After 72 h fermentation, the samples were taken out and introduce into distillation to separate the hydrous ethanol.

3.2.2.2 Ethanol Separation

Distillation is the final step in the production of ethanol. It is a purifications step. Distillation is the method used to separate two liquid based on their different boiling points. However, to achieve high purification, several distillations are required. Separation was carried out by simple distillation at 85°C for 2 hrs.

3.2.2.3 Specific gravity of ethanol

The specific gravity of the ethanol was determined by using 20°C pycnometer. The 25ml pycnometer was cleaned and dried first and then weighed (W_0), then after the bottle was filled with ethanol, stopper inserted and reweighed to give (W_1). The ethanol was substituted with water after washing and drying the bottle and weighed to give (W_2). The expression for specific gravity (Sp.gr) is:

$$sp.gravity \ = \frac{(W1 - Wo)}{(W2 - Wo)}$$

$$= \frac{mass\ of\ ethanol}{mass\ of\ an\ equal\ volume\ of\ water} \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots .. [3.3]$$

Where: W_0- weight (g) of empty bottle

W_1- weight (g) of bottle + sample

W_2 - weight (g) of bottle + water

Figure 3.4.Density measuring using pycnometer

Yield of ethanol

99.9% bioethanol yield from each fermented sample was determined as follows;

Volume of 99.9% Ethanol yield (V_{AE}) =$\frac{(VHE*\rho HE*XE)}{\rho AE}$ .... [3.4]

Where

V_{HE} = volume of hydrous ethanol formed.

V_{AE} = volume of anhydrous ethanol formed

ρ_{AE}= density of anhydrous ethanol which is 0.789g/ml

ρ_{HE} = density of hydrous ethanol which of the sample

X_E = mass fraction of ethanol

FT-lR determination of BSG Bioethanol

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

3.3 Design of the Experiment

Data analysis was carried out by DESIGN EXPERT version 6.0 software (central composite design) to evaluate the effects of the process variables; temperature (130^{o}C, 140^{o}C and 150^{o}C), reaction time (20 min, 25min and 30 min) and acid concentration (1.5, 1.75 and 2%w/w). 140^{o}C, 25 min and 1.75% acid concentration was used center point. A 2^3 full factorial experimental design with 20 experiments were employed, which includes 8 trails for factorial design, 6 trails for axial points and 6 trails for replication of the central points to estimate error based on the pattern generated through software. The response variable was sugar content after hydrolysis and ethanol yield after fermentation. This design of the experiment helps us to optimize of process parameters using Response Surface Methodology (RSM). Significance of the result was set from analysis of variance (ANOVA).

Table 3.1 Minimum, central and Maximum values of factors

Factor name	**Unit**	**low**	**Center point**	**High**
Temperature	^{o}C	130	140	150
Time	Min	20	25	30
Acid- concentration	%	1.5	1.75	2

4 RESULT AND DISCUSSION

4.1 Determination the sugar content of barley spent grain

4.1.1 Moisture Contents of the samples

The fresh BSG was collected from BGI Ethiopia brewery industry. Three samples (100 g, 125 g and 150 g) were prepared to determine the moisture content at 70^{o}C and the moisture content of the sample was obtained in Table 4.1 at 24h using equation 3.1 Drying process was terminated at 24 hr as the weight of the sample was approximately equal with the weight at 21h.

Table 4.1 Determination of moisture contents

Weight of sample (g)	Drying time (hours)					
	0	12	15	18	21	24
	100	62.45	56.05	53.67	50.89	50.87
	125	72.45	69.15	66.35	64.99	64.77
	150	86.87	83.99	80,66	78.20	78.15

The moisture content of the BSG sample with 100, 125, and 150 g was 49.13, 48.18 and 47.90w/w, respectively. Thus, the average moisture content of the three samples was 48.40%w/w.

4.1.2 Total reducing sugar content after hydrolysis

Total reducing sugar of the hydrolyzate sample was determined using calibration curve which was plotted from the known concentration of standard glucose reacted with Benedict solution reagent and absorbance of standard glucose-benedict solution after reaction,was measured using digital spectrophotometer at 540nm wavelength.

As we seen from the calibration curve below the absorbance and concentration of sugar has inverse relationship because in the sample which have less sugar concentration remain high amount of Cu (II) oxide (blue color) un react with the reducing sugar due to this absorbance of the sample increase but in the sample which have high sugar concentration Cu (II) oxide is completely react with the reducing sugar and form Cu (I) oxide (red color) precipitate and absorbance of the sample decreases.

Table 4.2. Concentration of standard glucose and its absorbance

Std.Glucose concentration (g/ml)	Absorbance
0	0.165
0.01	0.154
0.02	0.135
0.04	0.110
0.06	0.083
0.08	0.057
0.10	0.035

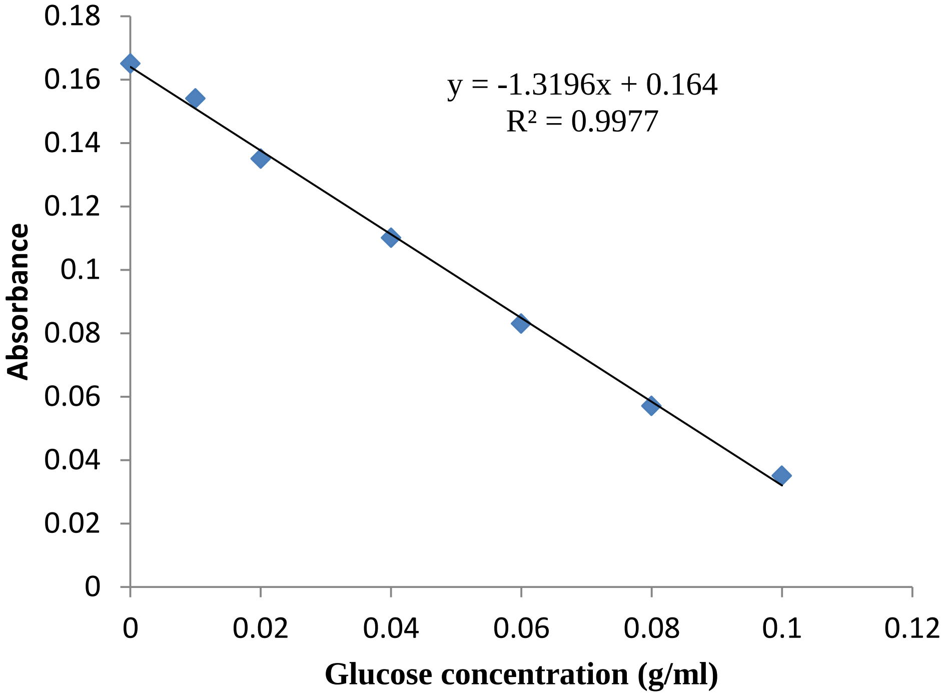


Figure 4.1. Calibration curve of standard glucose concentration

The concentration of total reducing sugar in the hydrolyzate samples was obtained from the caliberation plot in the above and expressed by %w/w in the table 4.3 below. The volume of hydrolyzate per sample was 400ml.

The highest yield of total reducing sugar (47.52%w/w (average)) was obtained at 140^{o}C temperature, 25 min hydrolysis time and 1.75% sulfuric acid concentration but the minimum yield (25.44%w/w) was obtained at 123.18^{o}C temperature as shown in the table 4.3 below. Because at low hydrolysis temperature,cellulose can not convert into glucose (Balat, 2011).

According to Dehnavi 2009, 60% by dry mass of BSG is cellulose and hemicellulose. From this total carbohydrate (cellulose and hemicellulose) 79.27% was converted into monomeric sugar in this current investigation.

Table 4.3 The yield of total reducing sugar (TRS) of samples

Run No	Factor1temp. (^{o}c)	Factor 2 time (min)	Factor 3 acid con. (%)	Absorbance	Con. Of TRS (%m/v)	Yield of TRS after hydrolysis(%w/w)
1	140.00	25.00	1.75	0.085	5.98	47.89
2	150.00	30.00	2.00	0.107	4.32	34.56
3	150.00	20.00	1.50	0.093	5.38	43.04
4	140.00	25.00	1.75	0.086	5.91	47.28
5	130.00	30.00	1.50	0.110	4.10	32.00
6	140.00	16.59	1.75	0.104	4.55	36.40
7	140.00	25.00	1.75	0.086	5.91	47.28
8	140.00	25.00	1.75	0.085	5.98	47.89
9	123.18	25.00	1.75	0.122	3.18	25.44
10	150.00	20.00	2.00	0.103	4.62	36.96
11	150.00	30.00	1.50	0.090	5.61	44.80
12	140.00	25.00	1.75	0.086	5.91	47.28
13	140.00	25.00	2.17	0.100	4.85	38.80
14	130.00	30.00	2.00	0.092	5.45	43.68
15	140.00	33.41	1.75	0.094	5.30	42.40
16	140.00	25.00	1.75	0.085	5.98	47.92
17	130.00	20.00	1.50	0.119	3.41	27.28
18	156.82	25.00	1.75	0.101	4.77	38.16
19	140.00	25.00	1.33	0.101	4.77	38.16
20	130.00	20.00	2.00	0.096	5.15	41.20

4.2 Evaluate the effect of hydrolysis process variables (time, acid concentration and temperature) in the yield of ethanol

4.2.1 Specific gravity (density) and yield of biothanol

The specific gravity of ethanol was determined using pycnometer cylinder and this Sp.gravity was related with concentration of ethanol using standard table from Perry chemical engineering hand book. The yield of ethanol from the experiment was calculated using equation (3.4) and listed below in table (4.4).

Table 4.4. Density and Yield of Ethanol

Run No	Volume of hydrous ethanol(ml) after distillation	Density Of hydrous ethanol(g/ml)	Ethanol Con. (w/w %)	Yield of anhydrous ethanol(ml/50g BSG)
1	14.60	0.91634	48.86	8.29
2	14.20	0.94431	35.33	6.00
3	16.00	0.93601	39.56	7.50
4	18.40	0.93919	38.00	8.31
5	14.50	0.94828	33.18	5.80
6	13.64	0.93613	39.53	6.40
7	19.54	0.94419	35.40	8.28
8	15.12	0.92014	47.12	8.31
9	8.00	0.91613	48.96	4.50
10	14.90	0.94214	36.50	6.50
11	19.50	0.94811	33.27	7.80
12	18.40	0.93918	38.00	8.31
13	19.62	0.95612	28.60	6.80
14	19.67	0.95023	32.08	7.60
15	21.30	0.95600	28.68	7.40
16	15.13	0.92018	47.10	8.26
17	13.67	0.95612	28.60	4.74
18	18.17	0.95204	31.04	6.80
19	13.67	0.93211	41.50	6.70
20	16.96	0.94401	35.49	7.20

As shown from the figure 4.2 below the yield of TRS and ethanol have direct relationship that means, the run which has maximum sugar content has maximum yield of ethanol. The same is true in the run which has less sugar content also less yield of ethanol. This situation indicates the efficiency of fermentation process. The fermentation parameters are fixed for all runs.

The maximum sugar content 47.52%w/w (average) and yield of ethanol 8.29 mL/50g dry BSG (average) were obtained at run 1, 4, 7, 8, 12 and 16 (25 min, 140^{o}C and 1.75% acid concentration). After fermentation 54% sugar conversion was achieved. This indicates conversion of sugar into ethanol is small. The reason for this result is the drawback of *Saccharomyces cerevisiae* which can not able to produce ethanol from 5-carbon sugars (Galbe and Zacchi 2002)..

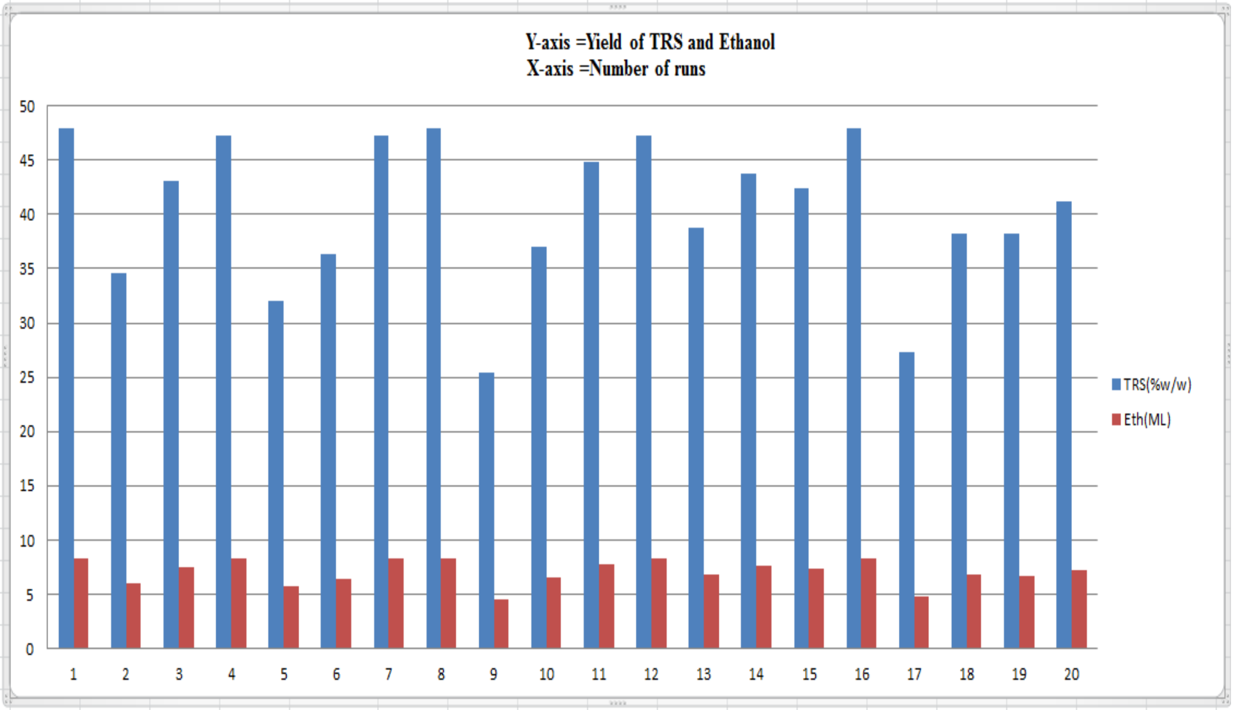


Figure 4.2. Relationship between total reducing sugar and the produced ethanol from BSG

4.2.2 Statistical Analysis of the Experimental Results

4.2.2.1 Analysis of variance (ANOVA)

The analysis of variance of the quadratic regression model was a significant model, from evident of Fisher's F test with a very low probability value [(P-model > F) =0.0001]. From Table 4.5 it was observed that the Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B, A^2, B^2, C^2, AC are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. The coefficient for the linear effect of temperature and time was highly significant, and that of acid concentration was least significant. It was also observed that there is an interaction effect between temperature and acid concentration.

Table 4.5. Analysis of variance (ANOVA) for Response Surface Quadratic Model

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F	
Model	25.10	9	2.79	29.46	< 0.0001	significant
A	2.93	1	2.93	30.97	0.0002	
B	0.63	1	0.63	6.69	0.0271	
C	0.19	1	0.19	2.05	0.1827	
A^2	10.88	1	10.88	114.90	< 0.0001	
B^2	2.63	1	2.63	27.74	0.0004	
C^2	3.32	1	3.32	35.06	0.0001	
AB	0.34	1	0.34	3.64	0.0856	
AC	6.23	1	6.23	65.80	< 0.0001	
BC	0.27	1	0.27	2.81	0.1244	
Residual	0.95	10	0.095			
Lack of Fit	0.95	5	0.19	0.36	0.788	not significant
Pure Error1.	200E-003	5	2.400E-004			
Cor Total	26.05	19				

The regression coefficients and the corresponding 95% CI (Confidence Interval) High and Low were presented in table 4.6 below. If zero was in the range High and Low 95% Confidence interval, the factors has no effect. From the 95% CI High and Low values of each model term, it could be concluded that the regression coefficients of temperature, time and the interaction terms of temperature and acid concentration have highly significant effect in ethanol production.

Table 4.6. Regression coefficients and the corresponding 95% CI High and Low

Factor	Coefficient Estimate	DF	Standard Error	95% C Low	95% CI High	VIF
Intercept	8.29	1	0.13	8.01	8.57	
A-temperature	0.46	1	0.083	0.28	0.65	1.00
B-time	0.22	1	0.083	0.030	0.40	1.00
C-acid con..	0.12	1	0.083	-0.066	0.30	1.00
A^2	-0.87	1	0.081	-1.05	-0.69	1.02
B^2	-0.43	1	0.081	-0.61	-0.25	1.02
C^2	-0.48	1	0.081	-0.66	-0.30	1.02
AB	-0.21	1	0.11	-0.45	0.035	1.00
AC	-0.88	1	0.11	-1.12	-0.64	1.00
BC	-0.18	1	0.11	-0.42	0.060	1.00

The following second order polynomial model was derived to explain the yield of ethanol produced from BSG.

Final Equation in terms of actual factors:

Yield = -311.99983 +3.20071 * Temperature +1.73346 * Time +80.42542 * Acid con. -8.68883E^{-003} * Temperature^2 -0.017078 * Time^2 -7.67958 * Acid con.^{2} -4.15000E^{-003} * Temperature * Time -0.35300 * Temperature * Acid con. -0.14600 * Time * Acid con. ....(4.1)

Table 4.7. Model adequacy measures

Std. Dev.	0.31	R-Squared	0.9637
Mean	7.08	Adj R-Squared	0.9309
C.V.	4.35	Pred R-Squared	0.8246
PRESS	7.17	Adeq Precision	17.677

The regression coefficient (R^2) quantitatively evaluates the correlation between the experimental data and the predicted responses. Results of R^2= 0.9637 and Adj-R^2= 0.9309 obtained explicates that the predicted values were found to be in good agreement with experimental values. Since the R^2 value is closer to 1.0 it indicates that the regression line perfectly fits the data. Similar to that in this investigation, R^2 obtained was 0.9637, which was close to 1. Results imply that the predicted values were found to be in good agreement with experimental values (R^2= 0.9637and Adj-R^2= 0.9309), indicating the achievement of the RSM. The model's goodness of fit was checked by regression coefficient (R^2). In this case, the value of the coefficient (R^2 =0.9637) from Table 4.7 indicated that only 3.63% of the total variance was not explained by the developed regression model. The obtained R^2 values suggest good adjustments to the experimental results. The adjusted determination coefficient (Adj-R^2= 0.9309) was also satisfactory for confirming the significance of the model. Pred R-Squared indicating that the model will probably explain a high percentage (about 82.46%) of the variability in new data. "Adeq precision" measures the signal to noise ratio. a ratio greater than 4 is desirable. In this study 17.677 indicates an adequate signal.

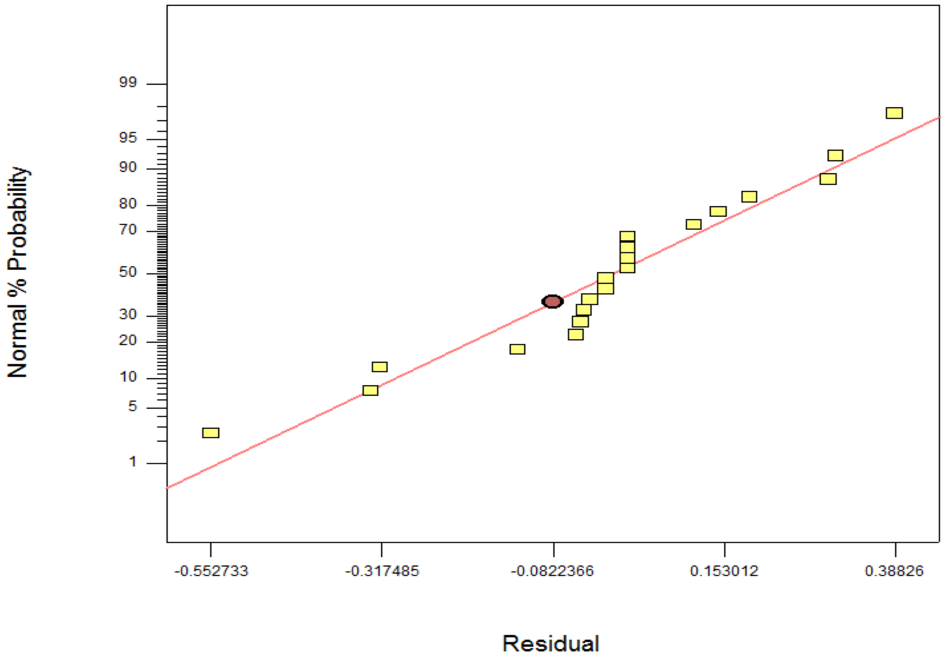


Figure 4.3. Normal plots of residuals

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

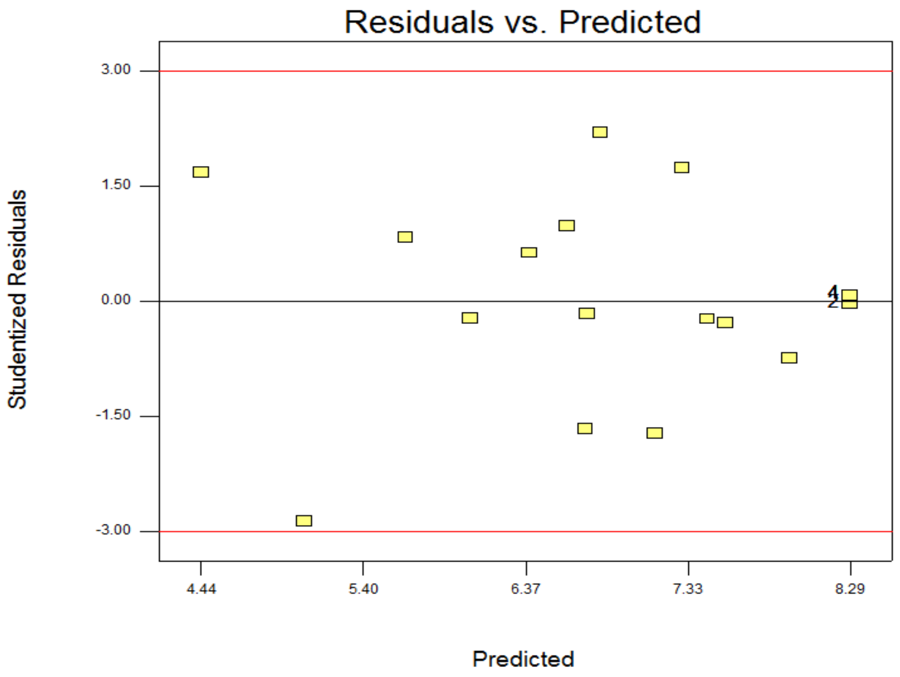


Figure 4.4. Residual versus predicted values

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

4.2.2.2 Response Surface and Contour plot on the Experimental Variables

In order to analyze the regression equation of the model, three-dimensional surface and 2D contour plots were obtained by plotting the response (yield of ethanol) on the Z axis against any two variables while keeping the other variable at zero level. These plots are created to analyze the change in the response surface. Conical shape response surface plot indicates optimum operating conditions. The response optimized value for the production of bioethanol was based on the two process variables described on the response surface plot. The effect of the independent variables and their mutual interaction on the yield of ethanol can be seen in Figures (4.5-4.16) below.

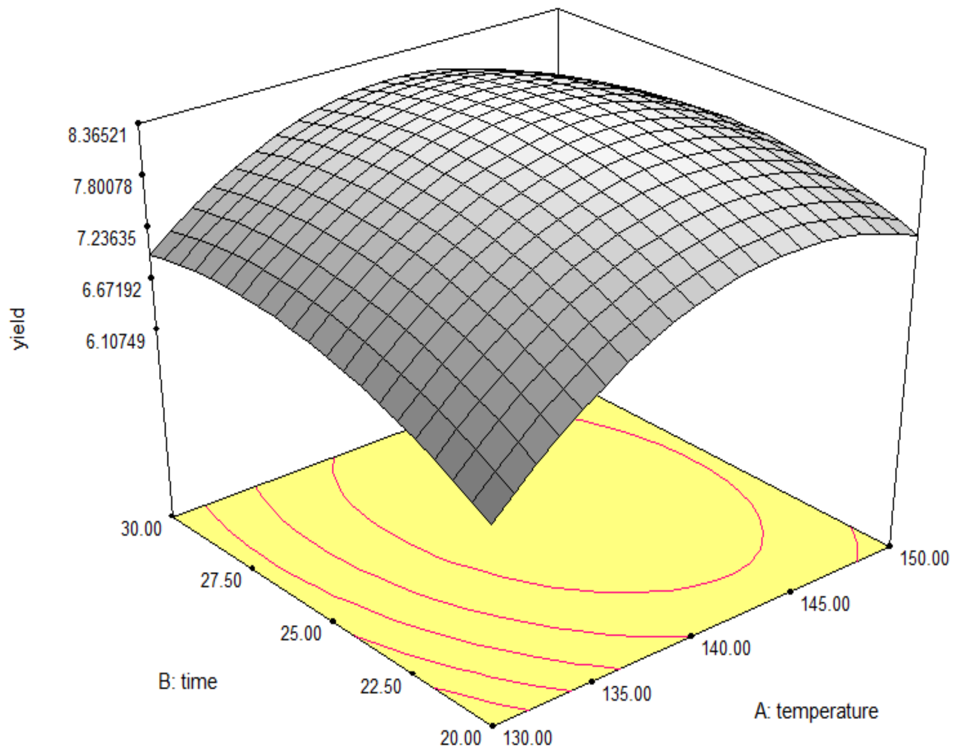


Figure 4.5. Response surface plots of the effect of temperature and time on the yield of Ethanol at fixed acid concentration.

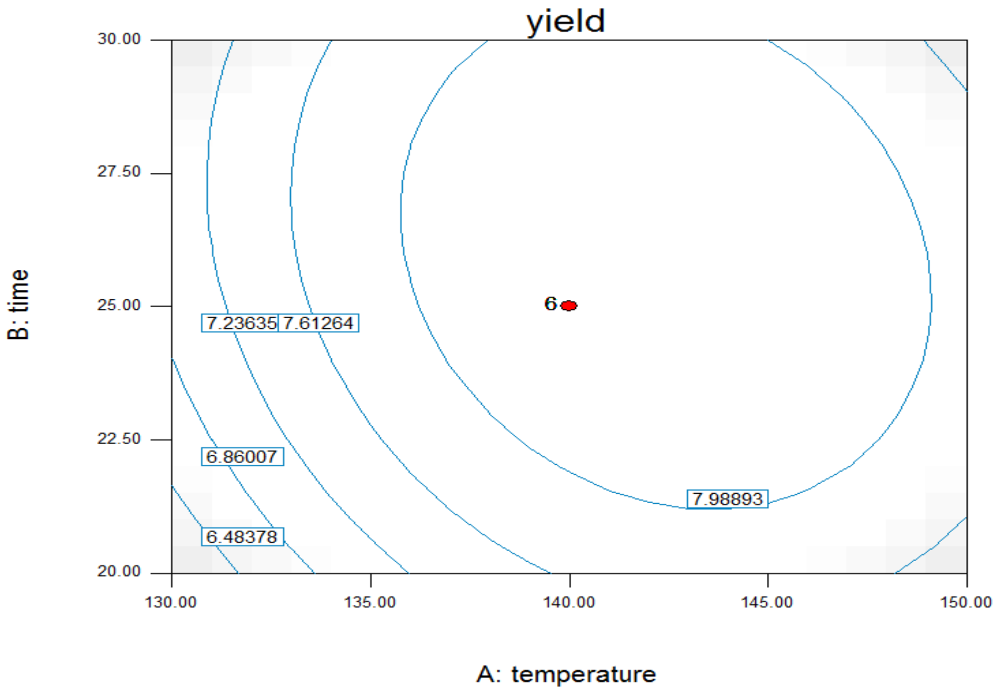


Figure 4.6 Contour plots of the effect of temperature and time on the yield of Ethanol at fixed acid concentration

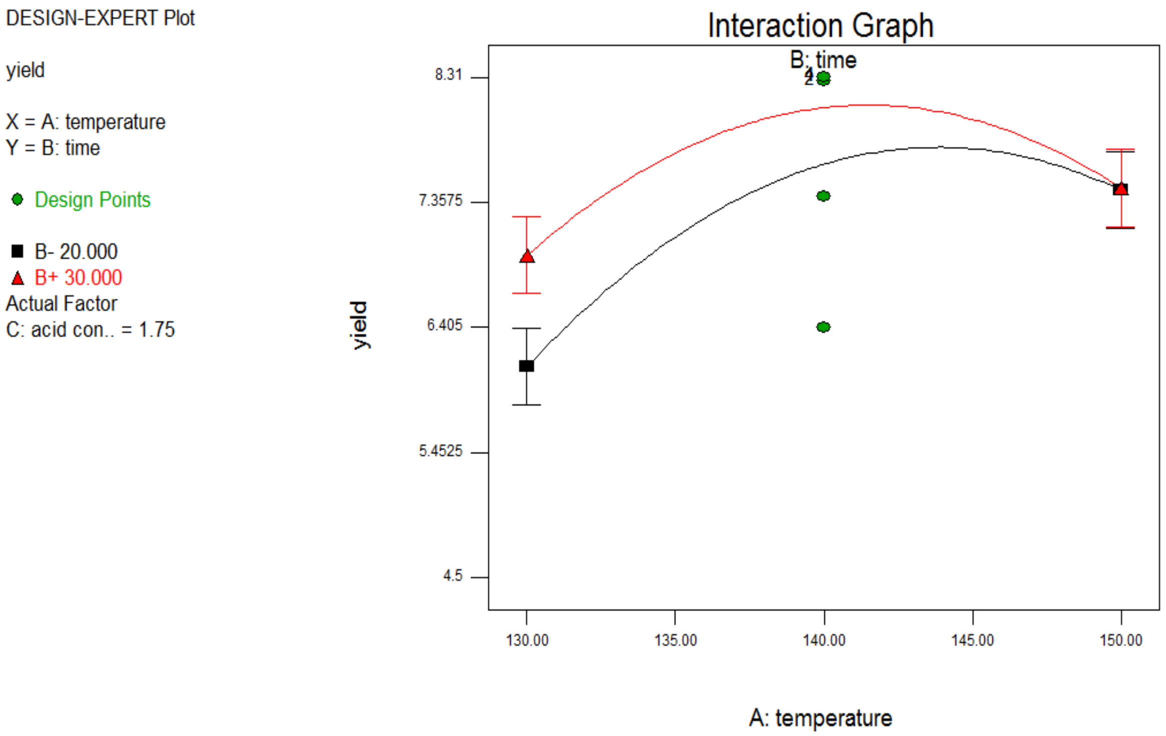


Figure 4.7. Effect and interaction of temperature and time (fixed) on the yield of ethanol at center of acid concentration

The response surface and contour plots of the quadratic model are shown in Figure (4.5, 4.8 and 4.11). It was reported that Response surface plots provide a method to predict the yield of ethanol for different parameter values of the tested variables and the interaction plots help in identification of the type of interactions between these variables. The axes of the contour plot are the experimental variables and the area within the axes is termed the response surface.

Figure (4.5) and (4.6) shows the response surface and contour plots respectively developed as a function of temperature and time, while the acid concentration was kept constant at 1.75%. It was observed that the yield of ethanol was more sensitive to temperature change, when the temperature change from 130-142.5^{o}C the yield of ethanol reaches at the peak and beyond 142.5^{o}C the yield slightly decreased. The reason for this observation is due to the fact that when the cellulose expose to high temperature, the sugar which obtained from cellulose degraded into not fermentable product and gives low yield of ethanol.

As we observed from figure (4.7) there is no an interaction between time and temperature at the given interval. Positive yield was observed at 30 min and around 142.5^{o}C reaction temperature. The yield of ethanol at 20 min reaction time is less than at 30 min in the same temperature.

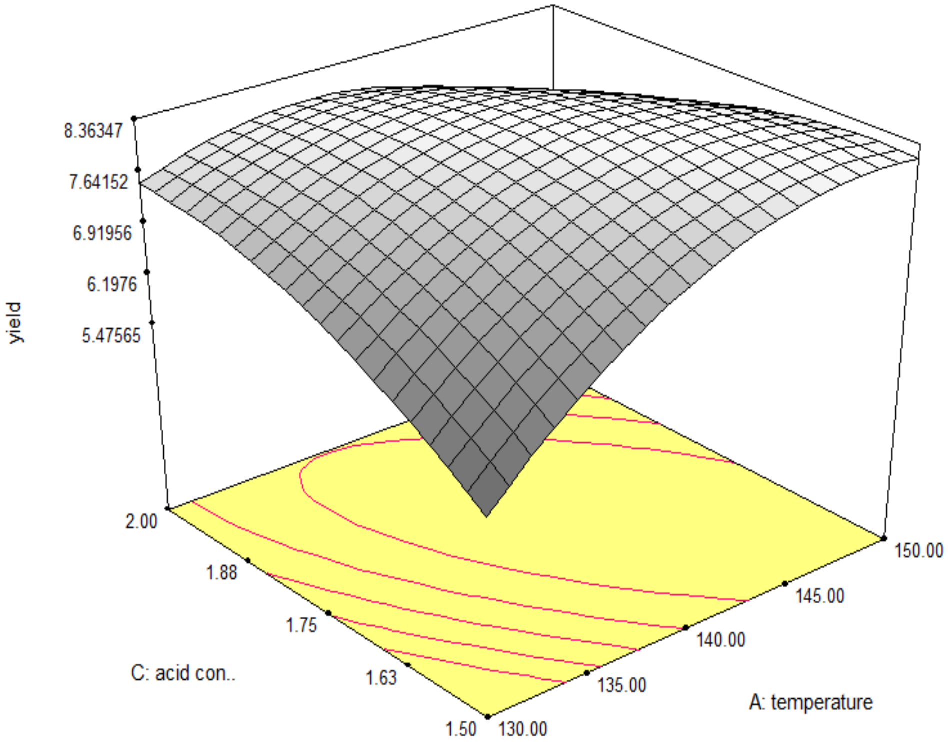


Figure 4.8. Response surface plot of the effect of temperature and acid concentration at constant time in the center

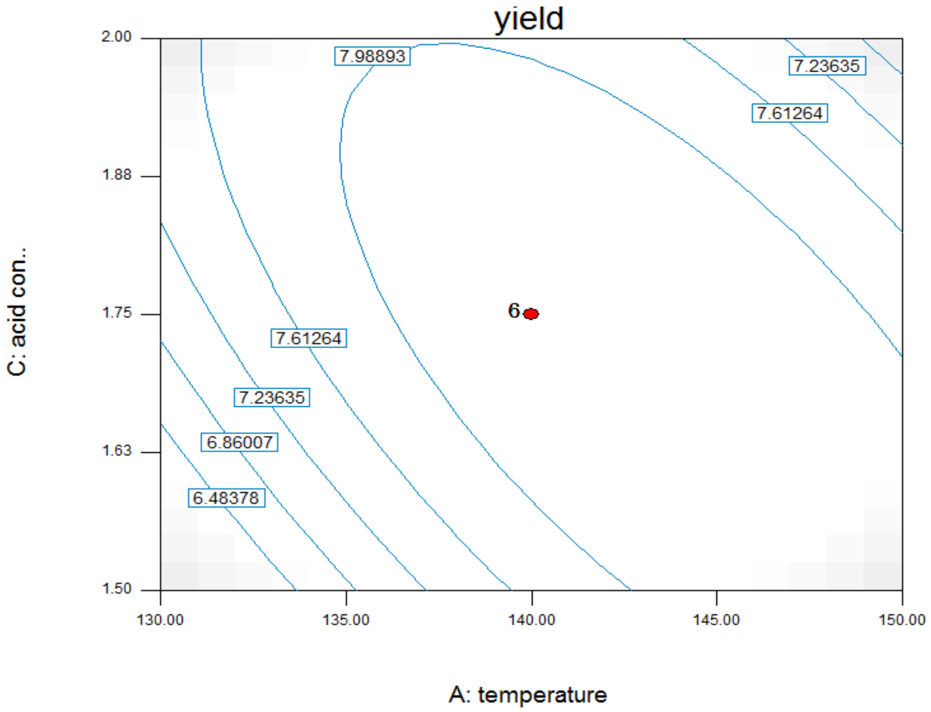


Figure 4.9 Contour plot of the effect of temperature and acid concentration at constant time in the center

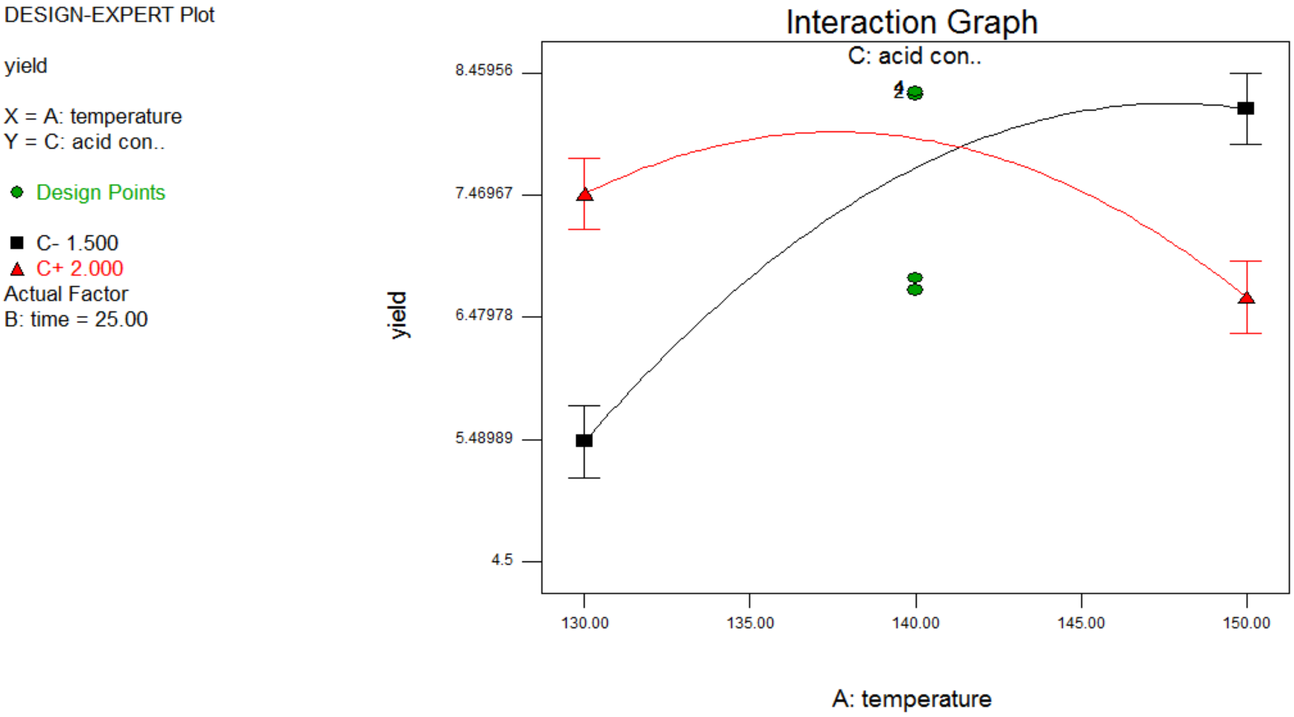


Figure 4.10. Effect and Interaction of temperature and acid concentration (fixed) on the yield of ethanol at center of time

Figure (4.8) and (4.9) represents the response surface and contour plots developed as a function of acid concentration and temperature respectively, while time was kept constant at 25min. At a definite acid concentration, the yield of ethanol increased slightly with hydrolysis temperature from 130 to 145^{o}C and nearly reached at a peak. However, upon increasing the hydrolysis temperature beyond 145^{o}C, there was a gradual decline in the yield, because sugar degrade might be occurred (Yang and Wyman, 2008). As shown from the contour plot the maximum yield of ethanol was occur in the region of 145^{o}C temperature and 1.64% acid concentration.

As we observed from figure (4.10) there is an interaction between acid concentration and temperature. Positive yield was observed at low temperature and high acid concentration and also at high temperature and low acid concentration. Because acid is used as a catalyst in the hydrolysis process so that at low acid concentration high temperature is required in order to obtain positive result the same is true at low temperature and high acid concentration. At lower temperature and acid concentration the cellulose might not hydrolysis to simple glucose and at higher acid concentration and temperature the cellulose might convert to other product which is not fermentable. Hence both temperature and acid concentration have strong interaction effect in hydrolysis process. The highest yield of ethanol was observed at 147°C hydrolysis temperature and 1.5% acid concentration.

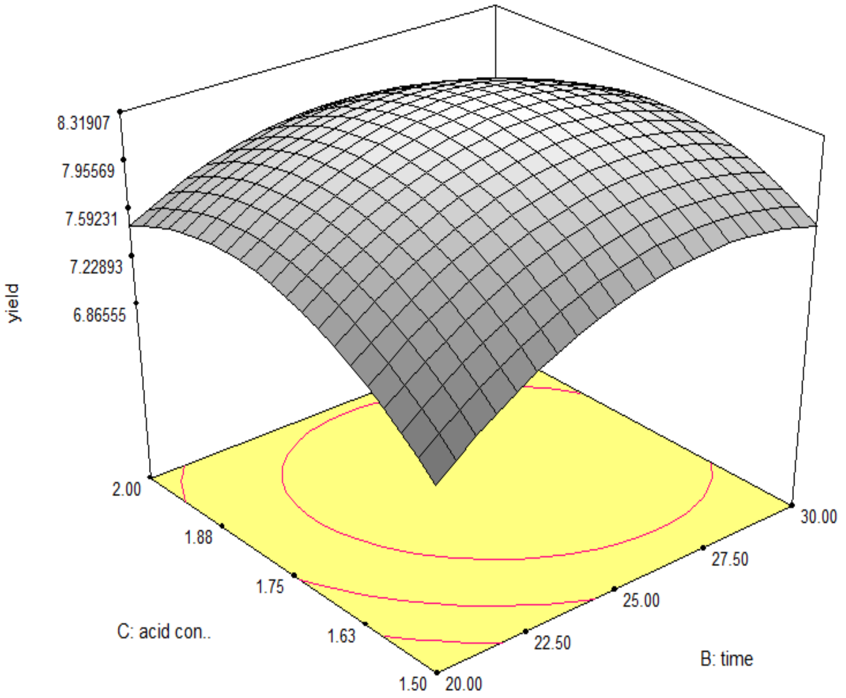


Figure 4.11. Response surface plot of the effect of time and acid concentration on the yield of ethanol at constant temperature

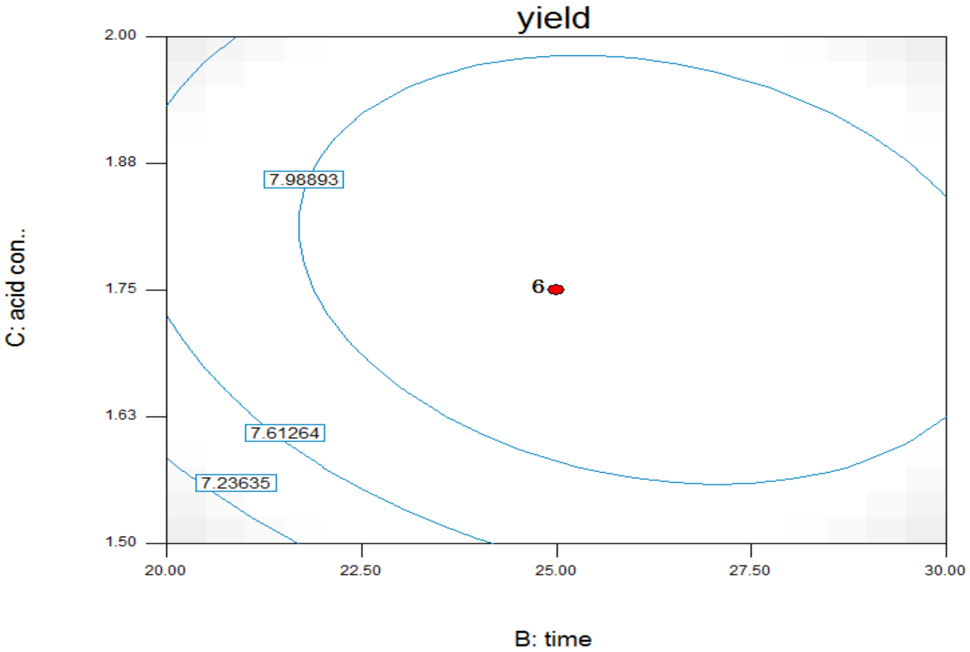


Figure 4.12 Contour plot of the effect of time and acid concentration on the yield of ethanol at constant temperature

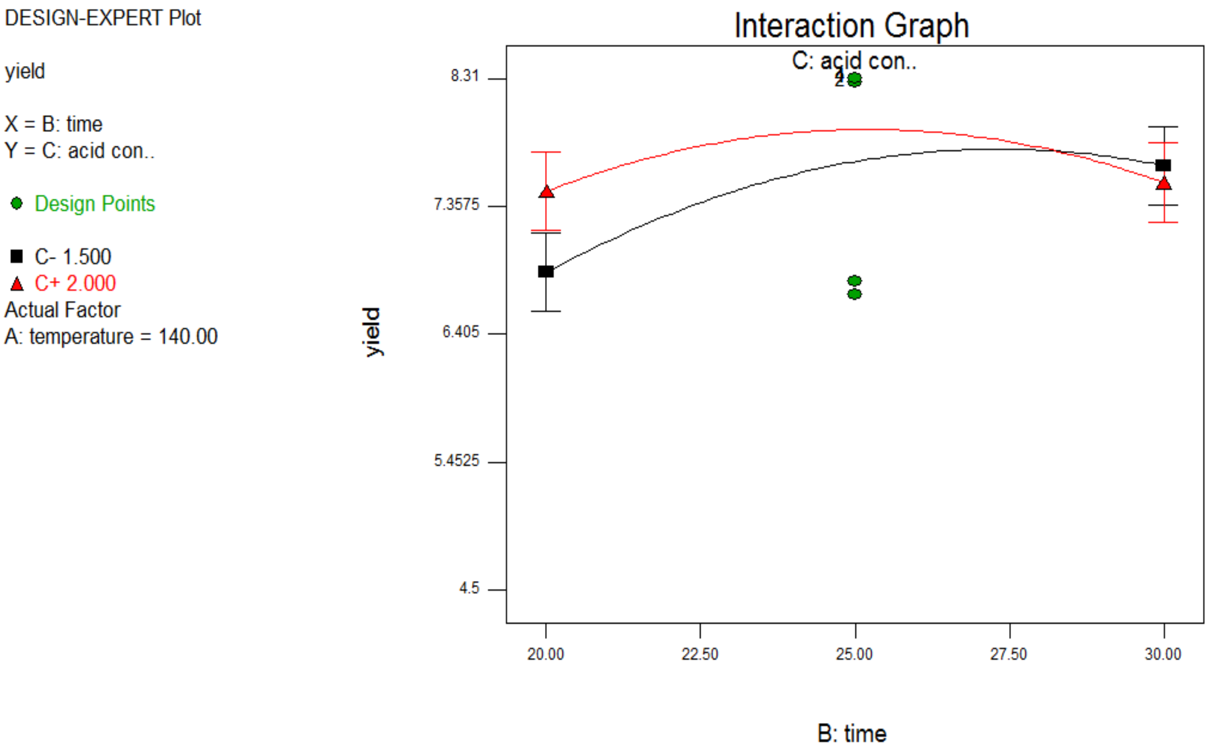


Figure 4.13. Effect of time and acid concentration (fixed) on the yield of ethanol at center of temperature

Figure (4.11) and (4.12) shows the response surface and contour plots developed as a function of time and acid concentration respectively, while the temperature was kept constant at 140^{o}C. Upon increasing the acid concentration from 1.5 to 1.75% with an increase of hydrolysis time from 20 to 26 min, the yield of ethanol increased in small extent. Beyond this time the yield of ethanol was gradually decreased. The highest yield was obtained at 26 min hydrolysis time and 1.75% acid concentration. The decrement of ethanol yield with increasing of acid concentration from 1.75-2 % is due to the decomposition of sugar and the formation of some inhibitor such as Furfural and 5-Methylhydroxy furfural. These substances are toxic substances for yeast and can inhibit the yeast growth (Yang and Wyman, 2008).

As it observed from figure (4.13) the time and acid concentration have small interact beyond 27.5 min and it has positive effect on the yield of ethanol at high acid concentration until the time reach 26 min. but, Beyond 26 min reaction time at fixed acid concentration the yield of ethanol slightly decreases due to the fact that lignocellulosic material is decomposed in to unwanted product or not fermentable molecules if it exposed into high temperature for long time.

4.2.2.3 Individual effect of experimental variables on the yield of ethanol

Effect of temperature

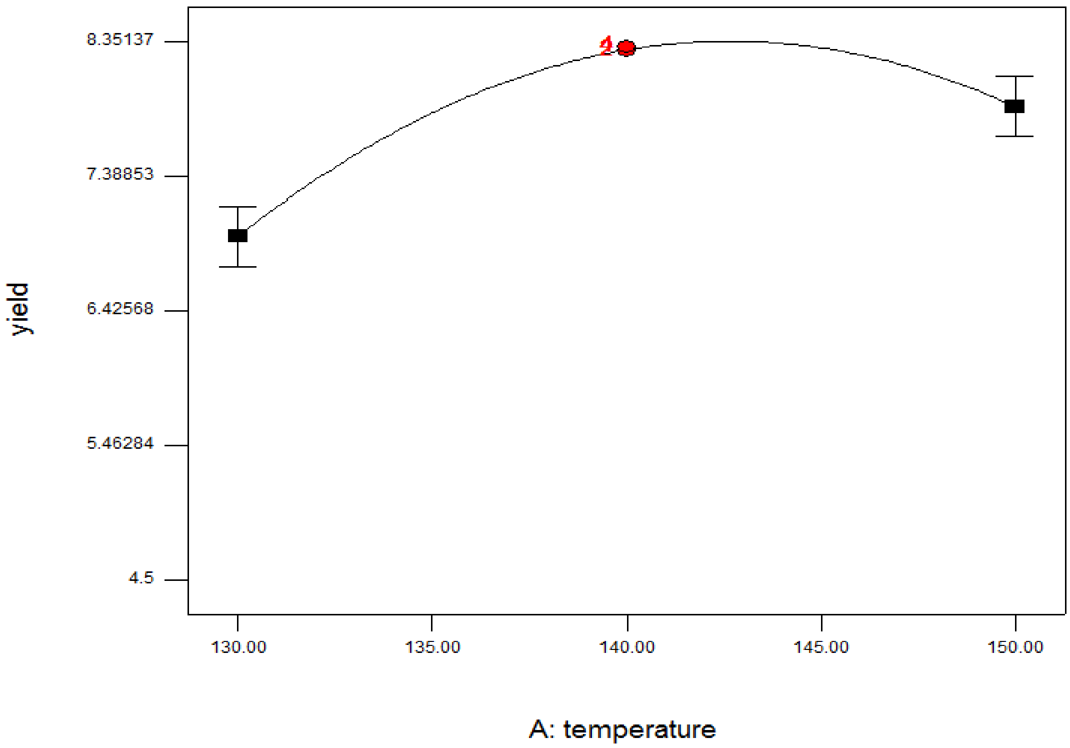


Figure 4.14. Effect of hydrolysis temperature on the yield of ethanol

Figure 4.14 represents the effect of hydrolysis temperature on the yield of ethanol at constant time and acid concentration at the center point. As shown on the figure 4.14 the yield of ethanol is very sensible to the hydrolysis temperature. Yield of ethanol was highly increased as temperature increase from 130°C to 144°C. Optimum yield of ethanol was obtained around 144°C hydrolysis temperature. Beyond 144°C hydrolysis temperature the yield of ethanol is slightly decrease, because at high temperature cellulose is degraded into not fermrmentale product such as 5-Methylhydroxy furfural (Yang and Wyman, 2008).

Effect of time

Figure 4.15 shows the effect of hydrolysis time on the yield of ethanol at constant temperature and acid concentration in the center point. As it observe from figure 4.15 below the yield of ethanol is slightly affect by hydrolysis time, as the hydrolysis time increase from 20min to 26min the yield slightly increase. Beyond 26 min hydrolysis time the yield of ethanol slightly decreases.

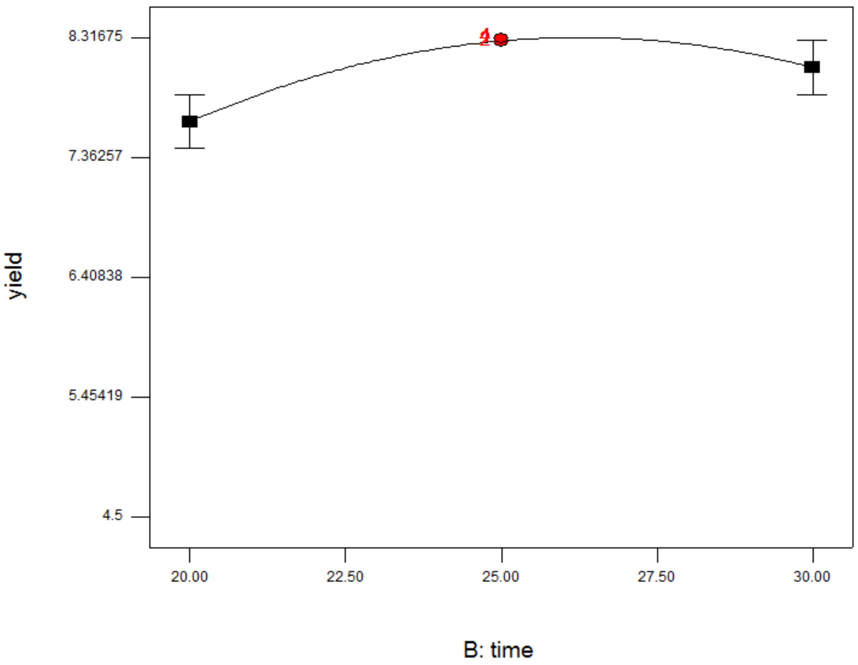


Figure 4.15. Effect of hydrolysis time on the yield of ethanol

Effect of acid concentration

Figure 4.16 shows the effect of acid concentration on the yield of ethanol at constant temperature and time in the center point.

As shown in figure 4.16 below the yield of ethanol was affected slightly by acid concentration, as the concentration of acid increase from 1.5% to 1.77% the yield slightly increase, beyond 1.77% the yield of ethanol slightly decreases,because high concentrations of acid may decompose the sugar and producing inhibitor molecules for the fermentation process (Taherzadeh and Karimi 2007).

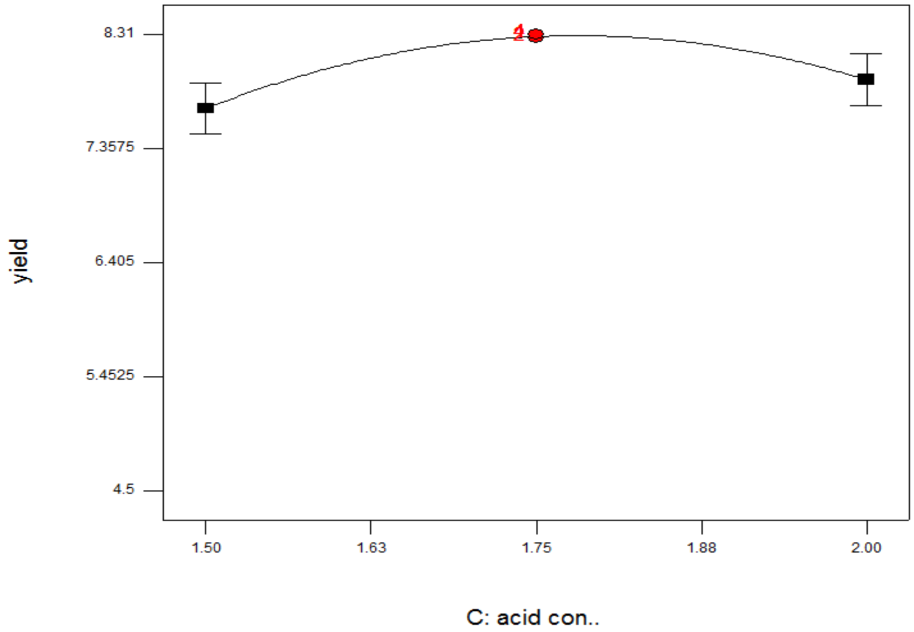


Figure 4.16. Effect of acid concentration on the yield of ethanol

4.3 Optimization of operating process variables in hydrolysis process using RSM

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

Table 4.8. Goal of optimization and limits of process parameters

Parameters	Goal	Minimum	Maximum
Temperature (^{o}C)	In range	130	150
Reaction time (min)	In range	20	30
Acid concentration (%w/w)	In range	1.5	2
Ethanol yield (ml/50 g BSG)	Maximum	4.5	8.31
TRS (%w/w)	Maximum	25.44	47.89

The optimum possible solutions in hydrolysis of Barley spent grain for production of TRS and bioethanol are presented in table 4.9 and in Figure (4.18-4.20) in the form of response surface plot and the contour plot. The predicted optimum yield of TRS and ethanol was

48.02%w/w and 8.38mL/50g dry BSG respectively observed at the process variables 144.29^{o}C, 26.26 min and 1.68%.

Table 4.9. Optimum possible solutions

No	Temperature (^{o}C)	Reaction time (min)	Acid.con (%)	Ethanol yield (ml/50 g BSG)	TRS	Desirability	
1	143.50	25.18	1.73	8.36058	47.9042	1.00	
2	142.56	26.48	1.70	8.37519	47.983	1.00	
3	144.25	26.69	1.65	8.37333	47.9458	1.00	
4	143.24	25.97	1.73	8.37434	47.9872	1.00	
5	144.46	26.10	1.67	8.38128	48.0045	1.00	
6	144.34	25.49	1.68	8.37607	47.9811	1.00	
7	144.92	25.71	1.64	8.37177	47.9435	1.00	
8	144.55	26.77	1.70	8.36231	47.9027	1.00	
9	144.68	26.36	1.65	8.3765	47.9664	1.00	
10	144.29	26.26	1.68	8.38117	48.0215	1.00	selected
11	143.70	27.00	1.68	8.37027	47.9401	1.00	
12	142.53	25.60	1.73	8.37196	47.9768	1.00	
13	143.49	25.26	1.73	8.36479	47.9286	1.00	
14	142.18	26.54	1.70	8.36896	47.9467	1.00	
15	142.45	25.64	1.72	8.37259	47.979	1.00	
16	143.93	25.79	1.72	8.36887	47.9496	1.00	
17	143.99	26.95	1.69	8.36889	47.9374	1.00	
18	142.81	26.50	1.75	8.35656	47.8968	1.00	
19	141.51	26.29	1.74	8.36043	47.914	1.00	
20	144.10	25.61	1.69	8.37889	47.9999	1.00	

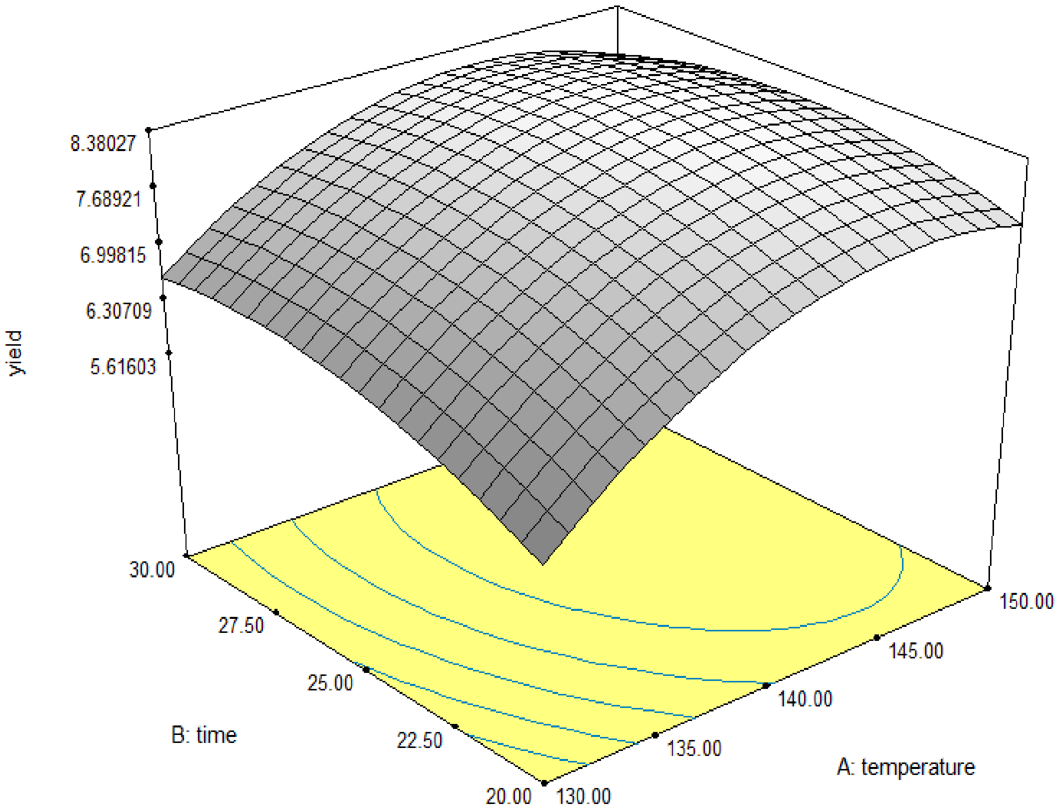


Figure 4.17. Response surfaces of predicted ethanol yield at fixed acid concentration

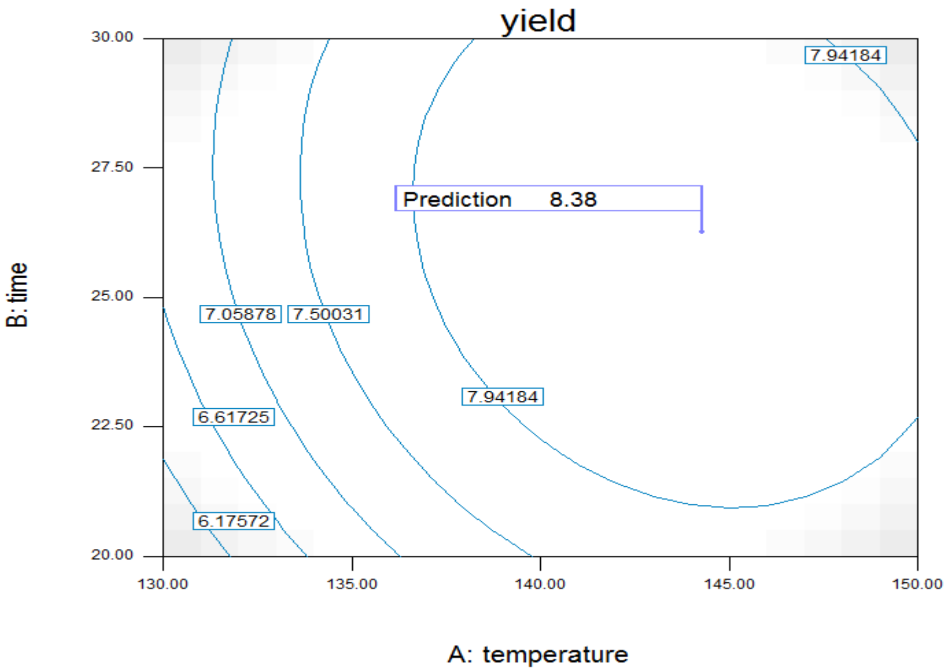


Figure 4.18 Contours plot of predicted ethanol yield at fixed acid concentration

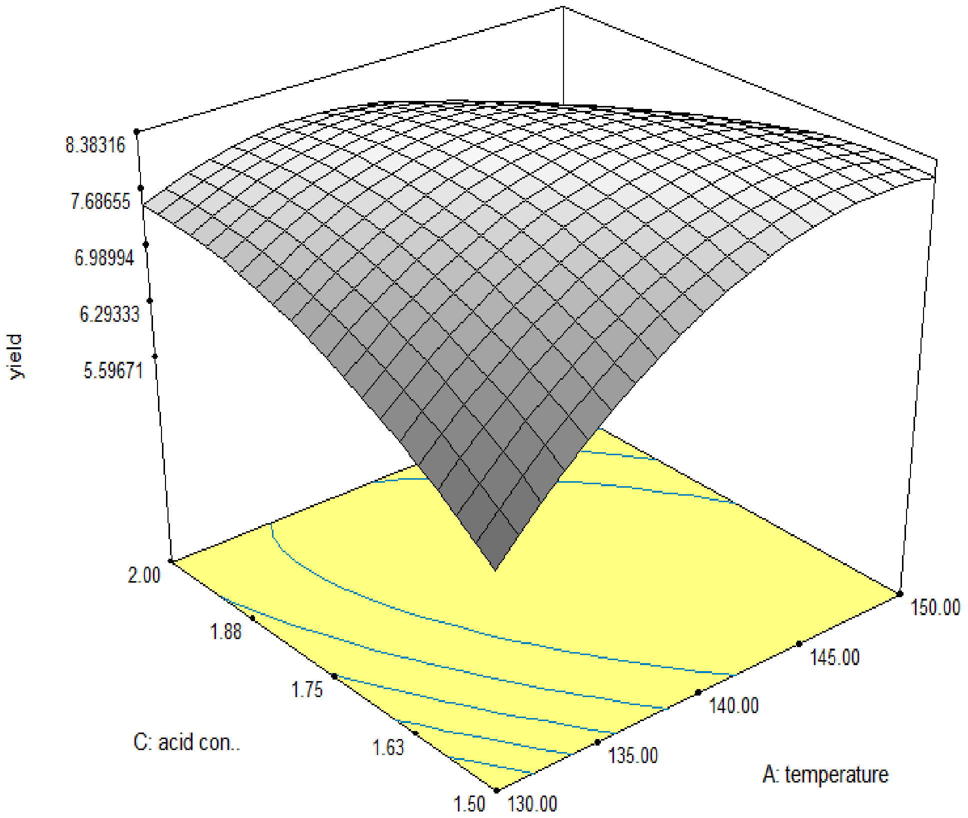


Figure 4.19. Response surfaces of predicted ethanol yield at fixed time

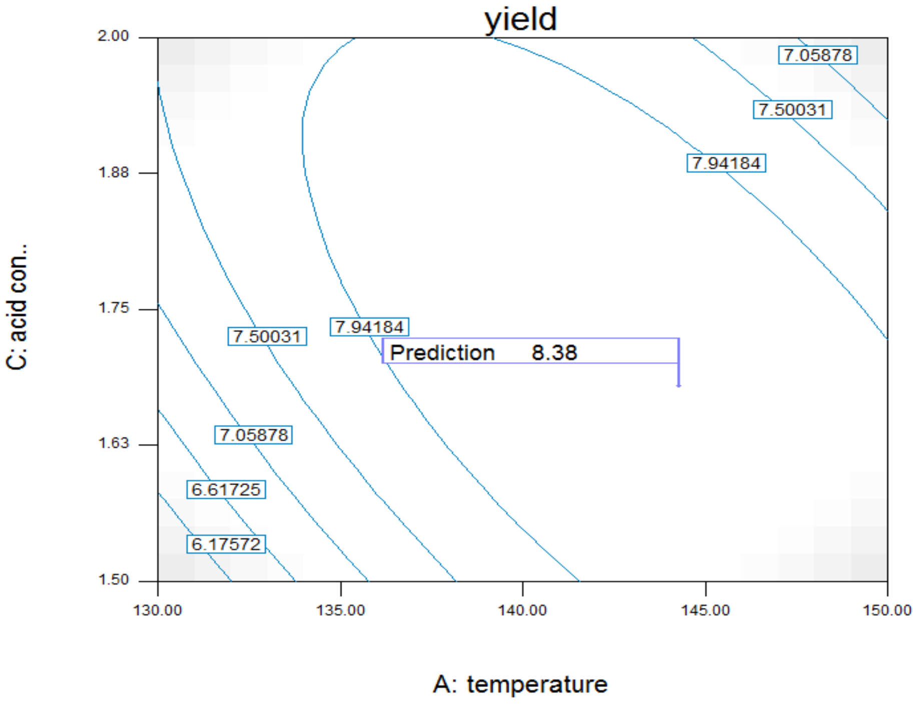


Figure 4.20 Contour plot of predicted ethanol yield at fixed time

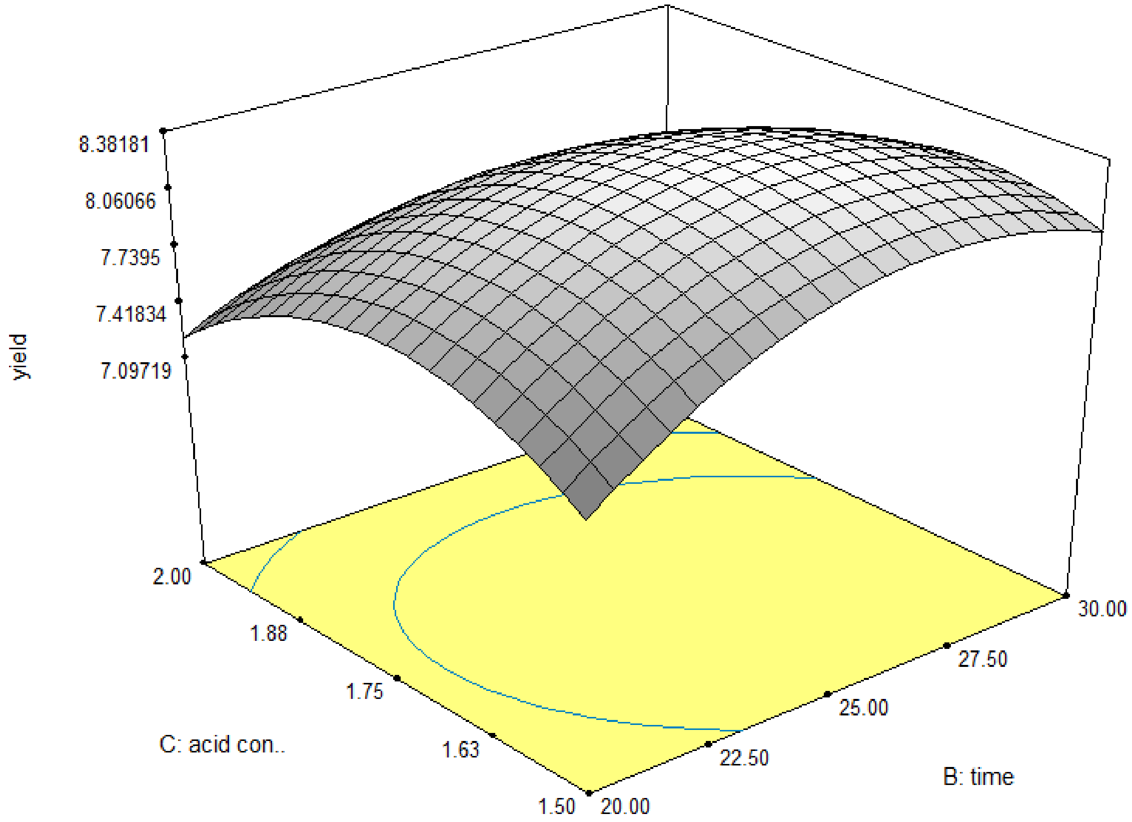


Figure 4.21. Response surfaces of predicted ethanol yield at fixed temperature

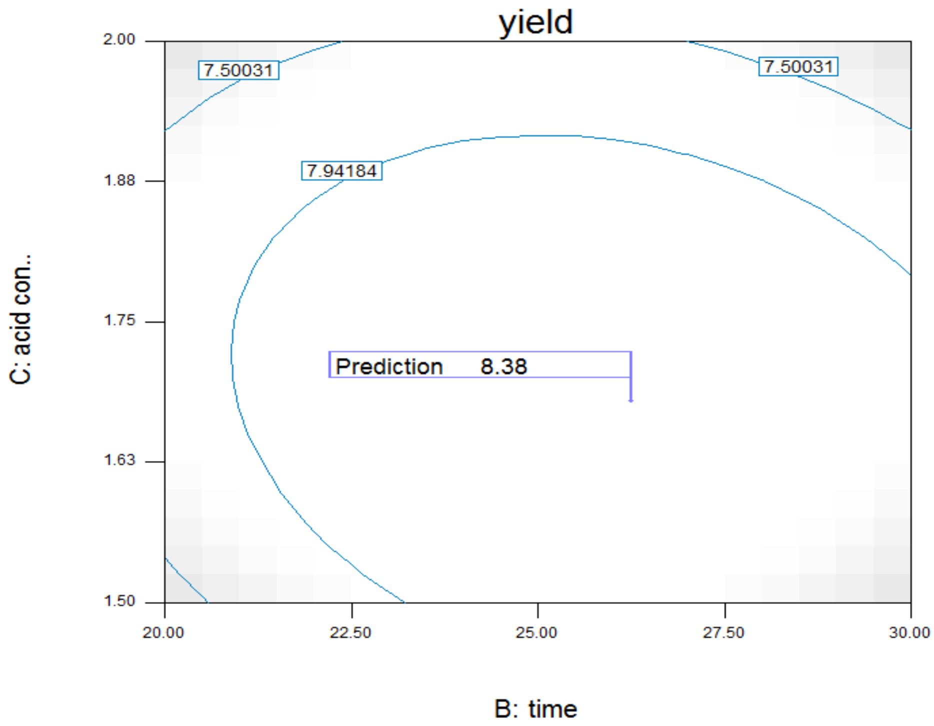


Figure 4.22 Contour plot of predicted ethanol yield at fixed temperature

4.4 Model validation

Based on the results of RSM the optimum process conditions in the hydrolysis of BSG were 144.29^{o}C, 26.26min and 1.68% acid concentration. In order to confirm the validity of the RSM model results a confirmation experiment with triplicate set was conducted at the above-specified optimum process conditions predicted by the model. Under these conditions the yield of ethanol and TRS were found 8.33 ml/50g BSG (average) and 47.60%w/w (average) respectively, which were close to the RSM result of 8.38ml/50g of BSG yield of ethanol and 48.02%w/w of TRS. This shows the experimental values were found to be close to the predicted values and hence the model was validated. Thus the model was useful to predict the yield of ethanol and also to obtain optimum process parameters for hydrolysis of Barley spent grain.

The fermentation process was carried out in shaker incubator at 30 °C, pH 5.0 and 175 rpm, for 72h using 5g/L concentrations of *S. cerevisiae*. The TRS to ethanol yield coefficient could reach a maximum of 0.276 g g^{-1}.

Table 4.10. Fermentation parameters for BSG biothanol production by *S. cereviseae*

Intial concentration of TRS (g/L)	Maximum Ethanol concentration (g/L)	Fermentation time (h)	Ethanol yield coefficient(g ethanol/g TRS)	Production rate of ethanol(g/L.h)
59.5	16.425	72	0.276	0.228

4.5 FT-IR Characterization of the Produced Bioethanol

Alcohols have characteristic IR absorptions associated with the O-H, C-O and the C-H stretching vibrations. When run as a liquid film the region 3500-3200 cm-1 with a very intense and broad band indicated the O-H stretch of alcohols, while the region 1260-1050cm^{-1} confirms the C–O stretch. The bands at around 2880 and 2930 cm^{-1} were assigned as the symmetric stretching modes of the $–CH_2$ and$–CH_3$ groups, respectively (Coates and Meyers, 2000; Yu et al, 2007). This ascertains that the product obtained from Barley spent grain (BSG) is definitely ethanol due to the confirmation of these regions (Figure 4.9).

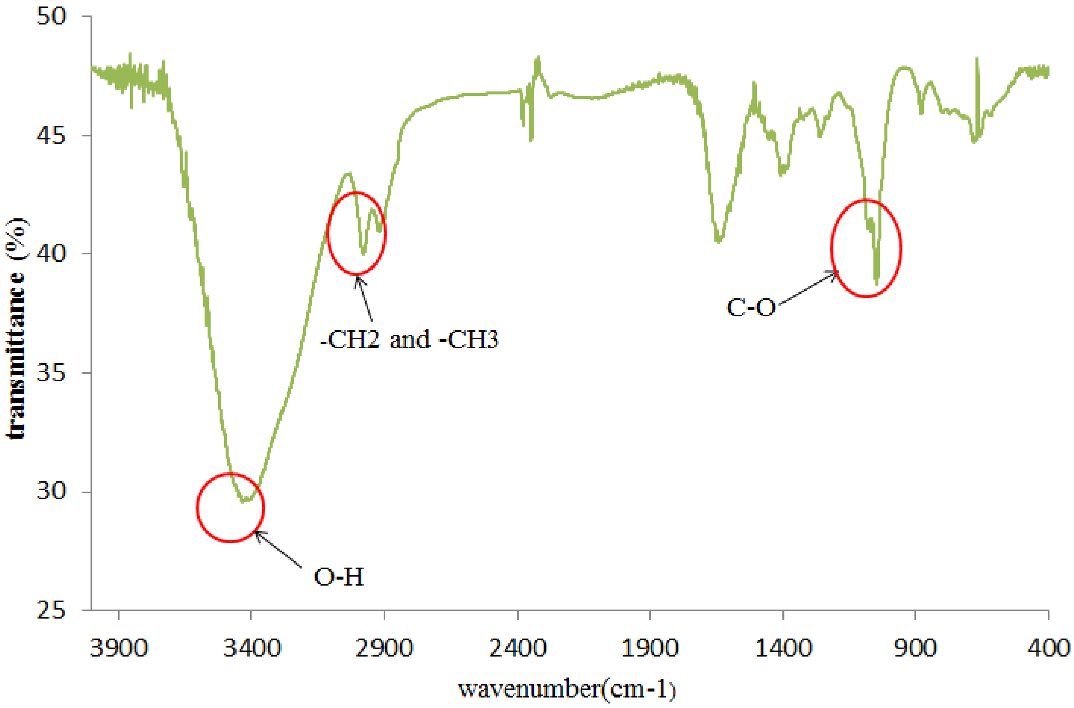


Figure 4.23. Fourier transform Infrared spectra of the produced bioethanol from Barley spent

5 MATERIAL AND ENERGY BALANCE OF BIOETHANOL PRODUCTION

Material and energy quantities, as they charge and discharge into and from process operations, can be described by material and energy balances. Such balances are statements on the conservation of mass and energy. If there is no accumulation, what goes into a process must come out. This is true for continuous operation. If no input and output during the operation carried out the process is batch process.

Material and energy balances are very important in an industry. Material balances are fundamental to the control of processing, particularly in the control of yields of the products. The first material balances are determined in the exploratory stages of a new process, improved during pilot plant experiments when the process is being planned and tested, checked out when the plant is commissioned and then refined and maintained as a control instrument as production continues. When any changes occur in the process the material balances need to be determined again.

The energy balance determinations are also made to determine the energy requirements of the process, the heating, cooling and power required. In this plant operation it is thought that an energy balance (energy audit) on the plant will show the pattern of energy usage and suggest areas for conservation and savings.

5.1 Material Balance

Basis: one year/340 days
Production rate: $5x10^6$lit/year of ethanol (99.9%) with plant operation of 340 days per year and the Plant attainment (i.e. the percentage of the available hours in a year) was calculated as

$$\% \text{ Plant attainment} = \frac{operation\ hours}{hours\ of\ the\ year} x100\%$$

$$= \frac{8160}{8760} x100\% = 93.15\%$$

The percent of plant attainment was acceptable because the recommended % of plant attainment for chemical industry is between 90-95%.
The production rate in term of mass flow rate was

$\varrho = m/v$ .... (5.1)

$m = \varrho \times v = 0.789\text{kg/lit} * 5\text{x}10^6\text{lit/yr}$

=3,945,000 kg ethanol per year or 3,945 ton/yr

Production flow diagram of bioethanol from BS

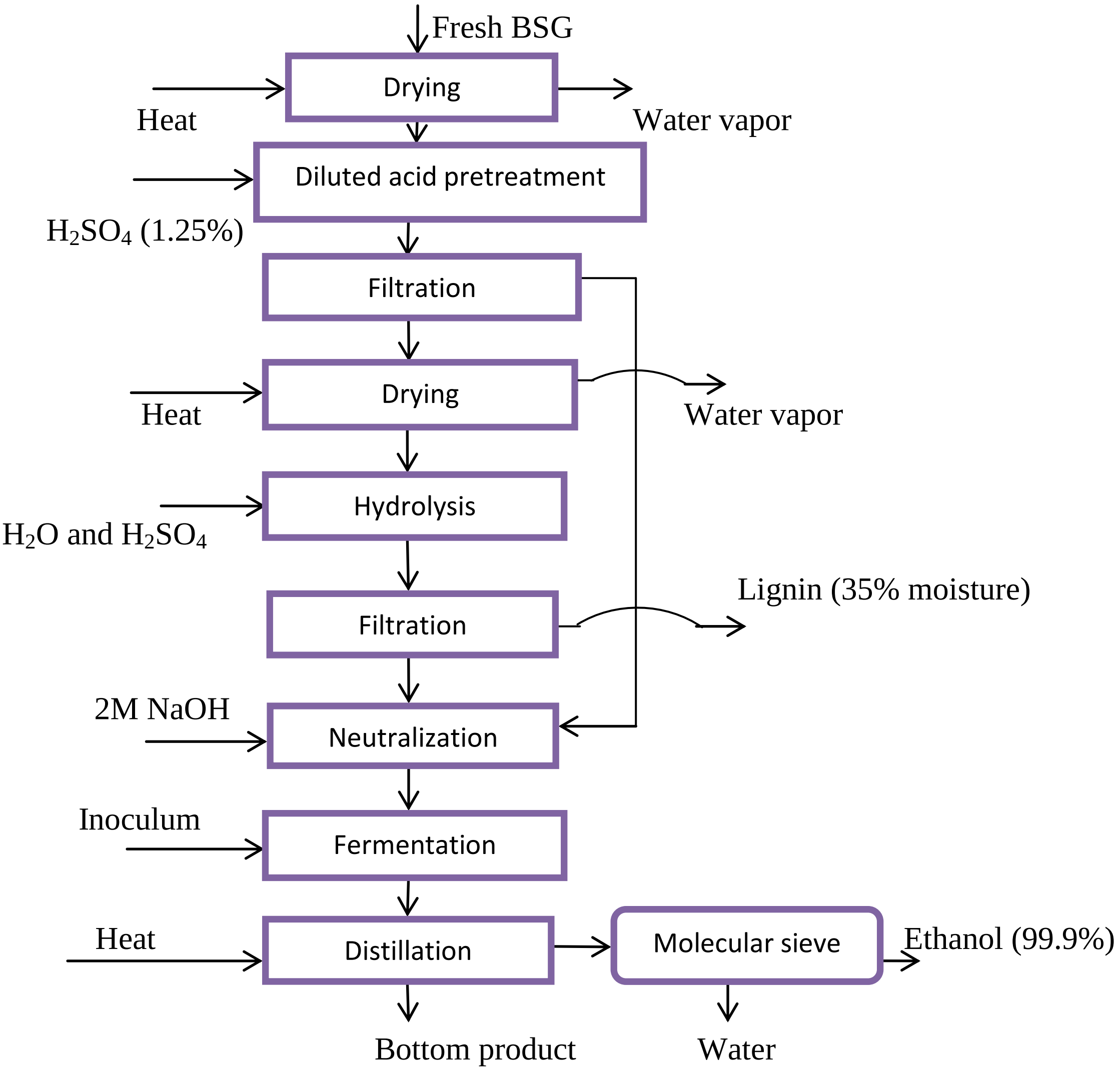
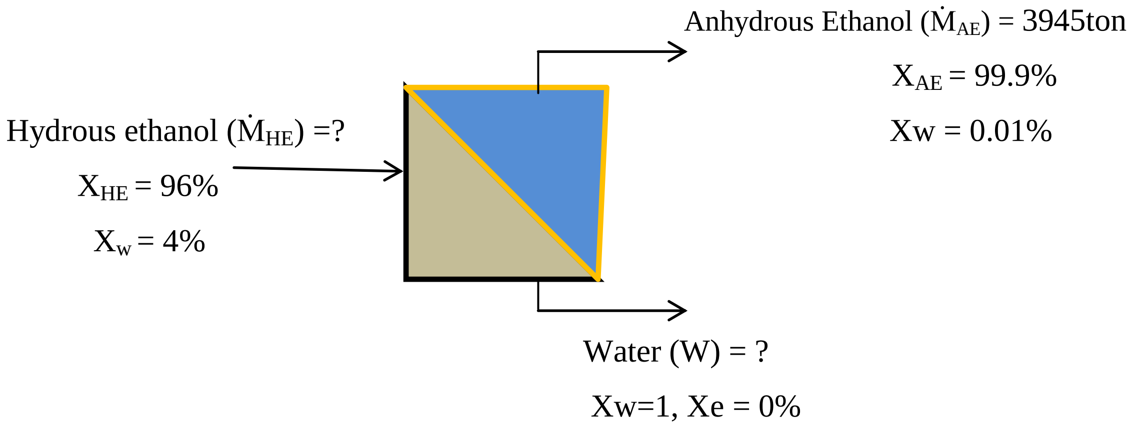


Figure 5.1. Production flow diagram of bioethanol from BSG

Material balance for Molecular sieve

Molecular sieve is an equipment which following the distillation column and bring the ethanol concentration from 96% to 99.9%.



Where Xe= Percentage of Ethanol

Xw= Percentage of water

Over all mass balance

$$\dot{M}_{HE}=W+\dot{M}_{AE} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (5.2)$$

Ethanol mass balance

$$\dot{M}_{HE}*X_{HE}=\dot{M}_{AE}*X_{AE}\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (5.3)$$

From equation 5.3

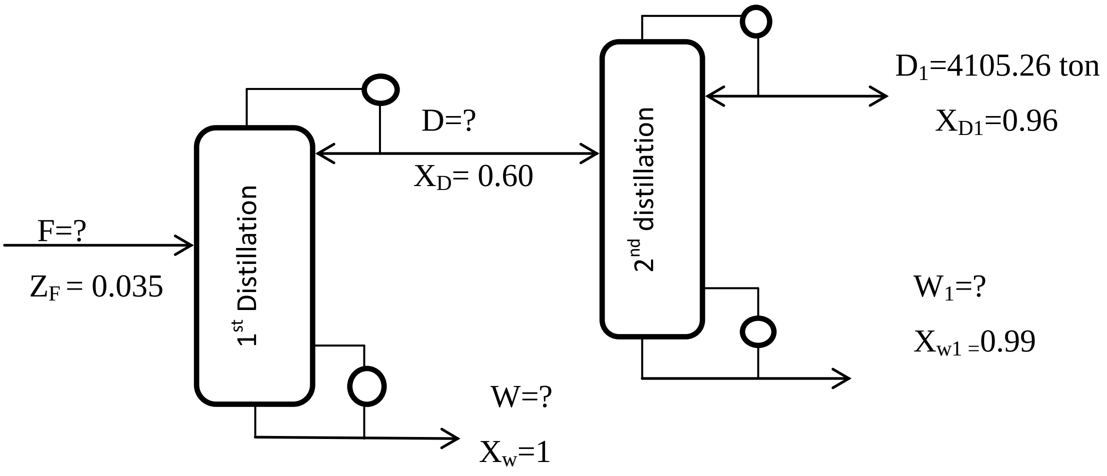
$$\dot{M}_{HE}=\frac{\dot{M}AE*XAE}{XHE}=\frac{3{,}945\ x\ 0.999}{0.96}=\ 4105.26\ ton$$

The water that is trapped by the molecular sieve is calculated from equation 5.2

$$W=\dot{M}_{HE}-\dot{M}_{AE}=4105.26-3{,}945=160.26 \text{ ton of water}$$

Material balance on distillation

Distillation is used for recovery of ethanol from water-ethanol mixture. The mixture of ethanol water which obtained from fermentation process was separated using two-stage distillation column to recovered 96% ethanol from the mixture of 3.50% ethanol.



Where: F=feed to the 1st distillation

D=distillate from 1st distillation

Z_F =feed composition of light component

D1= distillate from 2nd distillation

W= bottom product

Over all mass balance on the 2nd distillation

$D=D1+W1$

$D= 4105.26 +W1$ .... (5.4)

Ethanol mass balance on the 2nd distillation

$0.60D=0.96D1 +0.01W1$ ....(5.5)

Substitute equation 5.4 in to 5.5

$0.6(4105.26 +W1) = 0.96*4105.26 +0.01W1$

W1=2505 ton

D=4105.26 + 2505 =6610 ton

Over all mass balance on the 1st distillation

$F = D+W$

$F= 6610 +W$ ....(5.6)

Ethanol mass balance on the first distillation

$0.035 F = 0.60D +0$ ....(5.7)

Substitute equation 5.6 in to 5.7

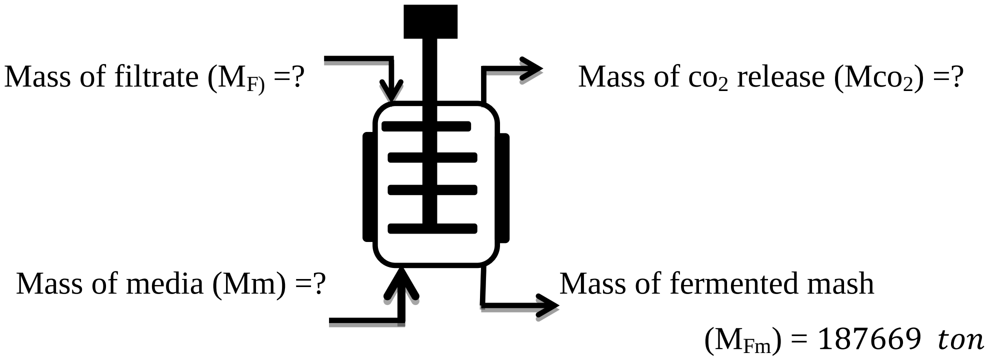
F=187669 ton and W=173145 ton

Material Balance on Fermentation

Material balance in the fermenter was carried out based on the fermentation reaction

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

180 g/ mol $\longrightarrow$ 92g/mol ethanol + 88 g/mol CO_2 for 100% conversion.



Assume 90% glucose can converted into ethanol if the fermentation process very effective from literature.

$$\frac{0.90MGF}{180} = \frac{\text{HEx}0.96}{92}$$

$0.90M_G= (4105.26\ x0.96x180)/92$

$M_G= 8567.5$ ton of glucose is required annually

Where

HE =hydrous ethanol

M_G=mass of glucose

The amount of carbon dioxide produced is

$M_{CO2} = (8,567.5\ x88)/180$

= 4188.55 ton of CO_2 released per year

Over all mass balance on the fermentation process is

$M_F + M_M = M_{FM} + M_{CO2}$

$M_M = 0.1M_F$

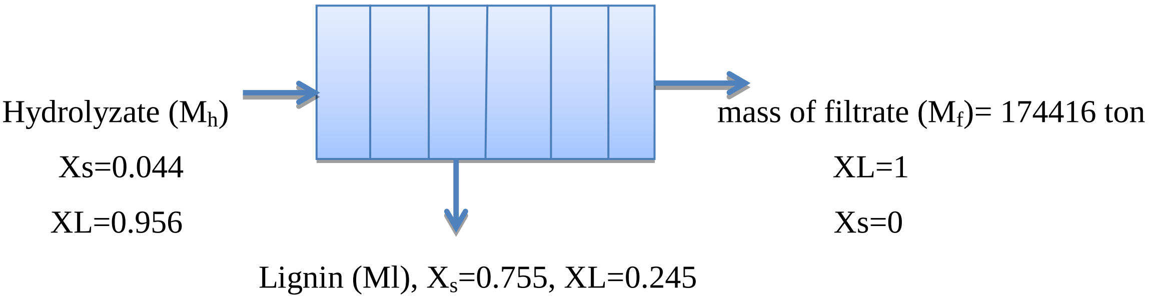
$M_F + 0.1M_F = 187669 ton + 4188.55$ ton

$M_F = 174415.98$ ton of filtered Hydrolyzate is required

Mm = 17441.60 ton of inoculum is required

Material balance on pressurized bag filter

This is used for separating insoluble solid (lignin) from liquid (sugar solution). Moisture content of sludge (lignin) was 24.5%.



Over all mass balance

$Mh=Ms + M_f$.. (5.4)

Mass balance on the solid

$Mh * 0.044 = Ms * 0.755$

$Mh=17.16\ Ms$... (5.5)

Substitute equation (5.5) into equation (5.4) to calculate mass of lignin or total solid remain after hydrolysis

16.16 Ms =Mf

Ms = (174416ton)/16.16

= 10793 ton of solid or lignin was generated per year.

The amount of hydrolyzate is calculated from equation (5.4)

M_H=185209 ton of hydrolyzate is need per year

Material balance in dilute acid treatment (Hydrolysis)

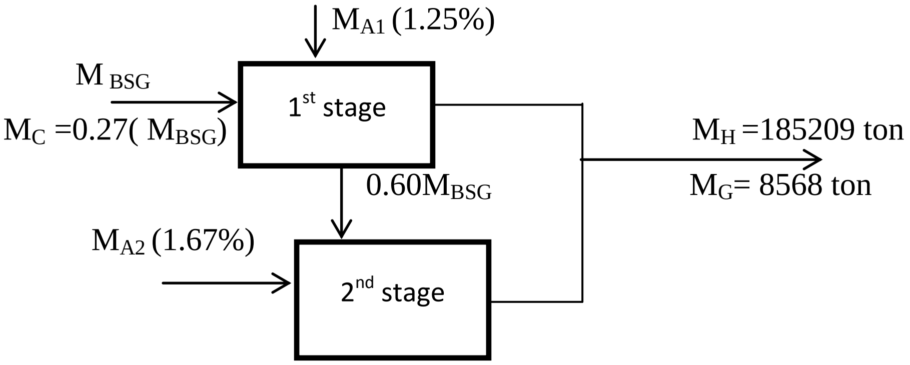
The mass balance was based on the laboratory result obtained; the liquid solid ratio was fixed at 8:1, in the first stage hydrolysis 1.25%w/w acid concentration was used to decomposed hemicellulose and in 2nd stage 1.68 %w/w acid concentration was utilized to decomposed cellulose in to its monomer which is called glucose. The amount of solid charged in to the 2nd stage was 60% of the total solid charged into the 1st stage and the, remain 40% was decompose in the 1st stage hydrolysis.

Hydrolysis reaction is

$$C_6H_{10}O_5 + H_2O \xrightarrow{H_2SO_4} C_6H_{12}O_6$$

162g/ mole of cellulose → 180g/ mole of glucose for 100% conversion

But not 100% conversion and assume that 95% of cellulose converts to glucose. According Dehnavi, (2009) 27% of barley spent grain is cellulose.



By using stoichiometry equation the required dry barley spent grain (M_{BSG}) is calculated as

$$\frac{0.27 * 0.95\ \mathrm{Mbsg}}{162} = \frac{MG}{180}$$

0.27*0.95* M_{BSG} = (162* 8568)/180

M_{BSG}=30062 ton of dry barley spent grain is required per year.

Mass balance on the 1st stage

The liquid solid ratio in the 1st stage was (8:1)

$$\frac{Mbsg}{MA1}=\frac{1}{8}$$

$$M_{A1}=8*M_{BSG}$$

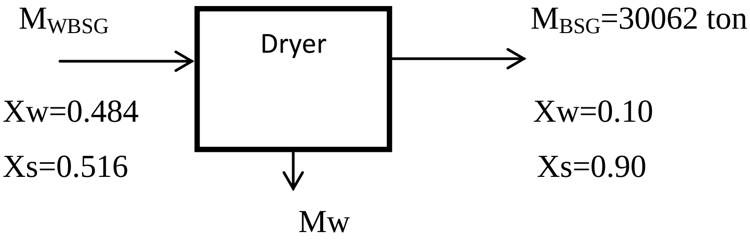
M_{A1}=240491 ton per year of 1.25% diluted sulfuric acid is required per year in the 1st stage hydrolysis. The acid required in this stage is 3006 ton/year.

The same is true for the 2nd stage hydrolysis; liquid solid ratio was (8:1)

$$M_{A2}=8*0.60M_{BSG}$$

M_{A2}= 144294 ton per year of 1.67% diluted sulfuric acid is required per year in the 2nd stage hydrolysis. The acid required in this stage is 2410 ton/year.

Mass balance on dryer



Over all mass balance

$$M_{WBSG}=Mw + M_{BSG}$$

Component mass balance on the solid BSG

$$0.516\ M_{WBSG}=0.90\ M_{BSG}$$

M_{WBSG}= (0.90*30,061.4)/0.516

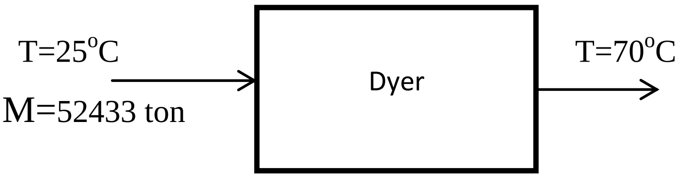
=52,432.67 ton of wet BSG is required

From the above over all mass balance, mass of water is 22,371.27 ton

5.2 Energy balance

The energy balance was performing based on the laboratory results.

The basic of the calculation is one hour.



The required energy is calculated as

$Q = m\ Cp\ \Delta T$

Where

Q= required energy

M=mass of wet BSG

Cp=specific heat of wet BSG, Cps =0.44 Cpw

Cp = CPw*Xw + Cps *Xs

= 4.18*0.484 +4.18*0.44*0.516

=2.97kJ/kg k

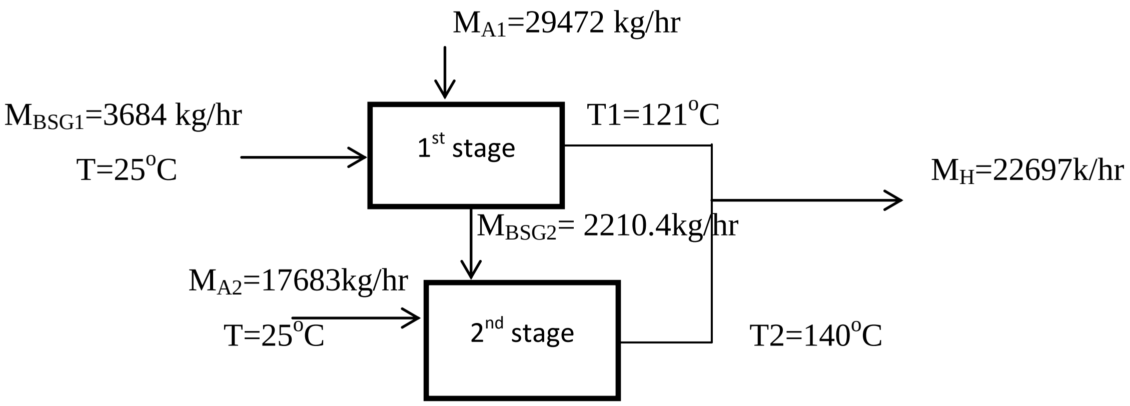
Q = m Cp ΔT

= 52,432.67 *2.97 (70-25)

Q=5,450,376 kJ/year =668kJ/h

Energy balance on hydrolysis

The energy used for hydrolysis process is generated from water steam.



Where

M_{BSG1}=mass of BSG input to 1st hydrolysis stage

M_{BSG2}=mass of BSG input to 2nd hydrolysis stage

M_{A1}=mass of water and acid solution input to 1st stage hydrolysis

M_{A2}= mass of water and acid solution input to 2nd stage hydrolysis

The amount of heat required for the hydrolysis process

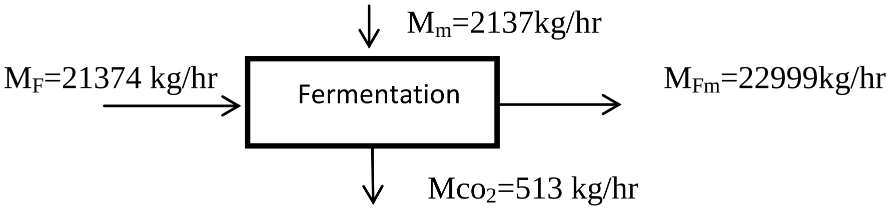
$$Q = M_1Cp_1\Delta T_1 + M_2Cp_2\Delta T_2$$

$Cp_1= \frac{3684*3.79+29472*4.18}{3684+29472}$=4.14kJ/kg k

$Cp_2= \frac{2210.4*3.79+17684*4.18}{2210.4+17684}$=4.136kJ/kg k

Q=33156*4.140(120-25) + 19894*4.136 (140-25 =2250282 kJ /hr

Energy balance on fermentation



Exothermic energy is released during fermentation; the amount of heat generated can be calculated. The outlet temperature is 30°C

Cp of mix at 30°C = 4.14kJ/kg k

Cp of CO_2 at 30°C = 0.846kJ/kg k

$Q_{MIX} = QCO_2 + Q_{Fh} + Q$

$Q = Q_{MIX} - QCO_2 - Q_{FM}$

$Q = M_{mix}Cp_{mix}\ \Delta T - Mco_2Cpco_2\ \Delta T - M_{Fm}Cp_{Fm}\ \Delta T$

$Cp_{Fm} = Cp_{mix}*X_{mix} + Cpco_2*Xco_2$

$Xco_2 = 513/\ (21374+2137) = 0.0218$

$X_{Fm} = 1-0.0.0218 = 0.978$

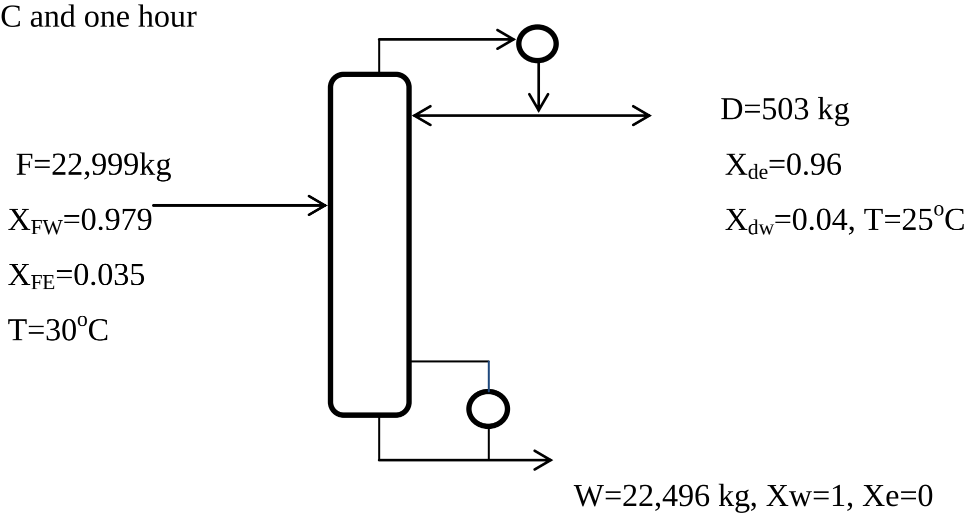
$Cp_{Fm} = 3.781$kJ/kg k

$Q = 23511.4*4.14*(30-25) - 513*0.846*(30-25) - 22999*3.781(30-25)$

= 49,826.61kJ/h

Energy balance on distillation boundary

Basis; 25°C and one hour



Specific heat capacity of distillate (Cpd)

Cp = 0.96*2.72+0.04* 4.18=2.778kJ/kg k

Specific heat capacity of the bottom product is specific heat capacity of water

Cpw =4.18kJ/kg k

Mass balance on the condenser, assume reflux ratio i.e. $R=L/D=2.5$

$L=2.5D=2.5*503$ kg/hr = 1257.5 kg/hr

$V=L+D=1257.5$ kg/hr + 503 kg/hr = 1760.5 kg/hr

From equilibrium data, boiling point of ethanol at 96% is 78.13^oC

At steady state condition

INPUT=OUTPUT

$HF = H_D + H_L + Q_C$

$Q_C=H_F-H_D-H_L$

Assume complete condensation, therefore

Enthalpy of Vapor = Latent + Sensible heat

$H_V = m_v\lambda_v + m_vcp\Delta T$

$=1760.5 * 789+1760.5 * 2.778(78.13-25) =1,648,876$ kJ/hr

Overall balance to determine Q_B

Input = Out put

$Q_B + H_F = Q_C + H_D + H_W$

$Q_B=Q_C+H_D+H_W-H_F$

$H_F = m_FCp\Delta T$

$Cp=0.021*2.72+0.979*4.18 = 4.15$ kJ/kg k

$H_F=22999$ kg/hr$*4.15*(30-25) = 477229.25$ kJ/hr

$H_W = m_wCp\Delta T=22,496 *4.18 (100-25) =7,052,496$ kJ/hr

$Q_B = Q_C + H_D + H_W - H_F$

$Q_B=1,648,876$ kJ/hr + 0 + 7,052,496 kJ/hr -477229.25 kJ/hr =8,224,143 kJ/hr

Amount /mass of steam required

Latent heat of steam at 274KN/m^2, $\lambda v=2174$kJ/kg, therefore mass of steam required,

$Q_B=Ms\ \lambda s$

$Ms=Q_B/\lambda s$ = (8,224,143 kJ/hr) / (2174 kJ/kg) = 3783 kg/hr

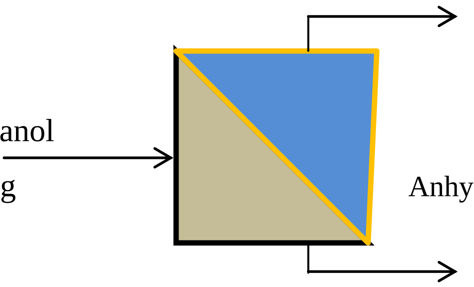
Mass of water that is able to condense is removed with temperature rise of 30^oC

$Q_C=Mw\ Cp\ \Delta T$, $M= \frac{Qc}{Cp\Delta T} = \frac{1,648,876}{4.18*30}$ =13149 kg/hr

Energy balance on molecular sieve

Molecular sieve is a mass transfer unit operation that is used to adsorb a small constituent molecule of water which is present in the ethanol to increase the concentration of ethanol and raise the concentration of ethanol to around 99.9%.

Basic; one hour



mass of water =19.64 kg

Xw=1, Xe=0%

Hydrous ethanol

($\dot{M}_{HE}$) = 503kg

X_{HE}=96%

X_w=4%

T_o=25°C

Anhydrous Ethanol ($\dot{M}_{AE}$) =483.3kg

X_{AE}=99.9%

Xw=0.01%

T=95°C

Cp is calculated based on their percentage of the input mass of hydrous ethanol

Cp = 0.96*2.72+0.04*4.18 = 2.78kJ/kg k

The temperature entering the molecular sieve is 25°C and exit is at a temperature of 95°C

$$Q = M\ Cp\ \Delta T$$

$$= 503 * 2.78 * (95-25)$$

$$= 97884 kJ$$

6 SIZING OF MAJOR EQUIPMENTS

Assumption:

- All tanks are 85% full or 15% safety factor.
- Sizing of equipment is depending on the material balance calculated on the above section.
- Basis : one day

Sizing of dried spent grain storage tank

Material of construction	carbon steel
Material handled	dried spent grain
Density of spent grain:	0.336g/cm3=336kg/m3
Required mass per day	88,415 kg

Density = mass/volume

The required volume of storage tank is for 3 days

Volume dried spent grain = (88,415 kg/day)/336kg/m^3

= ($263m^3$)*3=$789.4m^3$

V_{vessel} = (789.4/0.85) = $929m^3$

Sizing of water storage tank

Material of construction	carbon steel
Material handled	water
Density	1000 kg/m^3
Mass of water	1,115,453kg/day

$V\ of\ water\ = \frac{1{,}115{,}453\text{kg/day}}{1000kg/m3}$ = $1115.45m^3$/day

Volume of vessel = ($1115.45m^3$)/ (0.85)

= 1312.29 m^3

Sizing of mixer

Material of construction	carbon steel
Material handled	milled spent grain and water
Type of agitator	top entering

Density	888 kg/m^3
Mass of mixing material	79,735kg/day

V of slurry = mass of slurry/density = 795,735 kg /day/888 kg m^3 = 896 m^3

42 batches are operated per day, so that the volume of mixture per batch is 22 m^3.

V_{vessel}=22m^3/0.85= = 26 m^3 including 15 % safety factor

Sizing /design of agitator in the mixer

Assume Length to diameter ratio. I.e. L/d=2, L= 2D

Volume of the tank/mixer= 26m^3

$V=\frac{\pi D2}{4}*L$

26 m^3 = $\frac{\pi * D2 * 2D}{4}$

D=2.5 m and L=5 m

Diameter of agitator = 0.8*Dv=0.8*5 = 2 m

Sizing of hydrolysis reactor

Materials of construction	carbon steel
Material handled	slurry of BSG and water mixture
Density of slurry	888 kg /m^3
Temperature of the slurry	144.29°C
Mass of slurry	544,732.35kg/day

Capacity

$$v = m/ϱ = \frac{(544{,}732\ kg/day\ )}{888kg/m3}$$

$$= 614\ m^3 \text{ per day}$$

24 batches are carrying out per day, so that the volume of one batch is 25.58 m^3.

Required volume of vessel

$$V_{vessel} = (25.58m^3)/0.85$$

$$= 30\ m^3$$

Sizing /design of agitator in the hydrolysis reactor

Assume the length to diameter ratio. I.e. L/d= 2, L=2 D

Volume of the tank/mixer =30 m^3

$$V = \frac{\pi D2}{4} * L$$

$$30\ m^3 = \frac{\pi * D2 * 2D}{4}$$

D=3 and L= 6 m

Diameter of agitator = 0.8*Dv=0.8* 4= 2.4 m

Sizing of hydrolyzate storage tank

Materials of construction	rubber-linked cs
Material handled	hydrolyzate of BSG
Density of liquid	880 $kg\ /m^3$
Temperature of the slurry	20^{o}C
Mass of slurry	544,732.35kg/day

Capacity

$$v = m/ϱ = \frac{(544{,}732\ kg/day\ )}{880 kg/m3}$$

$$= 619\ m^3 \text{ per day}$$

The hydrolyzate storage tank is design to store for three days, so that the volume of vessel is calculated as

$V=619/0.85=728m^3*3=2185m^3$

Sizing of Fermenter

Material construction	carbon steel
Material to be handled	Hydrolyzate
Shape	dome shaped
Density of liquid	880kg/m3
Temperature	30^{0}C
Mass of feed	564288 kg/day

The residence time of one batch is 72 hour or 3 days, so that three fermenters are required for the ensuing fermentation days.

$$V \text{ of fermenter } = \frac{m}{ϱ} = \frac{564{,}288\ kg/batch}{880\text{kg/m3}} = 641\text{m}^3$$

Required volume of vessel with 15% safety factor is

$$V_{\text{vessel}} = \frac{v}{0.85} = \frac{641\ m3}{0.85} = 755\text{m}^3$$

Sizing of ethanol Storage tank

Material of constriction	Carbon steel
Material handled	Ethanol
Density	0.789kg/lit=789kg/m3
Temperature of liquid	20^0C
Mass of ethanol per day is	11603kg/day

$$v=\frac{m}{\varrho}=\frac{11603kg/day}{789kg/m3}=14.7\ m^3/day$$

Required volume of vessel

$$V_{vessel}=\frac{v}{0.85}=\frac{14.7}{0.85}=17.3m^3$$

The product may be stay for around one week or 7 days, so that the total volume of vessel required for one week is 122 m^3.

Sizing of distillation column

Specification

Mode of operation	continuous
Degree of separation	96% ethanol and 4% water @ the distillate
Contacting device	plate type
Condenser type	total condenser
Reboiler type	partial Reboiler
Operating pressure	0.5 bar
Vapor velocity	0.75 m/s
Feed flow rate	1780 kg/h or 81 kmol/h
Feed composition	60 % ethanol and 40% of water

Boiling point of ethanol = 78.13^oC = 351.13k

Boiling point of water = 100^oC = 373k

Reflux ratio = 2.5

Calculation

Over all mole balance

$$F = D + B$$

$$81\ kmol/h = D + B$$

Ethanol mole balance

$$81*0.60 = 0.96D + 0.01B$$

Combine the two equations and we got

D = 50.3 kmol/h and B = 30.7 kmol/h

L/D= 2.5

V= L + D =3.5 D =176.05 kmol/h

Molecular weight of feed = 0.6*46 + 0.4 * 18 = 34.8 g/mol

Mole fraction of ethanol in the feed of distillation (X_{EF})

$$X_{EF} = \frac{\frac{60}{46}}{\frac{60}{46}+\frac{40}{18}} = 0.37$$

Mole fraction of water in the feed of distillation (X_{WF})

$$X_{WF} = 1-X_{EF} = 0.63$$

Mole fraction of ethanol in the distillate (X_{ED})

$$X_{ED} = \frac{\frac{96}{46}}{\frac{96}{46}+\frac{4}{18}} = 0.904$$

Mole fraction of water in the distillate (X_{WD})

$$X_{WD} = 1-X_{ED} = 0.096$$

Water mole fraction in the bottom (X_{wB})

$$X_{wB} = \frac{\frac{99}{18}}{\frac{1}{46}+\frac{99}{18}} = 0.996$$

Ethanol mole fraction in the bottom (X_{EB})

$$X_{EB} = 1-X_{WB} = 0.004$$

Specific heat of feed (Cpf) = 0.6*2.72 +0.4*4.18= 3.304 kJ/kgK

Molar latent heat of the feed is

λ= 0.60*790 + 0.40*2376.7

= 1424.68kJ/kg

=1424.68 kJ/kg*(1kg/0.021kmol) = 67842.9kJ/kmol

= 67841.9 kJ/kmol*0.23 kcal/kJ

=1503.64 kcal / kmol

Average boiling point of feed

= ΣXiTi

= 0.37*308 + (1- 0.37)*308 = 308k

Heat capacity of the feed

Cpf=3.304 kJ/kgK *0.23kcal/kmol

=0.760 kcal/kgK

Vapor liquid equilibrium data of ethanol water solution given below in table

Table 6.1 VLE data for ethanol water solution

X(Liq)	0.0190	0.0721	0.0966	0.1238	0.1661	0.2337	0.2608	0.3273	0.3965
Y(vap)	0.1700	0.3891	0.4375	0.4704	0.5089	0.5445	0.5580	0.5826	0.6122

X(Liq)	0.5079	0.5198	0.5732	0.6763	0.7472	0.8943
Y(vap)	0.6564	0.6599	0.6841	0.7385	0.7815	0.8943

Quality of feed (q)

$$q=\frac{cpdT+\lambda}{\lambda}=\frac{0.760*(308-298)+1503.64}{1503.64}=1.00$$

The slope of the q-line is $=\frac{q}{q-1}=\frac{1.00}{1-1.00}=\infty$

The slope of the operating line for the rectifying section is

$$=\frac{R}{R+1}=\frac{2.5}{2.5+1}=0.714$$

Y-intercept of operating line for the rectifying section is $=X_{ED}/(R+1)=0.904/(2.5+1)=0.26$

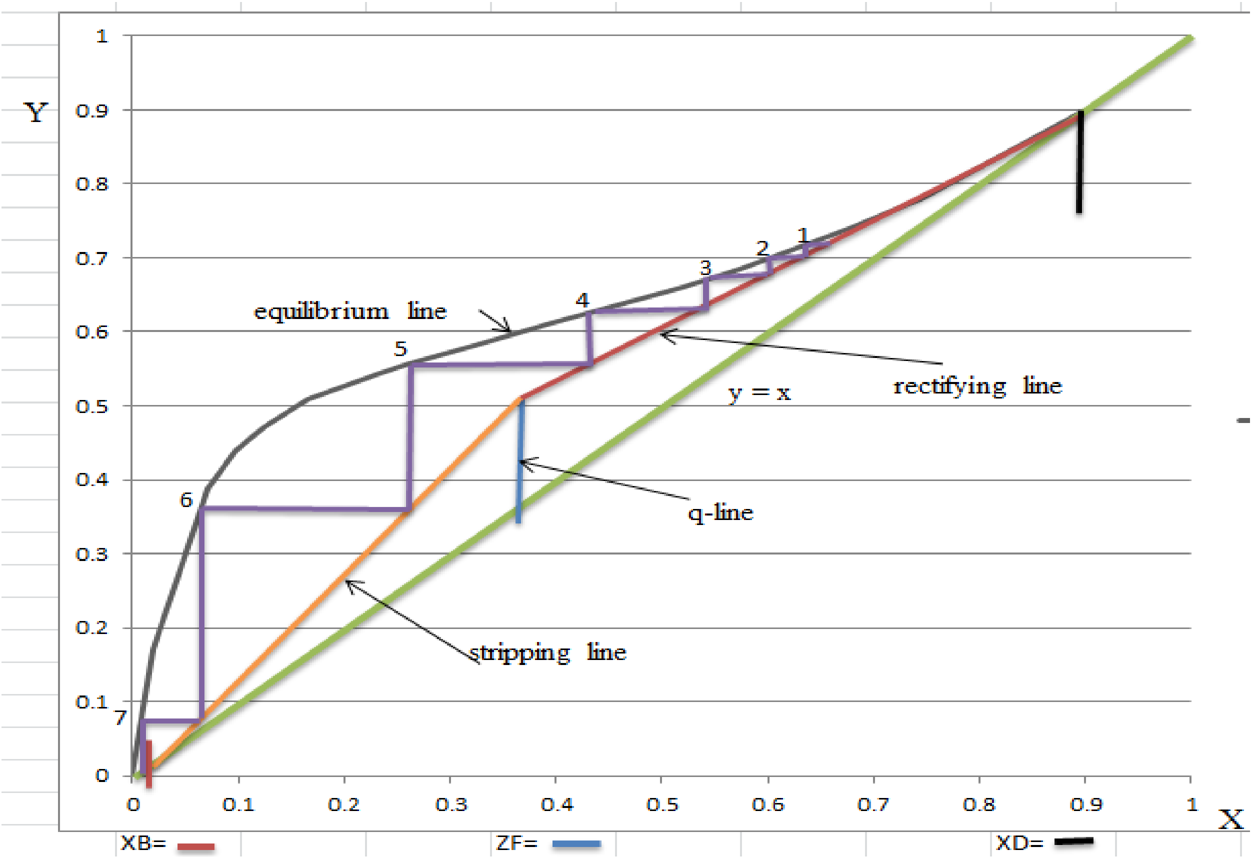


Figure 6.1 McCabe-Thiele diagrams to determine number of stage

The operating line for the stripping section is drawn to pass through the point $x = x_B = 0.004$ on the 45^o line and join to the point of intersection of the q-line and the operating line for the rectifying section.

With the above information, number of theoretical plate is 7 and feed is introduced at the 5 stages.

Number of stages =theoretical plate – 1=7-1= 6 thus 4 stages are on the rectifying section and 2 stages are present on the stripping section.

Diameter of distillation column

Assume diameter of column is constant in the stripping and rectifying section. Temperature at the top is 85^oC (358k) and Assuming an ideal gas behavior solution

$V=\frac{nRT}{p}$, where n is molar flow rate of vapor n =81.76 kmol/h

$R= 0.082 atm.m^3/kmol.k$

$P= 0.5atm$

$$V=\frac{\frac{81.76kmol}{h}*0.082*358}{0.5} = 2400\ m^3/hr$$

Cross sectional area

$$As=\frac{Volume\ flow\ rate}{linear\ velocity}=\frac{2400m3/h}{\frac{0.75m}{s}*3600\ s/h}=0.889m^2$$

Diameter of column

$$D=\sqrt{(4As/\prod)} =1.06\ m$$

Pump for delivering of water

Type	centrifuge pump
Operating condition	
Head	4m
Slurry to be handled	distilled water
Density	1000 kg/m3
Temperature of liquid	25^oC
Materials of construction	carbon steel
Capacity	$0.04m^3/s$

Pump for delivering filtered hydrolyzate

Type	centrifuge pump
Operating condition	
Head	4m
Slurry to be handled	hydrolyzate
Density	888 kg/m3
Temperature of liquid	25^{o}C
Materials of construction	carbon steel
Capacity	0.004m^3/s

Pump for delivering hydrolyzate

Type	diaphragm
Operating condition	
Head	4m
Slurry to be handled	hydrolyzate
Density	880 kg/m3
Temperature of liquid	25^{o}C
Materials of construction	carbon steel
Capacity	0.025m^3/s

Pump for delivering hydrolyzate

Type	centrifuge pump
Operating condition	
Head	4m
Slurry to be handled	hydrolyzate
Density	880 kg/m3
Temperature of liquid	25^{o}C
Materials of construction	carbon steel
Capacity	0.004m^3/s

Flow sheet of bioethanol production from Barley spent grain

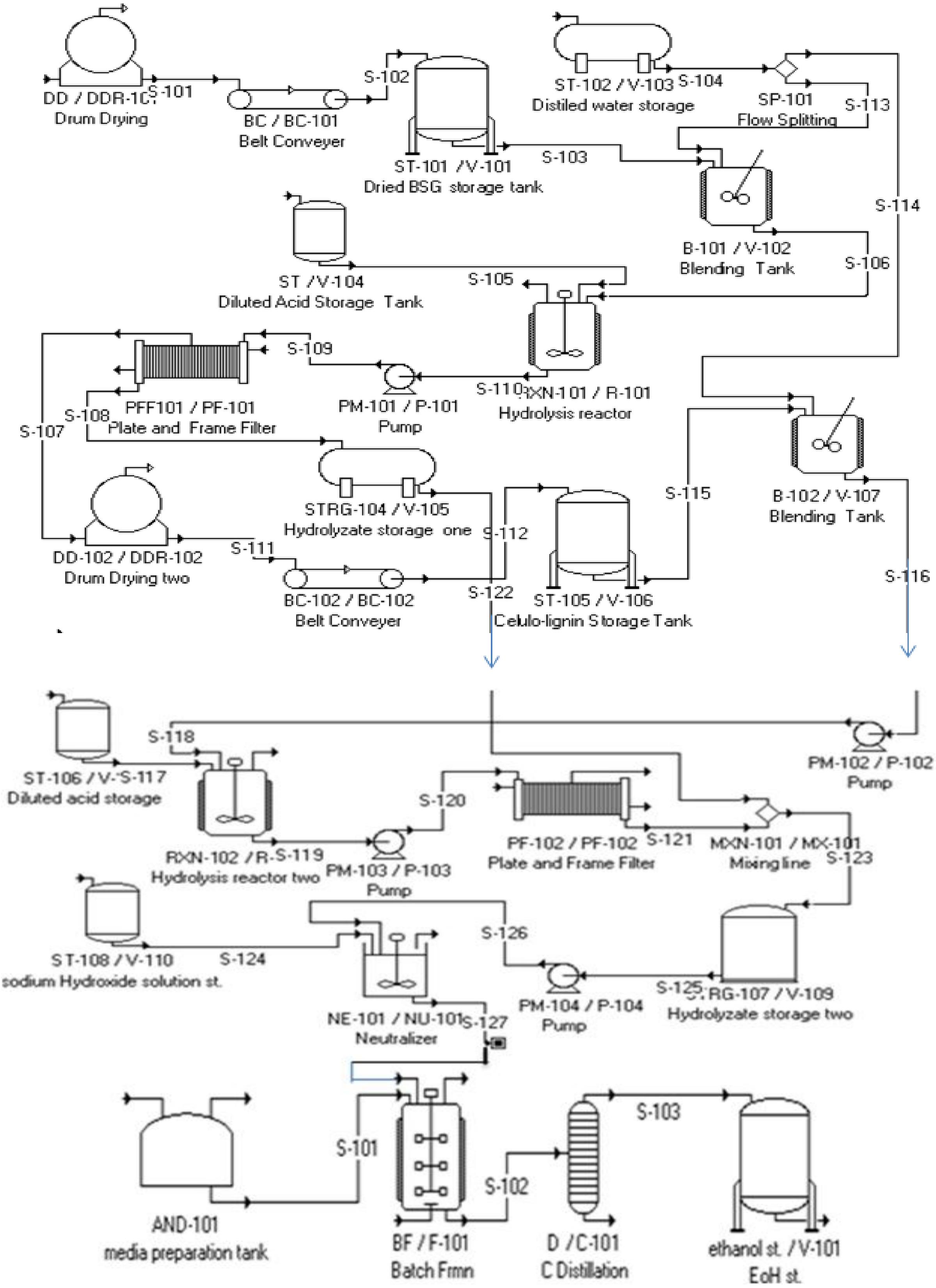


Figure 6.2 flow sheet of bioethanol production from BSG

Table 6.2 Summary of equipment sizing (based on the material balance)

Equipment name	capacity	quantity	material construction	specification
Drum drier	$30m^2$	2	carbon steel	
Dry BSG storage tank1	929 m^3/3days	1	carbon steel	
Dry BSG storage tank 2	$557m^3$/day	1	carbon steel	
Water St. Tank	$1312m^3$/day	2	carbon steel	
Filter (plate and frame)	$16m^2$	2	carbon steel	
Mixing tank	26 m^3/batch	2	carbon steel	with agitator
Hydrolysis reactor	15 m^3	2	stainless steel	with agitator
Hydrolyzate St. Tank	$2185m^3$	1	rubber-linked cs	
Neutralizer	$755m^3$/batch	1	stainless steel	with agitator
Fermenter	755 m^3/batch	3	carbon steel	with agitator
Distillation	diameter=1.06m No. stage 7	2	carbon steel	
Molecular sieve	45.27kg/hr	1	carbon steel	
Ethanol St. Tank	$122m^3$/week	1	carbon steel	with agitator
ST yeast propagation	$22m^3$/batch	1	carbon steel	
H_2SO4 St. Tank	$8m^3$	1	rubber-linked cs	
NaOH St. Tank	$5m^3$	1	rubber-linked cs	

Table 6.3 Sizing of pump summery

Fluid to be transport	Pump Type	Quantity	Capacity (m^3/s)
Water	Centrifugal	1	0.04
Filtered Hydrolyzate	Centrifugal	2	0.004
Fermented mash	Centrifugal	1	0.004
Mixture of BSG and water	diaphragm	2	0.025

7 PRELIMINARY ENGINEERING ECONOMIC ANALYSIS

Total Capital Investment

Total Capital Investment = Fixed Capital Investment + Working Capital Investment

For this case, capital investment items are calculated based on the purchased equipment cost of the plant.

Fixed capital cost = *f* (purchased equipment cost)

Table 7.1Total purchased cost

Equipment name	capacity	quantity	unit price ($)
Drum drier	30m^2	2	134980
Dry BSG storage tank	929m^3/3days	1	76661
Dry BSG storage tank	557m^3/3days	1	57232
Fermenter	755 m^3/batch	3	67708
Water St. Tank	1312m^3/day	2	95552
Filter (plate and frame)	16 m^2	2	31374
Mixing tank	26 m^3/batch	2	36454
Hydrolysis reactor	15 m^3	2	88906
Hydrolyzate St. Tank	2185m^3/batch	1	134288
Neutralizer	755 m^3/batch	1	67708
Ethanol St. Tank	122 m^3/week	1	33142
Distillation	diameter=1.06m		
	No. stage=7	2	58894
Molecular sieve	45.27kg/hr	1	6000
ST yeast propagation	75.5m^3/batch	1	48042
H_2SO4 St. Tank	8.00m^3	1	47008
NaOH St. Tank	5.00m^3	1	42308
Centrifugal pump	0.004m^3/s	4	46810
Diaphragm pump	0.025m^3/s	2	15725
	Total purchased cost		**1,458,484**

Data Sources: http://www.mhhe.com/engcs/chemical/peters/data/

Estimation of fixed capital investment

Table 7.2 Estimation of fixed capital investment

Direct cost (DC)	Percentage (%)	Price ($1000)
Purchased equipment	E	1,458,484
Purchased equipment installation	25%E	364,621
Instrumentation and control	10%E	145,848
Piping (installed)	15%E	218,773
Electrical(installed)	10%E	145,848
Building (including service)	11%E	160,433
yard improvement	5%E	72,924
Service facilities	35%E	510,469
A. Total direct cost (TDC)		**3,077,400**
Indirect cost (IC)		
Engineering and Supervision	14%E	204,188
Construction expenses and Contractor’s fee	15%E	218,773
Contingency,	8%E	116,679
B. Total indirect cost		**539,640**
Fixed-capital investment (A+B)		**3,617,040**

TCI=FCI+WC, since working capital cost is (10-20) % of total capital investment

TCI =FCI+0.15TCI

TCI = FCI/0.85= $4,255,341

Working cost (WC) = $(4,255,341-3,617,040) = $638301

Total production cost

Total production cost =manufacturing + general expense

Manufacturing cost =direct production cost +fixed charge +plant overhead cost

General expense =administrative +distribution and selling costs+ interest

Table 7.3 Table raw material cost

Raw materials	Capacity (tonne)	Unit price ($)	Total cost ($) per year
Barley spent grain	52,433	2.38	124,790
H_2SO_4	5,416	200	1,083,200
NaOH	295	350	103,250
Total raw material cost			1,309420

I. Manufacturing cost

Manufacturing cost = Direct production costs (DPC) + Fixed charges (FC) + Plant overhead costs (POC)

A. **Direct production cost (60% TPC)**

1. Raw material and inputs = $1,309,420
2. Operating labor (10% of TPC) = 0.10 TPC
3. Direct supervisor and clerical labor (12% of operating labor) = 0.10 * 0.12 * TPC = 0.012 TPC
4. Utilities (15% of TPC) = 0.15 TPC
5. Maintenance and repair (6% of FCI) = $3,617,040* 0.06 = $217,022
6. Operating supplies = 0.5% FCI = 0.005* $3617040 = $18085

Total direct production cost =1,546,347 + 0.272 TPC

B. Fixed Charges

1. Depreciation (10% FCI) = $361704
2. Insurance (1% FCI) = $36170
3. Local taxes (2%FCI) = $72341

Total fixed charges = $470215

C. Plant Overhead costs

1. 5% of total product cost =0.05 TPC

Total Manufacturing cost = A + B + C

= 2016562+0.322 TPC

II. General expenses

1. Administrative cost (2% of total product cost) = 0.02 TPC
2. Distribution and selling cost (2% of total product cost) = 0.02 TPC

Total general expenses = 0.04 TPC

III. Total product cost = manufacturing cost + general expenses

TPC=$**2016562**+0.322 TPC +0.04 TPC

TPC (1-0.362) =$**2016562**

TPC= $3,160,755

Economic evaluation

Gross earn cost

Current price of 99.9% ethanol $0.85/lit based on the current price of world biofuel market

Annual revenue=$0.85/lit X 5,000,000 lit

=$4,250,000

Gross annual profit = Annual revenue –Total production cost

=$(4,250,000-3,160,755)

=$1,089,245

Net income

Net income= Gross annual profit –income tax (35 % Gross annual profit)

=$(1,089,245-1,089,245*0.35)

=$718,902

Percent of profit

$$\% \text{ profit} = \frac{net\ income}{TPC} \text{x}100\%$$

$$= \frac{\$718{,}902}{\$3{,}160{,}755} \text{x}100\%$$

=23%

Percent rate of return

The yearly profit divided by the total initial Investment necessary represents Return on Investment. Taking the risk factor Mar = 12%, to be the plant feasible RoI >Mar (must).

Net income =$718,902

Total capital investment (TCI) = $4,255,341

-Rate of return, % ROR= $\frac{net\ income}{TCI}$x100%

$= \frac{718902}{4255341}$x100% =17% > 12% it is acceptable

Payback period

The minimum length of time theoretically necessary to recover the original fixed capital investment in the form of cash flow is called payback period.

Assume 10 years Project service life and we use straight line method to calculate depreciation. Dep. $=\frac{FCI}{10}=\frac{3617040}{10}=$ \$361,704

Payback period $= \frac{FCI}{dep+net\ profit}$

$= \frac{3617040}{361{,}704+718{,}902}$

=3.3 year < 5 year, it is acceptable

Break-even point (BEP)

At BEP

Total production cost = total sales income

Direct production cost +fixed charges + plant overhead cost + General expense = Total sales income

n*unit direct production cost + fixed charges + plant overhead cost + General expense = n*unit sale income

Where

n= production capacity per year

Unit direct production cost =Total production cost / Total production capacity

Unit direct production cost = \$3,160,755/5,000,000 liter/year

=\$0.63/liter

Unit sale income = \$0.85/liter

n*\$0.63 +\$470215 +126,430.2 + 158,038 = \$0.85*n

n= 3,430,377 liter

Break-even production capacity = 68.6% of the total production capacity.

8 CONCLUSION AND RECOMMENDATIONS

8.1 Conclusion

Barley spent grain (BSG) is promising lignocellulosic feedstocks for production bioethanol fuel. It is the most abundant by-product generated from the beer-brewing process, representing approximately 85% of the total by-products obtained. Bioethanol production from such lignocellulosic material was carried out in four main stages such as pretreatment, hydrolysis (first and second stage), fermentation and distillations.

In this study two-stage diluted acid hydrolysis were used and the effect of the hydrolysis process variable (temperature, time and acid concentration) in the yield of ethanol was investigated and optimized using response surface methodology. Based on analysis of variance (ANOVA) hydrolysis temperature, time and interaction between temperature and acid concentration have significant effect on the yield of ethanol. Positive yield of ethanol was obtained at a high acid concentration and low temperature as well as at high temperature and low acid concentration. As the result of RSM optimization at 144.29^{o}C, 26.26 min and 1.68% hydrolysis temperature, time and acid concentration, respectively afforded 47.6 %w/w and 0.1314 g/g of BSG or 8.33 mL per 50 gram of Dry BSG yield of TRS and ethanol respectively. All points were located near to the central point of the design. Based on this study, it is evident that the chosen method of optimization was efficient, and reliable. From this result, it can be concluded that Barley spent grain (BSG) has the potential to serve as a low-cost feedstock for the production of ethanol and two-stage diluted acid hydrolysis process is very effective, but the yield of ethanol is not satisfactory as compared with the yield of total reducing sugar. The reason for this result is the drawback of *Saccharomyces cerevisiae* which can not able to produce ethanol from 5-carbon sugars (Galbe and Zacchi 2002).

Based on the rough economic analysis, production of bioethanol from Barley spent grain is profitable since the rate of return on investment was 37%, this show us the project returns its 37% of the initial investment in one year and the payback period is around four years.

8.2 Recommendation

Based on the current investigation the following recommendations are forwarded:

- Further researches have to be carried out to increase the yield of bioethanol from Barley spent grain by use other microorganisms which are capable of converting 5- and 6- carbon sugar into ethanol.

- Optimization of hydrolysis variables are carried out in this study. But further optimization of the Pretreatment, Fermentation and Distillation processes are recommended to maximize the yield of ethanol from BSG.

- Detailed economic feasibility studies in the production process is recommended, since it is critical for the rationale of commercialization.

- BSG is rich in cellulose and hemicelluloses, due to this it is used as a low valuable raw material in different applications, such as for production of xylitol, furfural, biogas and bioethanol. But in developing country this valuable by-product is used as low-cost animal feed and the remaining are deposited into landfills. Further research is required to utilize this largely available by-product, Barley Spent Grain.

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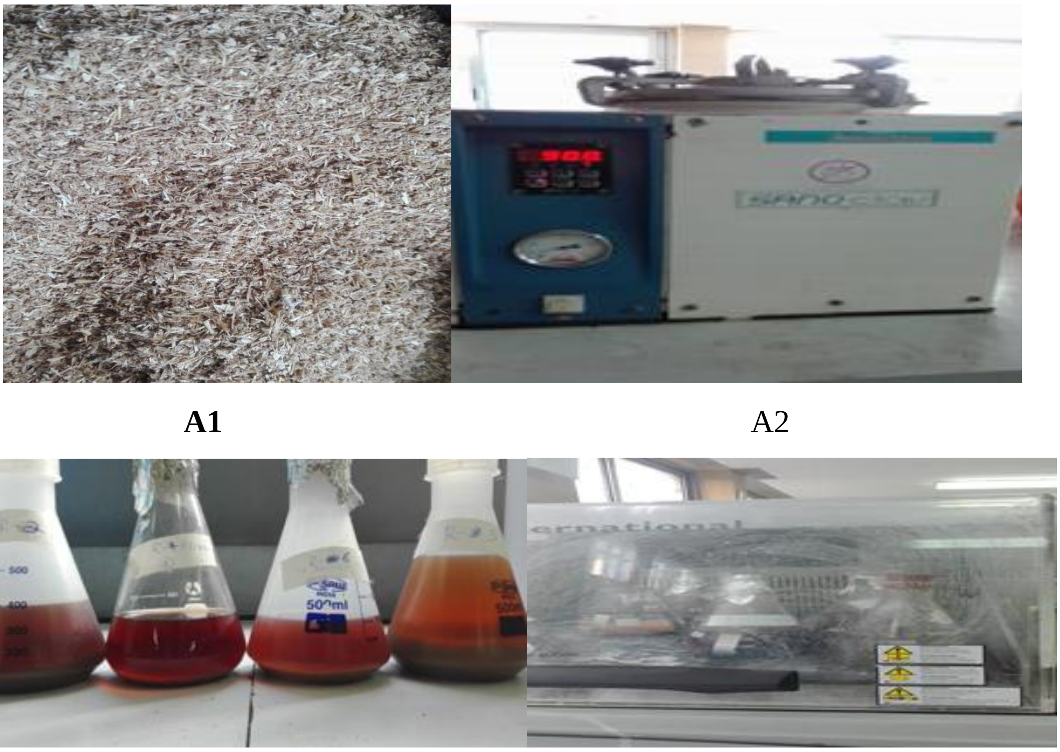
Appendixes

Appendix A: Laboratory equipments and sample photos



A1 A2

A2 A3

A1. Dry barley spent grain, A2. Auto clave unit, A3 hydrolyzate samples and A4 samples in shaker incubator

A4 A5

A4 simple distillation unit and A5 sample products

A6 waste after hydrolysis

A7 weight balance for density

A8 A9 A10

A8 Digital spectrometer, A9 Samples after mixing with benedict and A10 Reaction of reducing sugar with benedict solution at 90°C water bath

A11 A12

A11. Sample of standard glucose after reaction with benedict solution and
A12. Hydrolyzate sample after reaction with benedict solution

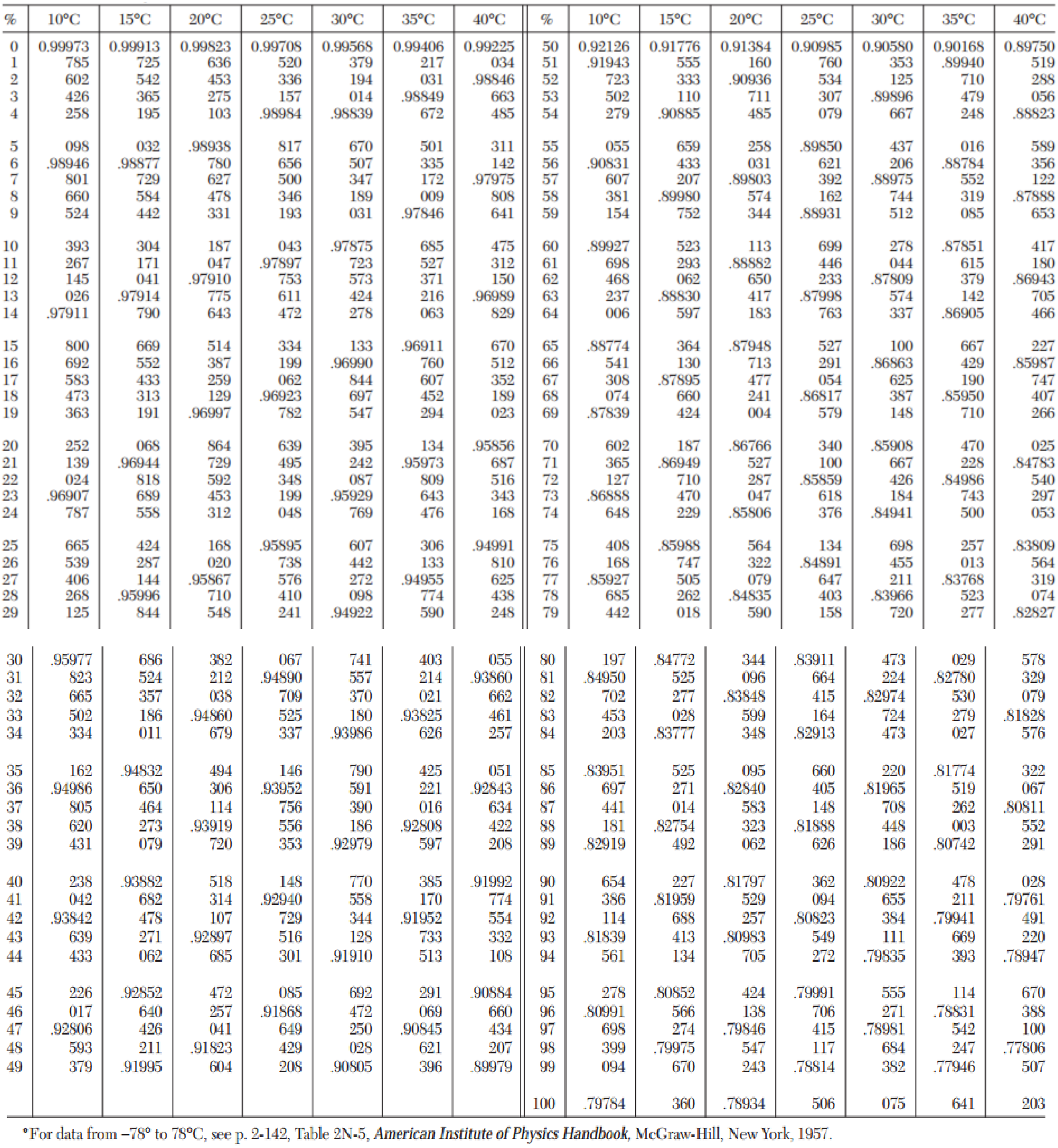
Appendix B: General Characteristics of bioethanol

Molecular formula	C_2H_5OH
Molecular mass	46.07g/mol
Appearance	colorless liquid
Water solubility	miscible
Density	0.789kg/lit
Boiling point	78.5^{o}C
Freezing point	-117^{o}C
Flash point	12.8^{o}C (lower temperature ignition)
Ignition point	425^{o}C
Explosion limit	lower 3.5%V/V: upper 19%V/V
Vapor pressure	38^{o}C 50mmHg
Lower heating value	@20^{o}C 29.800kJ/Kg
Higher heating value	@20^{o}C 21.09kJ/l
Specific heat	1 kcal/kg@60^{o}C
Acidity	@pka 15.9
Viscosity	1200mpa.s (20^{o}C)
Refractive index (nd)	1.36 (25^{o}C)
Octane number	99

Appendix C: Central composites design for hydrolysis

Std.	Run	Block	Factor1temp. (^{o}c)	Factor 2 time (min)	Factor 3 acid con. (%)
20	1	Block 1	140.00	25.00	1.75
8	2	Block 1	150.00	30.00	2.00
2	3	Block 1	150.00	20.00	1.50
18	4	Block 1	140.00	25.00	1.75
3	5	Block 1	130.00	30.00	1.50
11	6	Block 1	140.00	16.59	1.75
16	7	Block 1	140.00	25.00	1.75
19	8	Block 1	140.00	25.00	1.75
9	9	Block 1	123.18	25.00	1.75
6	10	Block 1	150.00	20.00	2.00
4	11	Block 1	150.00	30.00	1.50
15	12	Block 1	140.00	25.00	1.75
14	13	Block 1	140.00	25.00	2.17
7	14	Block 1	130.00	30.00	2.00
12	15	Block 1	140.00	33.41	1.75
17	16	Block 1	140.00	25.00	1.75
1	17	Block 1	130.00	20.00	1.50
10	18	Block 1	156.82	25.00	1.75
13	19	Block 1	140.00	25.00	1.33
5	20	Block 1	130.00	20.00	2.00

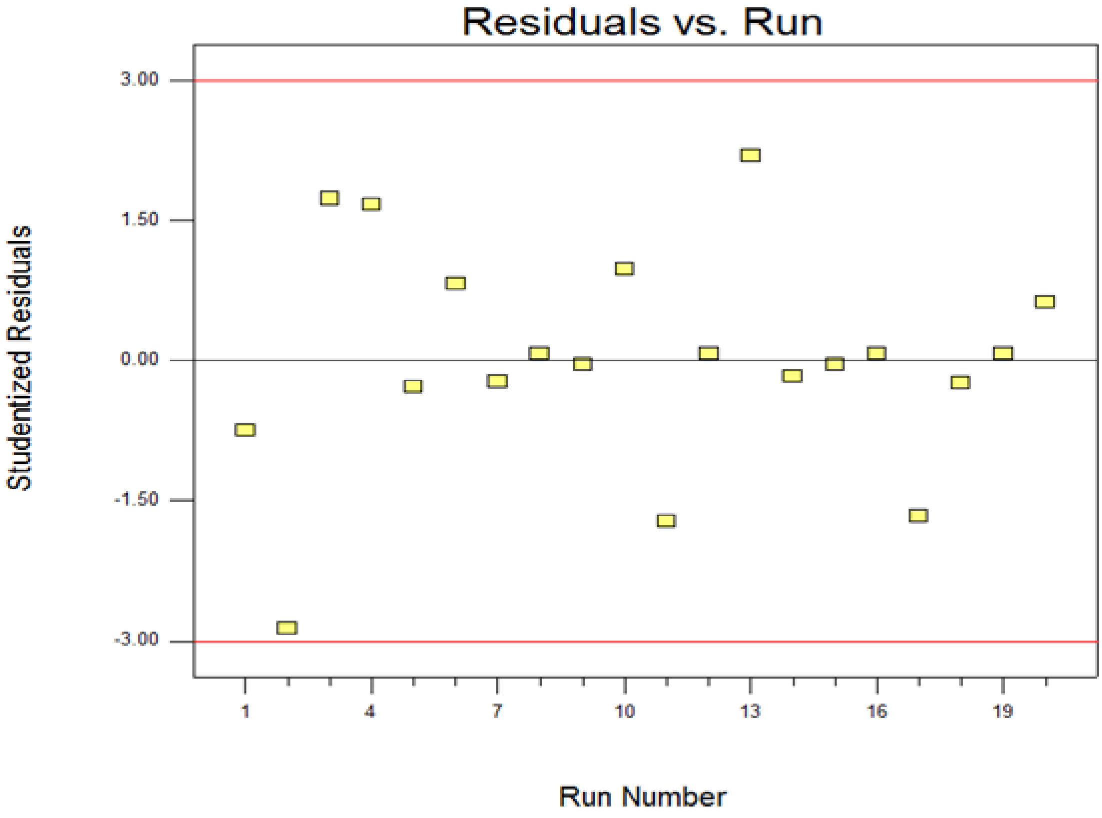
Appendix D: Densities of Mixtures of C_2H_5OH and H_2O at 20°C



%	10°C	15°C	20°C	25°C	30°C	35°C	40°C	%	10°C	15°C	20°C	25°C	30°C	35°C	40°C
0	0.99973	0.99913	0.99823	0.99708	0.99568	0.99406	0.99225	50	0.92126	0.91776	0.91384	0.90985	0.90580	0.90168	0.89750
1	785	725	636	520	379	217	034	51	.91943	555	160	760	353	.89940	519
2	602	542	453	336	194	031	.98846	52	723	333	.90936	534	125	710	288
3	426	365	275	157	014	.98849	663	53	502	110	711	307	.89896	479	056
4	258	195	103	.98984	.98839	672	485	54	279	.90885	485	079	667	248	.88823
5	098	032	.98938	817	670	501	311	55	055	659	258	.89850	437	016	589
6	.98946	.98877	780	656	507	335	142	56	.90831	433	031	621	206	.88784	356
7	801	729	627	500	347	172	.97975	57	607	207	.89803	392	.88975	552	122
8	660	584	478	346	189	009	808	58	381	.89980	574	162	744	319	.87888
9	524	442	331	193	031	.97846	641	59	154	752	344	.88931	512	085	653
10	393	304	187	043	.97875	685	475	60	.89927	523	113	699	278	.87851	417
11	267	171	047	.97897	723	527	312	61	698	293	.88882	446	044	615	180
12	145	041	.97910	753	573	371	150	62	468	062	650	233	.87809	379	.86943
13	026	.97914	775	611	424	216	.96989	63	237	.88830	417	.87998	574	142	705
14	.97911	790	643	472	278	063	829	64	006	597	183	763	337	.86905	466
15	800	669	514	334	133	.96911	670	65	.88774	364	.87948	527	100	667	227
16	692	552	387	199	.96990	760	512	66	541	130	713	291	.86863	429	.85987
17	583	433	259	062	844	607	352	67	308	.87895	477	054	625	190	747
18	473	313	129	.96923	697	452	189	68	074	660	241	.86817	387	.85950	407
19	363	191	.96997	782	547	294	023	69	.87839	424	004	579	148	710	266
20	252	068	864	639	395	134	.95856	70	602	187	.86766	340	.85908	470	025
21	139	.96944	729	495	242	.95973	687	71	365	.86949	527	100	667	228	.84783
22	024	818	592	348	087	809	516	72	127	710	287	.85859	426	.84986	540
23	.96907	689	453	199	.95929	643	343	73	.86888	470	047	618	184	743	297
24	787	558	312	048	769	476	168	74	648	229	.85806	376	.84941	500	053
25	665	424	168	.95895	607	306	.94991	75	408	.85988	564	134	698	257	.83809
26	539	287	020	738	442	133	810	76	168	747	322	.84891	455	013	564
27	406	144	.95867	576	272	.94955	625	77	.85927	505	079	647	211	.83768	319
28	268	.95996	710	410	098	774	438	78	685	262	.84835	403	.83966	523	074
29	125	844	548	241	.94922	590	248	79	442	018	590	158	720	277	.82827
30	.95977	686	382	067	741	403	055	80	197	.84772	344	.83911	473	029	578
31	823	524	212	.94890	557	214	.93860	81	.84950	525	096	664	224	.82780	329
32	665	357	038	709	370	021	662	82	702	277	.83848	415	.82974	530	079
33	502	186	.94860	525	180	.93825	461	83	453	028	599	164	724	279	.81828
34	334	011	679	337	.93986	626	257	84	203	.83777	348	.82913	473	027	576
35	162	.94832	494	146	790	425	051	85	.83951	525	095	660	220	.81774	322
36	.94986	650	306	.93952	591	221	.92843	86	697	271	.82840	405	.81965	519	067
37	805	464	114	756	390	016	634	87	441	014	583	148	708	262	.80811
38	620	273	.93919	556	186	.92808	422	88	181	.82754	323	.81888	448	003	552
39	431	079	720	353	.92979	597	208	89	.82919	492	062	626	186	.80742	291
40	238	.93882	518	148	770	385	.91992	90	654	227	.81797	362	.80922	478	028
41	042	682	314	.92940	558	170	774	91	386	.81959	529	094	655	211	.79761
42	.93842	478	107	729	344	.91952	554	92	114	688	257	.80823	384	.79941	491
43	639	271	.92897	516	128	733	332	93	.81839	413	.80983	549	111	669	220
44	433	062	685	301	.91910	513	108	94	561	134	705	272	.79835	393	.78947
45	226	.92852	472	085	692	291	.90884	95	278	.80852	424	.79991	555	114	670
46	017	640	257	.91868	472	069	660	96	.80991	566	138	706	271	.78831	388
47	.92806	426	041	649	250	.90845	434	97	698	274	.79846	415	.78981	542	100
48	593	211	.91823	429	028	621	207	98	399	.79975	547	117	684	247	.77806
49	379	.91995	604	208	.90805	396	.89979	99	094	670	243	.78814	382	.77946	507
								100	.79784	360	.78934	506	075	641	203

[*] For data from –78° to 78°C, see p. 2-142, Table 2N-5, *American Institute of Physics Handbook*, McGraw-Hill, New York, 1957.

Appendix E. Residuals Vs Run of ethanol

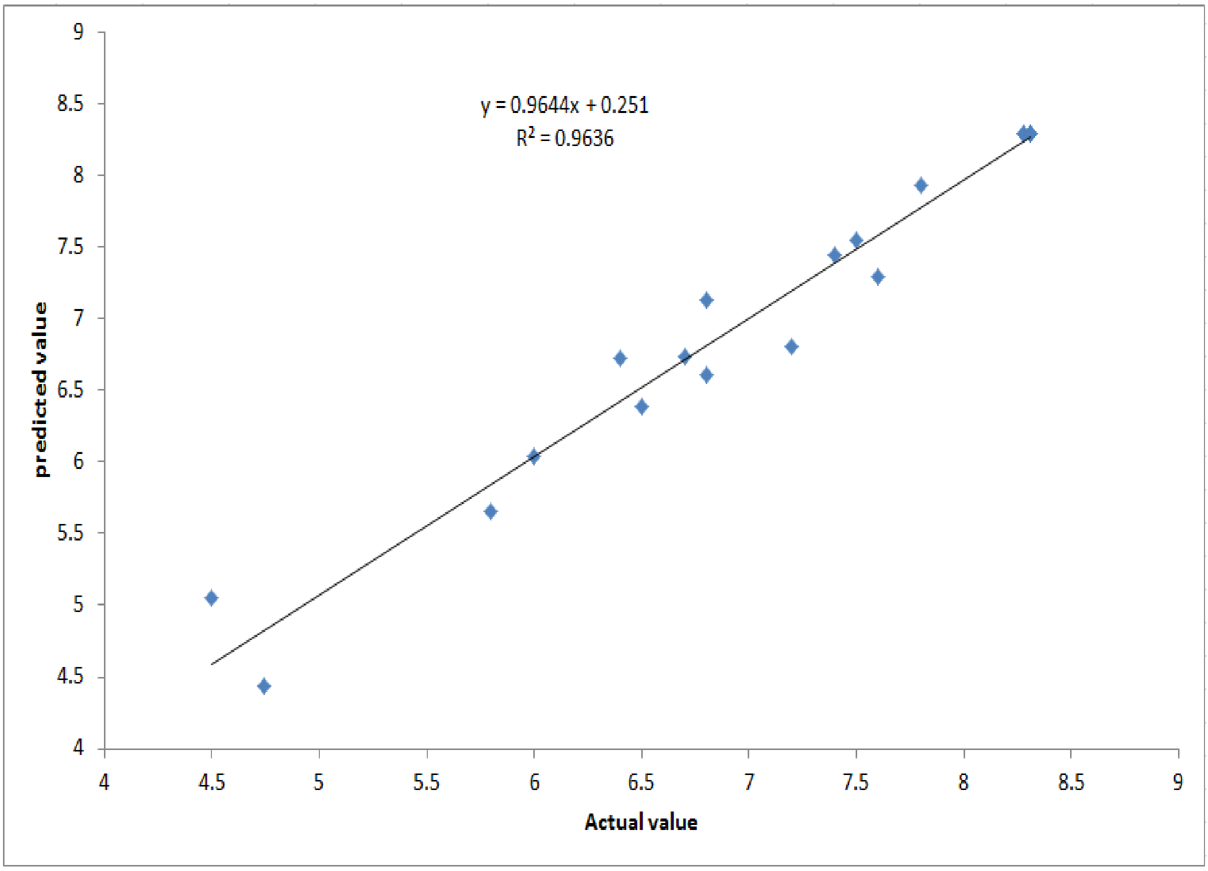


Appendix F. Diagnostics Case Statistics of ethanol

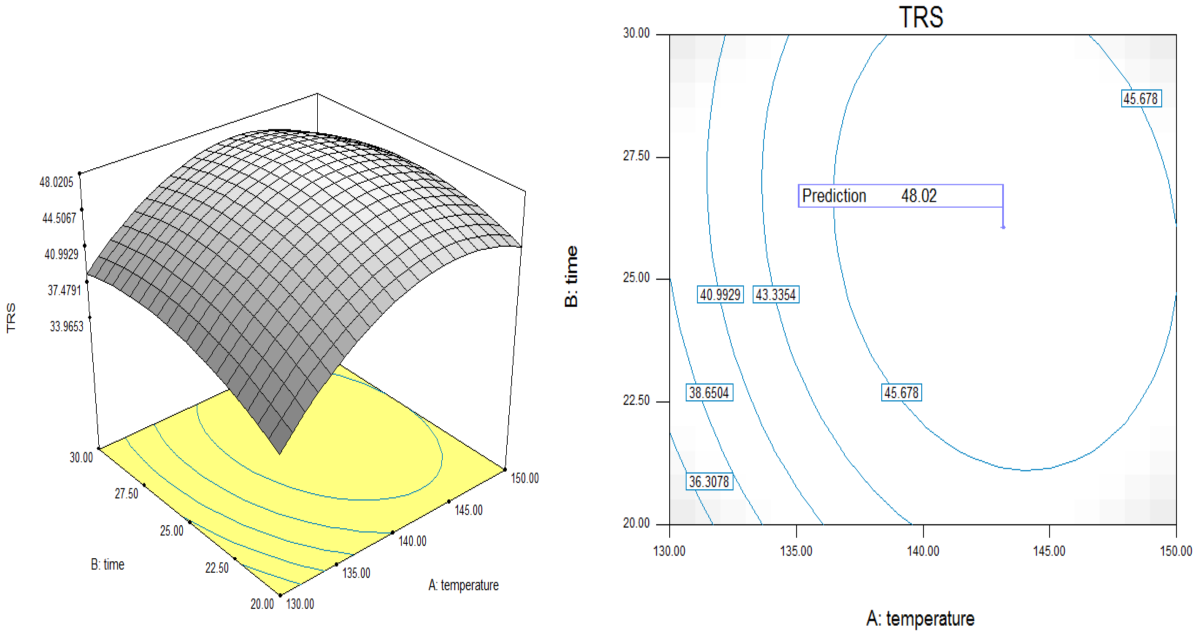
Std. Order	Actual Value	Predicte Value	Student Residual	Leverage	Residual	Cook's Distance	Outlier t	Run Order
1	4.74	4.44	0.30	0.670	1.678	0.571	1.878	4
2	7.50	7.55	-0.050	0.670	-0.283	0.016	-0.269	5
3	5.80	5.65	0.15	0.670	0.825	0.138	0.811	6
4	7.80	7.93	-0.13	0.670	-0.740	0.111	-0.722	1
5	7.20	6.81	0.39	0.670	2.196	0.978	2.894	13
6	6.50	6.39	0.11	0.670	0.631	0.081	0.611	20
7	7.60	7.29	0.31	0.670	1.739	0.613	1.975	3
8	6.00	6.04	-0.039	0.670	-0.222	0.010	-0.211	7
9	4.50	5.05	-0.55	0.607	-2.866	1.271	-6.438 *	2
10	6.80	6.61	0.19	0.607	0.979	0.148	0.976	10
11	6.40	6.72	-0.32	0.607	-1.658	0.425	-1.847	17
12	7.40	7.44	-0.044	0.607	-0.230	0.008	-0.218	18
13	6.70	6.73	-0.032	0.607	-0.163	0.004	-0.155	14
14	6.80	7.13	-0.33	0.607	-1.724	0.460	-1.952	11
15	8.28	8.29	-9.590E-003	0.166	-0.034	0.000	-0.032	9
16	8.28	8.29	-9.590E-003	0.166	-0.034	0.000	-0.032	15
17	8.31	8.29	0.020	0.166	0.073	0.000	0.069	8
18	8.31	8.29	0.020	0.166	0.073	0.000	0.069	12
19	8.31	8.29	0.020	0.166	0.073	0.000	0.069	19
20	8.31	8.29	0.020	0.166	0.073	0.000	0.069	16

* Case(s) with |Outlier T| > 3.50

Appendix G. actual values vs. predicted value

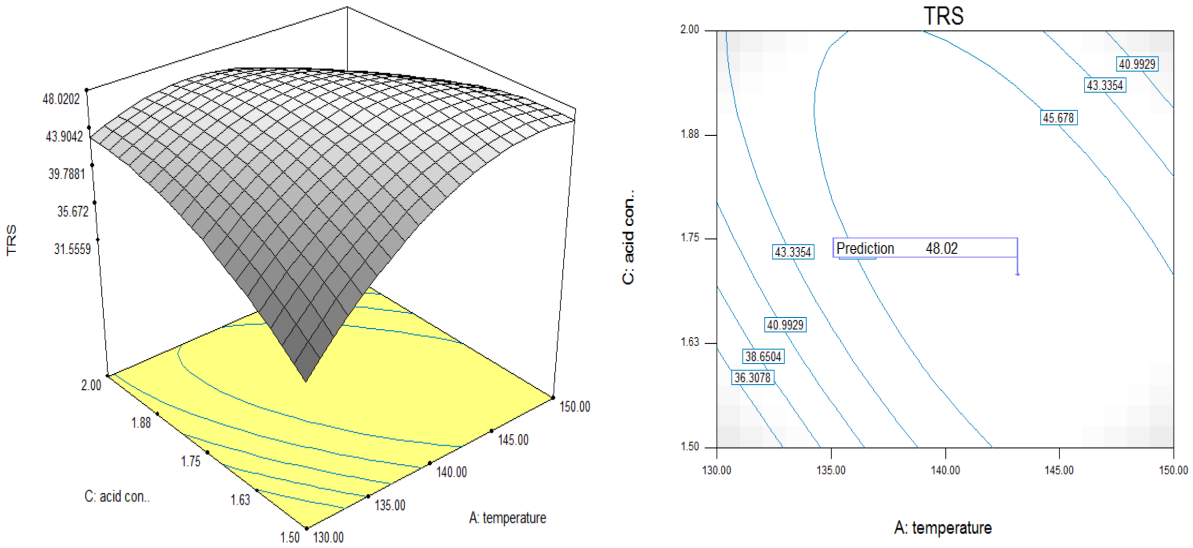


Appendix H. Response surface and Contour plot of optimized yield of TRS



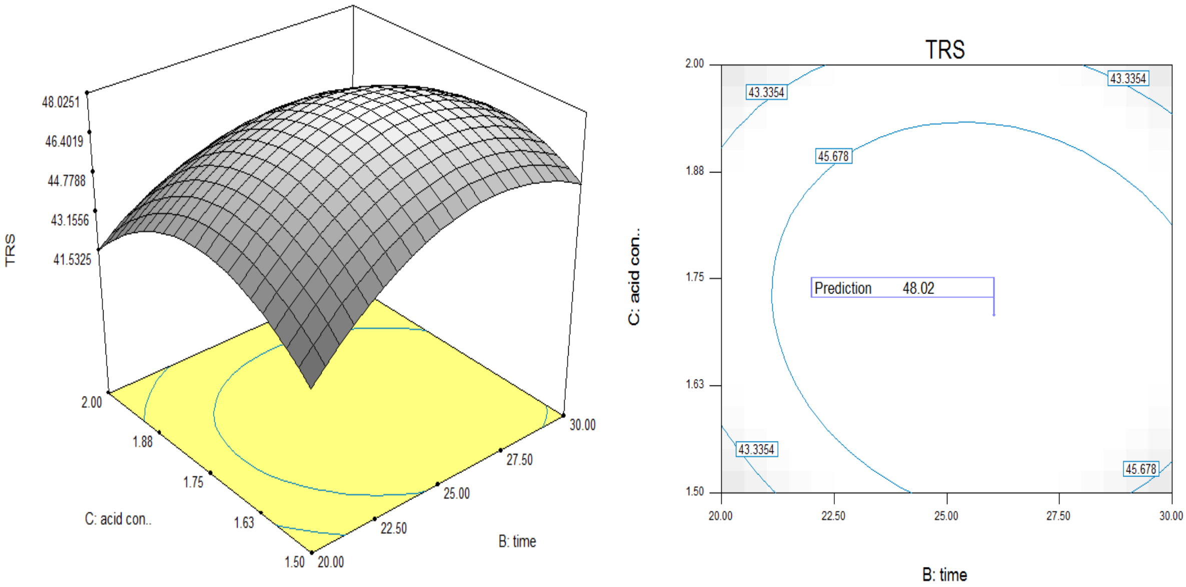
(a) (b)

(a) Response surfaces plot of predicted TRS yield at acid concentration (b) Contour plot of predicted TRS yield at fixed acid concentration



(c) (d)

(c) Response surfaces plot of predicted TRS yield a constant time (d) Contour plot of predicted TRS yield at constant time



(e) (f)

(e) Response surfaces plot of predicted TRS yield at constant temperature (f) Contour plot of predicted TRS yield at constant temperature