ADDIS ABABA UNIVERSITY

ADDIS ABABA INSTITUTE OF TECHNOLOGY

SCHOOL OF CHEMICAL AND BIO ENGINEERING

PROCESS ENGINEERING STREAM

PRODUCTION OF CELLULOSIC ETHANOL FROM WOOD SAWDUST

By

Gebreyohannes Gebrehiwot

July 2016

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

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Gebreyohannes Gebrehiwot

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

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Declaration

I declare that this thesis for the M.Sc. Degree at Addis Ababa Institute of Technology, Addis Ababa University, Ethiopia 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.

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List of Acronyms

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ANOVA----------Analysis of variance

CBP----------Consolidated bioprocess

ETB----------Ethiopian Birr

FT-IR----------Fourier transform infrared

GDP----------Gross domestic product

GHG----------Green house gases

HMF----------5-Hydroxymethyl furfural

LCM----------Lignocellulosic materials

LHW ----------Liquid hot water

SHF----------Separated hydrolysis and fermentation

SSF----------Simultaneous Saccharification and fermentation

TVA----------Tennessee Valley Authority

USDA----------United State of Development Agency

VLE ----------Vapor Liquid Equilibrium

Abstract

Production of cellulosic ethanol was investigated in this study. The wood sawdust has lignocelluloses content, which make it suitable as fermentable sugars, when hydrolyzed. The dried sawdust with uniform particle size was hydrolyzed with sulfuric acid for breaking the bond that binds sugar monomers together. The black residue or lignin was removed and the reduced sugars were neutralized for catalyst activity in the fermentation process. Four experimental parameters were investigated during hydrolysis. They are particle size (1-3mm), acid concentration (3-7%), temperature (90-110^{o}C) and time (120-240 minutes). The total reduced sugars content of hydrolyzed solutions were analyzed using phenol sulfuric acid method. The result of stastical analysis showed a maximum sugar content of 72.51% (w/w) during the hydrolysis of 10% (w/v) wood sawdust of 2mm particle size at 5% (v/v) sulfuric acid concentration at 100^{o}C and 180 minutes. The minimum yield of 41.24% (w/w) was achieved at 1mm particle size, 3% (v/v) sulfuric acid concentration, 90^{o}C and 120 minutes. The reduced sugars were fermented using *Saccharomyces cerevisiae* in anaerobic condition and the residual solutions were distilled every 150 minutes by rotary evaporator. The ethanol concentrations were measured by Alcoholmeter. And maximum and minimum ethanol concentrations of 73.10% (v/v) and 35.20% (v/v) with 90% (0.46 g/g) of the theoretical conversion (0.51g/g) in 72 hours were achieved respectively. Response surface methodology (RSM) employing the central composite design (CCD) was used to optimize the total reduced sugar using acid hydrolysis. The optimum result was obtained at 1.72mm particle size, 4.21% (v/v) acid concentration, 102.46^{o}C and 156.60 minutes. The total reduced sugar yield of 67.56% (w/w) was achieved under these conditions. The rough feasibility analysis evaluation indicates that, this project is beneficial. The cost estimation studies provide the rate of return on investment as 52.4%. The payback period shows the return of the total investment cost in 3 years with the net present value of 22.73 million Birr.

Keywords: Wood sawdust, Acid hydrolysis, Reduced sugars, Phenol-Sulphuric acid, Fermentation, *Saccharomyces cerevisiae*, Cellulosic ethanol, Alcoholmeter, Optimization.

1. INTRODUCTION

1.1. Background of the study

The world's energy supply is mainly dependent on nonrenewable, crude oil derived (fossil) liquid fuels, of which almost 90 percent are employed for energy generation and transportation. The problem of rapidly increasing population has caused many developing countries to expand their industrial base, resulting in increased energy demands [1]. It is inevitable that fossil fuels such as oil, coal and natural gas will be exhausted with time. Hence, there is need to explore the possibilities of using alternative energy source, which are as efficient as oil; ethanol fermentation is one such option [2].The transfer of crude oil based refinery to biomass based biorefinery has attracted strong scientific interest which focuses on the development of cellulosic ethanol as an alternative transportation fuel to petroleum fuels.

Biofuels are considered as a replacement for fossil fuels and an answer to poverty and climate crisis. They are presented as being both renewable and environment friendly. Increasing attention is being focused on the production of biofuels as the alternative that will contribute to global reduction in greenhouse gas emissions [3].

Biomass exploitation has great raw material availability challenges, particularly in the technological scheme of fuel bioethanol. Bioethanol is an environmentally friendly and directly exploitable fuel for substitution of petrochemicals, which today are used for the 97 percent of the transportation needs [4].

The increasing demand for ethanol for various chemical and motor fuel industrial purposes such as alternative source of energy, industrial solvents, cleansing agents, preservatives and its important role in reduction of green house gas emissions has necessitated increased production of this alcohol [5].

Ethanol production is usually accomplished by chemical synthesis of petrochemical substrates and microbial conversion of carbohydrates present in agricultural products. Owing to depleting reserves and competing industrial needs of petrochemical feed stocks, there is global emphasis on ethanol production by microbial fermentation process. Increased yield of ethanol production by microbial fermentation depends on the use of ideal microbial strain, appropriate fermentation substrate and suitable process technology [6].

Bioethanol is a fuel derived from renewable resources like locally grown crops and even waste product/waste paper or grass and tree trimmings etc [7]. These materials contain lignocellulose which includes cellulose, hemicelluloses and lignin.

Lignocelluloses biomass, including forestry residue, agricultural residue, yard waste, wood products, animal and human wastes etc., is a renewable resource that stores energy from sunlight in its chemical bonds [8]. It has great potentials for the production of affordable fuel ethanol because it is less expensive than starch (e.g. corn) and sucrose (e.g. sugarcane) producing crops and are available in large quantities. Lignocelluloses biomass typically contains 50 to 80 percent (dry basis) carbohydrates that are polymers of 5-C and 6-C sugar units. Most carbohydrates can be processed either chemically or biologically to yield biofuels such as ethanol. The lignocelluloses structure is more resistant to decay by organism and it is not perishable like soluble sugar and starch. The complex substance may be broken down into sugars by either acid treatment at various temperatures or by enzymatic treatment [9].

Ethanol is currently produced from sugars, starches and cellulosic materials. The first two groups of raw materials are currently the main resources for ethanol production, but concomitant growth in demand for human feed similar to energy could make them potentially less competitive and perhaps expensive feedstock in the near future, leaving the cellulosic materials as the only potential feedstock for production of ethanol [10]. Cellulosic materials obtained from wood sawdust and agricultural residuals, municipal solid wastes and energy crops represent the most abundant global source of biomass [11]. These facts have motivated extensive research toward making an efficient conversion of lignocelluloses into sugar monomers for further fermentation to ethanol. The use of ethanol as an alternative motor fuel has been steadily increasing around the world for a number of reasons.

Wood sawdust serves as a cheap substrate for ethanol production does not distort the human food chain and takes care of the environmental waste. The major difficulty in the hydrolysis of lignocellulosic contents from wood, to obtain fermentable sugars, lies in separating it from lignin that encloses it and makes it difficult to access. Acid hydrolysis is one of the pretreatment methods used for the lignocellulosic contents to make it susceptible to fermentation to obtain ethanol. Dilute acid hydrolysis requires high temperature and pressure which makes it expensive and inhibit yield.

The aim of this work is to investigate the effect of hydrolysis process variables including the sawdust particle size, hydrolysis acid concentration, hydrolysis temperature and hydrolysis time on the hydrolysis of lignocellulosic content of the sawdust to produce total reduced sugar and cellulosic ethanol.

1.2. Statement of the problem

The issue of energy security resulting from the decline of world petroleum reserves, increase of petroleum price and environmental concerns has encouraged governments and researchers to look for substitute renewable energy sources that are technically feasible, economically competitive and environmentally friendly. From the substitute energy sources, bioethanol derived from lignocellulosic biomass has been in advance increasing attention as a replacement for fossil fuel.

Nowadays, the production of ethanol from sugar and starch feedstock is often viewed as competing with food production and increasing prices of food, fuel and as well as scarcity. Therefore, production of cellulosic ethanol from lignocellulosic biomass has been attracting interest because using renewable non-edible biomass as a feedstock to produce ethanol to minimize competition with food industry and to negotiate the debate of “Food Vs Energy” controversy.

In another way, the manufacturing of wood products have been alarmingly increased and widely accepted, since the method of production and process is updated and simplified. During the manufacturing of wood products has highly extensive waste is obtained. This lignocellulosic waste (wood sawdust) is sometimes trashed away in to garbage. But lignocellulose contains carbohydrate polymers that can be easily converted in to usable form of energy without affecting the environment. This sawdust serves as a cheap substrate for cellulosic ethanol production, doesn’t distort the human food chain and takes care of the environmental waste.

The major complexity in the hydrolysis of lignocelluloses content from wood sawdust convert to reduced sugar lies in separating it from lignin. The acidic hydrolysis technology is perhaps currently seen as the most technologically matured and economical method of reduced sugar release from the lignocellulosic biomass.

1.3. Objectives of the study

1.3.1. General objective;

The general objective of this study was to investigate the production of cellulosic ethanol from wood sawdust using acid hydrolysis.

1.3.2. Specific objectives;

The specific objectives of the study were:-

- ✓ To investigate the effect of hydrolysis process variables (particle size, hydrolysis acid concentration, hydrolysis temperature and hydrolysis time) on the reducing sugar yield
- ✓ To quantify the reducing sugar content of wood sawdust of the hydrolysates solutions
- ✓ To specify the optimal operating conditions to maximize the reducing sugar yield
- ✓ To undertake the preliminary economic feasibility of cellulosic ethanol production from wood sawdust
- ✓ To prove the efficiency of a wood sawdust; as a potential source of viable renewable energy

1.4. Significance of the study

All energy sources have an impact on the environment. Concerns about the greenhouse effect and global warming, air pollution and energy security have led to increasing interest and more development in renewable energy sources such as biofuels, solar, wind, geothermal and hydrogen.

But it will need to continue to use fossil fuels and nuclear energy until now, cleaner technologies can replace them. In the worldwide economy much focus has been laid on the rising oil price which has become a hot topic. The rising oil price has increased the interest of finding other possible way to produce fuel and the production of cellulosic ethanol has grown steadily during the last 28 years.

The aim of this thesis is to transforming lignocellulosic waste (wood sawdust) to something valuable, namely cellulosic ethanol using the acid hydrolysis and subsequent fermentation by *Saccharomyces cerevisiae* respectively.

2. LITERATURE REVIEW

2.1. Over view of ethanol production from lignocellulosic materials

Lignocellulosic materials (LCMs) are produced in large quantities and without clear application and their use as raw material for bioethanol production shows the economic and ecologic benefits. LCMs are composed mainly of three polymers; Cellulose made up of glucose units, Hemicelluloses made up of several sugars (as xylose or arabinose) and Lignin made up of phenylpropane units, interconnected in a strong structure [12].

2.1.1. Cellulose

Cellulose was first discovered in 1838 by French chemist Anselme Payen, who isolated it from plant matter. He found that cellulose contains 44 to 45 percent carbon, 6 to 6.5 percent of hydrogen and the rest containing of oxygen. Based on these data, the empirical formula was deduced to be $C_6H_{10}O_5$ [11]. Cellulose is a homopolysaccharide composed of β-D-glucopyranose units linked together by (1 →4) glycosidic bonds. It provides structural support and chemical resistance for the plant. Considering the uniform hydrolysable glucose building blocks, the cellulose molecule would be the best carbohydrate source for the fermentation process. Major functional groups, hydroxyl groups are able to form intermolecular and intramolecular hydrogen bonds. Intermolecular linkages which form between different molecules are responsible for the formation of microfibril structures and highly ordered crystalline areas. Glucose anhydride, formed by removing water from glucose, is polymerized into long chains of cellulose containing 5,000 to 10,000 glucose units. The basic unit of the cellulose polymer consists of two anhydride glucose units called cellobiose units [12].

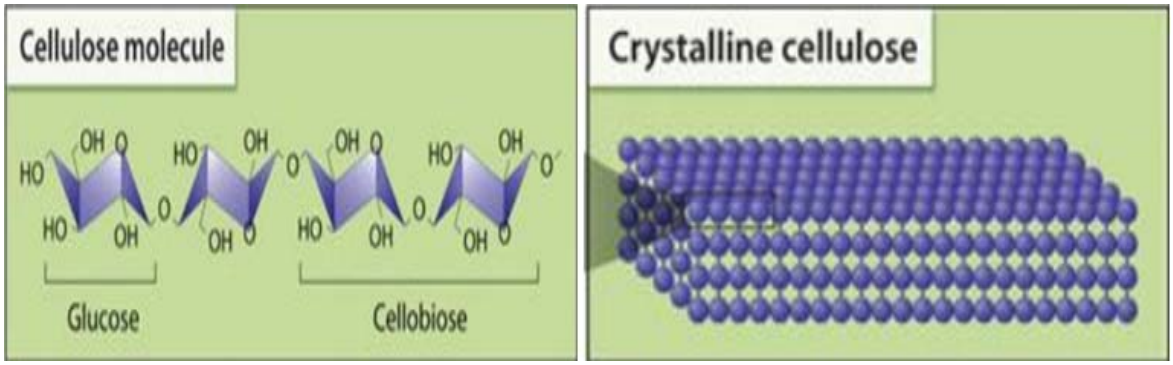


Figure 2.1: Structure and composition of cellulose fibers [12]

Cellulose is a linear high molecular weight polymer composed of β-D-glucopyranose units which are linked by 1-O-4 glycosidic linkages (Figure 2.2) [9]. It provides structural support and chemical resistance for the plant. Considering the uniform hydrolysable glucose building blocks, the cellulose molecule would be the best carbohydrate source for the fermentation process.

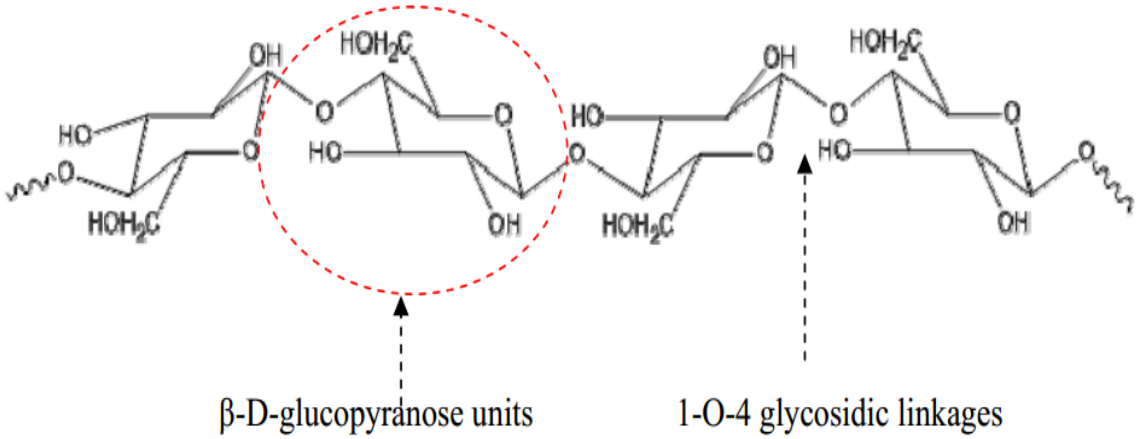


Figure 2.2: Cellulose molecular structures [9]

Major functional groups, hydroxyl groups are able to form intermolecular and intramolecular hydrogen bonds. Intermolecular linkages which form between different molecules are responsible for the formation of micro fibril structures and highly ordered crystalline areas (Figure 2.3) [4]. Cellulose in plant materials consists of both, crystalline and amorphous areas. Because of the high energy of a large amount of hydrogen bonds, cellulose crystalline areas are hard to degrade with enzymes or chemicals. Under normal conditions, due to the existence of hydrogen bonds, the cellulose is relatively insoluble. An efficient cellulose degradation technique, including effective enzymes, high temperatures, concentrated acid or alkaline, is necessary for both amorphous and crystalline cellulose in the conversion process.

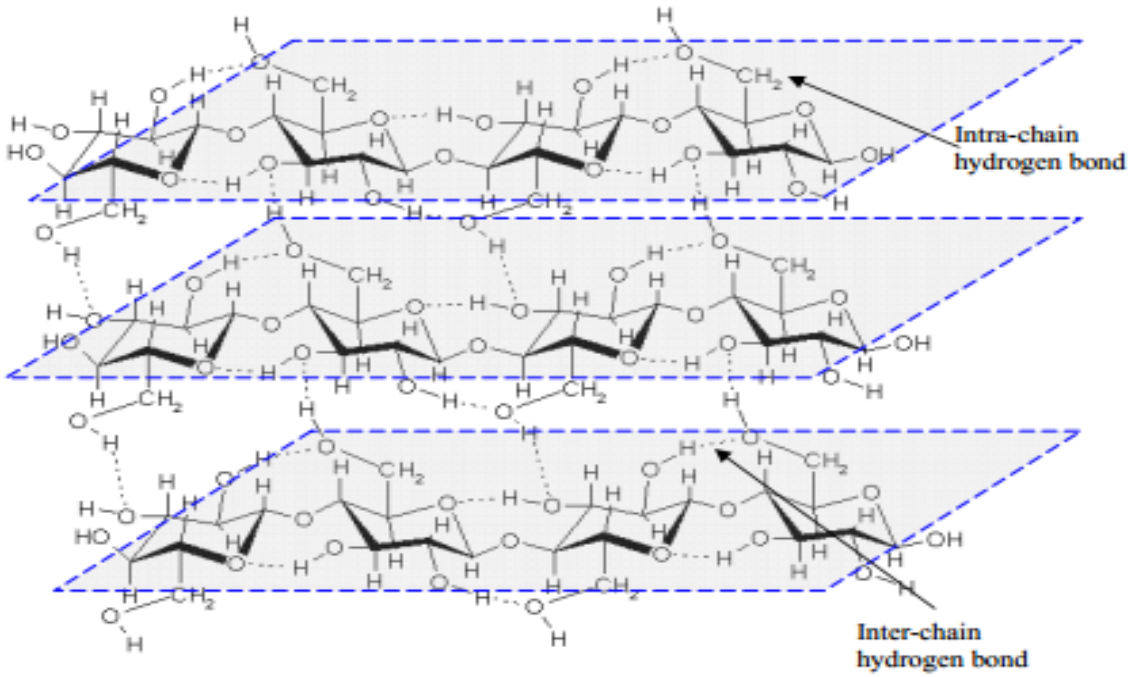


Figure 2.3: Cellulose crystalline arrays [4]

2.1.2. Hemicelluloses

Hemicelluloses is another important polysaccharide type in plant cell walls, it is a branched hetero-polysaccharide consisting of various pentose and hexoses units including xylose, mannose, galactose, rhamnose and arabinose. Besides these units, they may also contain acetic, uronic acid as well as 4-O-methyl ether. They are shorter chain molecules with covalent bonds to lignin to form cross-linked structures. Due to the linkages among hemicelluloses, cellulose and lignin, removal of hemicelluloses which is easier than removal of lignin and cellulose, is considered to be an efficient way to increase cellulose utilization. Xylose is the most important type of sugar within hardwood and grass type hemicelluloses. In hardwood, it comprises most of the hemicelluloses main chains. Thus, conversion of xylose is the key to utilize hardwood hemicelluloses [11, 12]. Due to the structural diversity, a wide range of enzymes including endoxylanase, exoxylanase, mannanase, arabinosidase, acetylesterase and glucoronisidase are needed. Pretreatments, especially alkaline pretreatment also were found to be effective in separating hemicelluloses from lignin and cellulose.

Hemicelluloses are a mixture of various polymerized monosaccharide such as glucose, mannose, galactose, xylose, arabinose, 4-O-methyl glucuronic acid and galacturonic acid.

2.1.3. Lignin

Lignin is a mononuclear aromatic polymer located in the cell walls of biomass and is connected to cellulose fibers [12]. As the second most abundant material in the plant cell wall, lignin provides additional strength and protection against fungi and insect attack. The high molecular weight phenylpropan structure of lignin leads to high insolubility in most solvents. Lignin significantly adds to the rigidity and moisture resistance of the biomass. Furthermore, the existence of the lignin has been considered as an inhibitor for cellulose, due to the non-productive binding of lignin and cellulose depending on the lignin types [12]. As a result, chemicals like surfactants, $MgSO_4$ and $CaCl_2$, and exogenous proteins like bovine serum albumin are added prior to enzyme or microbe loading to reduce the reaction between lignin and cellulose. In addition, many pretreatment methods have been developed to diminish the obstacle of lignin. Minimizing the effect of lignin inhibition depends on the source of lignocellulosic biomass, since lignin within the lignocellulosic materials is not uniform and varies in different plants and even in different locations of the plant. For example, in the middle lamella and primary cell wall, lignin content is higher than in the secondary cell wall [11, 12]. There are three monolignol building blocks for lignin: p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol (Figure 2.4) [12].

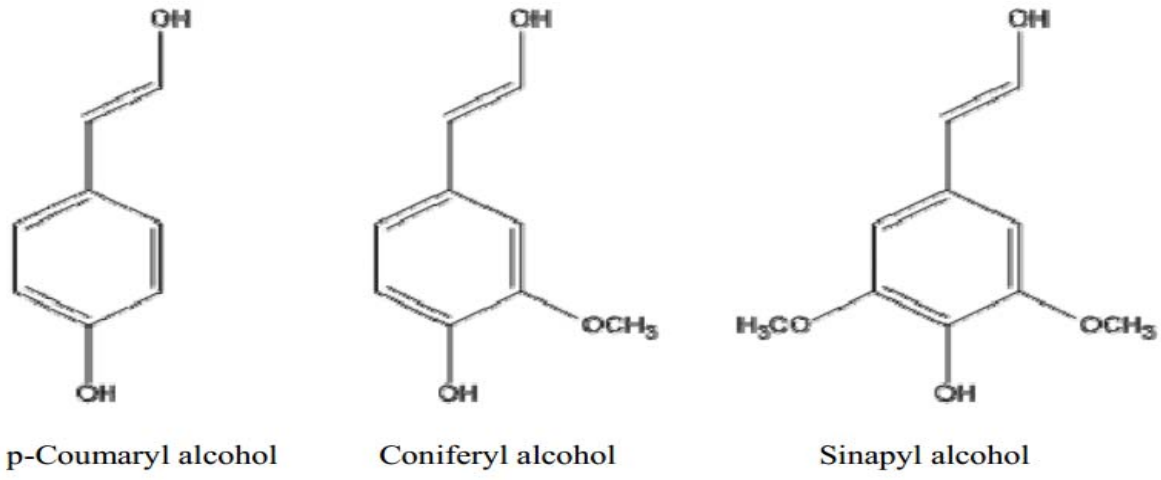


Figure 2.4: The structures of lignin building blocks [12]

LCMs can vary in composition and moisture content according to; region, fertilization practices, harvesting, storage and storage time in table 2 1.

Table 2.1: Composition of several LCMs for Ethanol production [12]

Feedstock's	Content (Dry weight percentage)		
	Cellulose	Hemicelluloses	Lignin
Hardwoods			
Eucalyptus	48	29.0	23.0
Globukus			
Acacia dealbata	52.0	24.1	23.9
Popular	49.05	27.71	23.24
Buck locust	47.6	27.66	24.74
Softwoods			
Salix	42.50	31.50	26.00
Spruce	44.00	24.60	32.00
Pine	44.55	23.81	27.67
Agro-industrial residues			
Corn stover	40.00	29.60	23.4.
Rice husks	36.70	21.00	21.30
Rice straw	36.20	19.00	9.90
Wheat straw	32.90	24.20	8.90
Cotton stalk	35.00	16.80	7.20
Sugarcane bagasse	50.00	27.00	20.00
Ethiopia mustard	32.70	21.90	18.90

2.1.4. Ethanol

Ethanol, also called ethyl alcohol, grain alcohol, or drinking alcohol, is a volatile, flammable, and colorless liquid with a slight chemical odor. A psychoactive drug and one of the oldest recreational drugs known, ethanol produces a state known as alcohol intoxication when consumed. Best known as the type of alcohol found in alcoholic beverages, it is also used in thermometers, as a solvent and as a fuel and, due to its low freezing point, the active fluid in post mercury thermometers. In common usage, it is often referred to simply as alcohol or spirits [5].

The molecule is a simple one, being an ethyl group linked to a hydroxyl group. Its structural formula, CH_3CH_2OH, is often abbreviated as C_2H_5OH, C_2H_6O or EtOH.

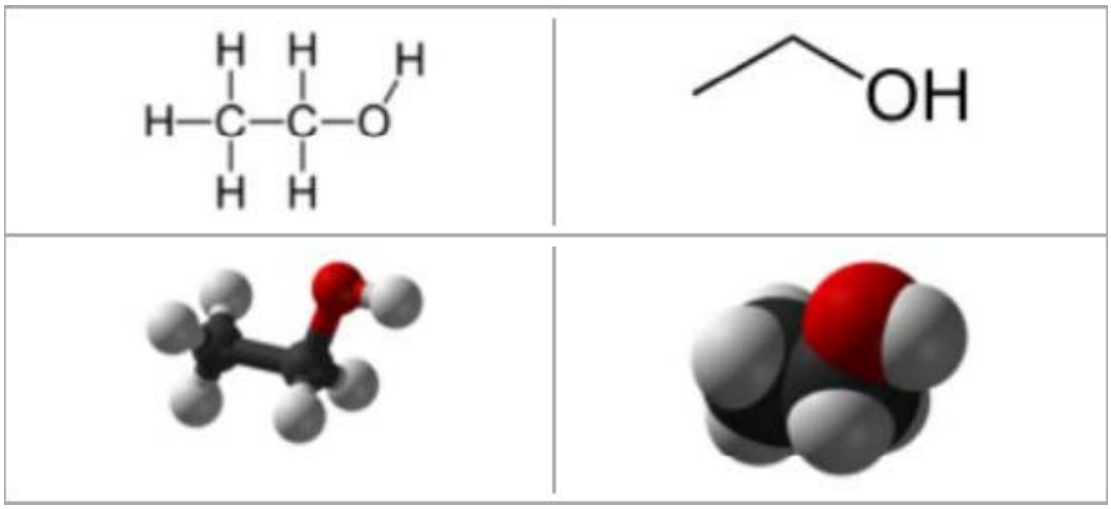


Figure 2.5: Chemical structure of ethanol [11]

2.2. World market of ethanol

Today, bioethanol is the most dominant biofuels and its global production showed an upward trend over the last 28 years with a sharp increase from 2000. As of 20014, worldwide production capacity for bioethanol fuel was about 45 billion liters per year, with approximately 15 percent annual growth between 2000 and 2014. This value increased to 49 billion liters in 2006, when the Americans produced 75 percent of the total world ethanol output, followed by Asia/Pacific, and Europe/Africa with respective values of 15 and 10 percent [13].

The industrial alcohol market showed a rather modest rate of growth similar to the increase in the gross domestic products (GDP) many countries. The market for beverage alcohol in most developed countries is stagnating, due to increased health awareness. Fuel ethanol production is predicted to have the strongest increase in the Americans, where the production is expected to rise to around 85 billion liters by 2017, representing about 52 billion liters increase in the projection period. In Asia this value is anticipated to increase to 12 billion liters during the same period and in Europe, with the policy of increasing the share of biofuels in the transportation sector, the production will raise strongly. Therefore, total output in 2017 is forecast to reach over 135 billion liters [13].

Table 2.2: Projected biofuels production in biofuels producing countries and in the world [13]

Country/ Region	Biofuels	Projected production or consumption in different years ($L*10^6$)				
		2013	2015	2017	2019	2021
World	Ethanol	113853.8	129015.6	140902.1	150728.4	162664.6
	Biodiesel	28507.8	29879.9	33314.7	36161.5	39636.9
Brazil	Ethanol	28684.5	37323.3	43483.7	45644.3	47929.6
	Biodiesel	2587.0	2744.0	2902.8	3069.9	3245.5
USA	Ethanol	55769.8	60536.2	63784.9	68825.1	75889.1
	Biodiesel	6057.5	5138.5	5192.0	5191.7	6390.9
Canada	Ethanol	1605.0	1512.5	1430.4	1459.6	1486.2
	Biodiesel	487.8	452.2	413.9	380.5	355.2
Europe	Ethanol	7048.5	7625.1	8528.8	9978.9	11565.8
	Biodiesel	11287.6	12151.7	14211.1	16374.1	17784.2
Argentina	Ethanol	497.3	625.9	742.2	853.2	963.6
	Biodiesel	2697.1	2956.4	3171.4	3136.4	3300.5
India	Ethanol	422.5	532.0	630.9	725.2	819.3
	Biodiesel	2292.7	2512.6	2695.7	2665.6	2805.4

2.3. Current ethanol production in Ethiopia

Ethanol is manufactured from microbial conversion of molasses through fermentation. The production process consists of conversion of biomass to fermentable sugars, fermentation of sugar to ethanol and the separation and purification of ethanol. Fermentation initially produces ethanol containing a substantial amount of water. Then this solution is distilled using distillation column the majority of water to yield up to 95 percent purity ethanol, the balance being water. This mixture is called hydrous ethanol. If the remaining water is removed in further process, the ethanol is called anhydrous ethanol and suitable for blending with gasoline. Ethanol is "denatured" prior to leaving the plant to make it unfit for human consumption by addition of small amount of products such as gasoline [15].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 estate (Fincha, Metehara, Wonji/Shoa and Tendaho; under operation) industries which are run and administered by Sugar Development Agency (SDA) [15].

Table 2.3: Projected ethanol production by year in million liters [15]

Industry	2011/2012	2012/2013	2013/2014	2014/2015	2015/2016	2016/2017
Fincha	17.2	21.8	24.1	30.5	33.4	35.7
Metehara	18.8	24.5	28.3	31.3	33.5	38.4
Wonji/Shoa	-	15.6	17.8	21.8	24.1	26.7
Tendaho	21.9	36.1	46.6	55.4	58.9	62.1
Total	**57.9**	**98.0**	**116.8**	**139.0**	**149.9**	**162.9**

2.4. Cellulosic ethanol

Cellulosic ethanol i.e. ethanol from forestry or agricultural waste is considered a way to prevent displacement of crops to feed humans. Only a small percentage of a plant can be used in the form of sugar or starch, consumed by animals or human beings, or fermented by yeast into ethanol. Most of the rest of the plant is cellulose. Using the bulky portion of the plant may be more efficient than using other portions of the plant. Some grasses have higher energy storage in the form of cellulose when compared to corn in the form of grain and can be grown efficiently with less application of N_2 based fertilizer, low pesticides use and less processed energy [11].

Cellulosic ethanol is a second generation biofuels, as opposed to ethanol made from corn which is considered a first generation biofuels. The important difference is that the second generation biofuels uses nonfood residual biomass including stems, leaves, husks, wood chips etc. Cellulosic feedstock's are under research and will be used for ethanol production in the upcoming years. Crop byproducts like corn stove, grain straw, rice hulls, paper pulp, sugarcane bagasse, wood cheeps (sawdust) and native grasses such as switch grass are major cellulose based feedstock's which can be converted easily into ethanol [11].

2.5. Factors for selecting wood sawdust as feedstock for cellulosic ethanol

2.5.1. Why wood sawdust is considered as a renewable energy source?

Many environmental problems such as greenhouse gases and pollution of air, water and soil originate from fossil fuels. Fossil fuels release greenhouse gases, like carbon dioxide, that contribute to global warming. Carbon dioxide from fossil fuel combustion accounted for nearly 80 percent of global warming in the 1990's [16]. The sawdust is obtained from sawn wood and probably other wood wastes. Sawdust can be wastes/residue from either hardwood or softwood

or the mixture of both. Softwood and hardwood vary in the percentages of cellulose, hemicelluloses and lignin (Table 2.4). The lignocelluloses fractions are the polysaccharide complex in LCMs. Lignocelluloses contents are not directly available for bioconversion because of their intimate association with lignin [16].

Table 2.4: Weight percent of cellulose, hemicelluloses & lignin in wood sawdust biomass [16]

Raw material	Cellulose	Hemicelluloses	Lignin
Wood sawdust	48.0%	29.0%	23.0%

Table 2.5: Percentage composition of sawdust biomass determined by thermal analysis [16]

Raw material	Carbon	Volatile matter	Ash content
Wood sawdust	25.5%	73.0%	1.5%

Sawdust serves as a cheap substrate for ethanol production, does not distort the human food chain and takes care of the environmental waste. The major difficulty in the hydrolysis of lignocelluloses contents from wood, to obtain fermentable sugars, lies in separating it from lignin that encloses it and makes it difficult to access. Acid hydrolysis is one of the pretreatment methods used for the lignocelluloses contents to make it susceptible to fermentation to obtain cellulosic ethanol [16].

The various possible feed stocks for production of cellulosic ethanol; corn stover, wood and switch grass have significant potential. However, the choice of sawdust for the experiment is because;

- ✓ Cheap and readily available
- ✓ Although corn stover may have tremendous potential as a feedstock, corn stover collection can result in damage to the soil such as erosion
- ✓ Perennial crops, cultivated specifically for use in bio energy production, were determined to potentially be available at quantities similar to corn stover. However, an increase in the production of such crops would require a decrease in the areas of other agricultural crops or pastureland.
- ✓ The previous study indicated that using waste products as an ethanol feedstock may be a more desirable option, since the production of both additional corn grain and switch grass for ethanol production increases GHG emissions by a substantial amount.

2.6. Process technology for cellulosic ethanol production

There are two primary routes for the production of cellulosic ethanol; biochemical and thermochemical routes. The biochemical route relies primarily on the use of enzymes and other microorganisms and the thermochemical route relies on the application of heat and chemical synthesis.

The biochemical route of cellulosic ethanol production from lignocellulosic biomass contains four major processes; including pretreatment, hydrolysis, fermentation and distillation.

2.6.1. Pretreatment techniques

Pretreatment is required to alter the biomass macroscopic and microscopic size and structure as well as its submicroscopic structural and chemical composition to facilitate rapid and efficient hydrolysis of carbohydrates to fermentable sugars. Lignocellulosic biomass, including forestry residue, agricultural residue, yard waste, wood products, animal and human wastes, etc., is a renewable resource that stores energy from sunlight in its chemical bonds [17].

The overall purpose of pretreatment is to break down the shield formed by lignin and hemicelluloses, disrupt the crystalline structure and reduce the degree of polymerization of cellulose. Pretreatment has been viewed as one of the most expensive processing steps within the conversion of biomass to fermentable sugar . With the advancement of pretreatment technologies, the pretreatment is also believed to have great potential for the improvement of efficiency and cost reduction [17] .

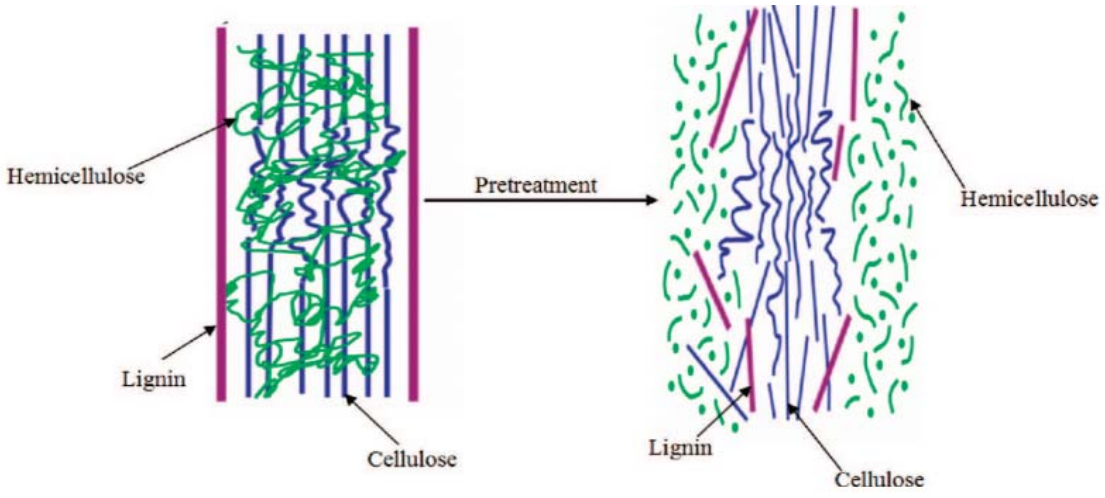


Figure 2.6: Schematic of the role of pretreatment in the conversion of biomass to ethanol [17]

Pretreatment techniques have been developed for various end uses of biomass feedstock's. The aim of this study emphasizes the biomass pretreatment in preparation for acid hydrolysis for producing of reduced sugars and microbial fermentation for cellulosic ethanol production. It primarily covers the impact of biomass structural and compositional features on the pretreatment, the action mode of different pretreatment methods, the pretreatment study status, challenges and future research targets [11,17].

Various pretreatment technologies have been extensively studied to process different biomass for cellulosic ethanol production. However, none of those can be declared a "winner" because each pretreatment has its intrinsic advantages and disadvantages. An effective pretreatment is characterized by several criteria; avoiding size reduction, preserving hemicelluloses fractions, limiting formation of inhibitors due to degradation products, minimizing energy input and being cost effective [11].

2.6.1.1. Physical pretreatment

Physical pretreatments are methods without addition of chemicals or micro-organisms. They use external forces to reduce the LCMs into fine particles in order to increase the surface area of the materials. According to the forces used, the physical pretreatment can be further divided into two sub-catalogs; mechanical (dry, wet, vibratory ball milling) [18] and non-mechanical method (steam explosion, irradiation and pyrolysis) [19].

2.6.1.1.1. Mechanical pretreatment

Mechanical pretreatments use shearing force to reduce biomass particle size, change the lignocelluloses structure and reduce degree of polymerization and crystallinity of cellulose [19]. Depending on the final size of the material, the mechanical pretreatment consists of milling, grinding or chipping. Chipping leads to 10 to 30mm particles and milling and grinding lead to 0.2 to 3mm [20]. Milling includes ball milling, two roll milling, hammer milling, compression milling agitation bead milling, pan milling, fluid energy milling and colloid milling [18, 21]. For aspen, vibratory ball milling is reported to be a more effective methods compared to ordinary ball milling [18]. However, according to the research performed by Cadoche et al, the energy input to reduce the biomass to fine particle is higher than the theoretical energy content held in biomass, which makes the milling not economical for most lignocellulosic biomass, especially for high moisture biomass [24]. Furthermore, these methods are species selective. Improper

application of mechanical pretreatment will lead to carbohydrate losses, in which case the final fermentable sugars and ethanol yield will be reduced [16]. Therefore, mechanical pretreatment is considered to be impractical to be applied exclusively. Combination of mechanical pretreatment and chemical size reduction is commonly used to make the pretreatment more cost efficient [25].

Extrusion, which utilizes heating, mixing and shearing to increase accessibility of the materials, is a novel promising pretreatment technology. Both physical and chemical modifications occur as the lignocellulosic biomass passes through the extruder. High efficiency makes this pretreatment method appealing [26].

2.6.1.1.2. Non-mechanical method

Irradiation pretreatment can be performed by Gamma rays, microwave, ultrasound, pulsed electrical filed, UV and electron beam. Irradiation will cause the disruption of beta-1, 4-glycosidic bonds and cellulose crystalline structures [19]. In addition, the high energy of these radiations will lead to the formation of free radicals, which leads to a further degradation of the lignocellulosic materials [27]. This method is widely used in waste water sludge pretreatment [27, 28]. For the application for lignocellulosic biomass, including rice straw, bagasse, sawdust, chaff, corn stalk, peanut huts and oil palm empty fruit bunch, was studied in pervious experiments by using ultrasonic irradiations [27]. Dramatic enhancements of the enzymatic hydrolysis efficiency were achieved [29, 30]. Unfortunately, irradiation pretreatments are reported to consume high levels of energy and require long process time with expensive high quality equipment. Irradiation pretreatment methods by themselves are currently limited to laboratory scale and not considered as a feasible solution for industrial applications.

Steam explosion is exposing biomass to steam under high pressure and temperature followed by a decompression at the end [31]. Liquid hot water (LHW) pretreatment (co-current, counter-current and flow through) is a pretreatment similar to steam explosion, except that, in LHW pretreatment, instead of steam, biomass is merged into hot water with certain pressure and temperature. Both these processes are able to cleave the acetyl groups and uronic acid groups from hemicelluloses and consequentially acidify the medium. Furthermore, water at high temperature acts as acid [31]. As a result, acidic condition will cause partially hydrolysis of hemicelluloses and amorphous cellulose to oligosaccharides and to fermentable sugars, also resulting in a more accessible material for the following hydrolysis or fermentation steps [21]. Moreover, for steam explosion, the lignin structure is partially modified which also leads to

higher digestibility of the biomass [32]. In addition, the shear forces caused by expansion contribute to the structural modification [33]. Both mechanisms are similar to acid pretreatment. However, the steam explosion is able to offer higher fermentable sugar concentrations due to the lower water content [18]. There are no chemicals added in both methods. In addition, size reduction is not required for lignocellulosic biomass since breakage of particles will occur during pretreatments [31], these methods are more economic feasible in lignocellulosic conversion application. The total reduced sugar yield after hydrolysis is increased over seven times with the pretreatment as compared to non treated aspen [34].

LHW was applied on yellow poplar wood sawdust with and without pH control [35].The pretreatment proved to involve the partial conversion of cellulose and hemicelluloses into polysaccharides and monosaccharide's. Nevertheless, during steam explosion and LHW, high temperature is reported to increase the lattice structure of the cellulose and consequentially increase the cellulose crystalline [36, 37]. In addition, higher pretreatment temperature also leads to increased material solubility adding to the pretreatment yield loss [35]. High severities also causes the production of various fermentation inhibitors, includes furfural, 5-Hydroxymethyl furfural (HMF), phenolic compounds and aliphatic acid [28], making this method not suitable for pretreatment. Monitoring and controlling the pH is considered to be an efficient improvement for LHW pretreatment. With controlled pH, cellulose solubility and formation of HMF, levulinic and formic acid are minimized [35].

2.6.1.2. Biological pretreatment

Because of the environmental issues, more and more studies turn to biological pretreatment methods, which use fungi, bacteria or their enzymes. Fungi are applied as delignification agent to digest or change the lignin structure and hydrolyze the hemicelluloses [20]. As a result, enzymes used for the hydrolysis process are able to approach polysaccharides such as cellulose and other hemicelluloses much easier. In the present literature, various species of brown rot fungi, white rot fungi and soft rot fungi have been studies [20, 38].

White rot fungi only attacks lignin, but brown rot and soft rot fungi mainly attack cellulose while slightly modifying the lignin structure. Among these species, white rot fungi are more commonly used in pretreatment since they produce lignin-degrading enzymes including laccases and peroxides [39]. *Pleurotus ostreatusis* reported to be able to convert over 35 percent of wheat straw cellulose into reduced sugars in five weeks [40]. *Stereum hirsutum* was found to be

very efficient in pretreated *Pinus densiflora*, a type of softwood [41]. Other white rot fungi like *Cyathus stercolerus, Phanerochaete chrysosporium, Phanerochaete sordid and Pycnoporus cinarbarinus* were also tested on several substrates for their delignification efficiency [39].

Researchers agree that there are significant advantages to the biological pretreatment methods, especially since these procedures are completely environmentally friendly and require low energy input [26]. However, the drawback of these methods outweighs their advantages. Almost all those fungi need more than one week to react with biomass. In addition, lignin degradation of white rot fungi requires a carbon source, mainly cellulose and hemicelluloses, since it is a co-oxidative process. Thus, while reacting with lignin, these fungi will reduce cellulose and hemicelluloses concentration in biomass, thereby decreasing the yield of fermentable sugars [42].

As a result, although enormous experiments have been carried out at lab-scale, none of the biological pretreatments have been applied to industrial-scale production [18]. Thus, these methods still need to be improved before they can be used for large-scale production. Development of genetically modified strains with high lignin degradation capacity and high cellulase activity are necessary [18].

2.6.1.3. Chemical pretreatment

Chemical pretreatments have been studied dated back to the early 1900's and several reports have been published since 1980's comparing response of enzymatic hydrolysis after different chemical pretreatment methods. Contradictory to the physical methods, chemical pretreatments are mainly used for modifying the lignin in the biomass, removing hemicelluloses and to change cellulose polymerization as well as cellulose crystalline structure [43]. Acids, alkali, salts, organic solvents as well as oxidizing agents are all considered to be effective pretreatment agents [44].

2.6.1.3.1. Acid pretreatment

Acid pretreatment is one of the oldest and most commonly used methods. 72% sulfurous acid was considered to be the first concentrated acid used in the pretreatment process in the US and 42% hydrochloric acid hydrolysis was experimented in Germany [45]. Organic acids including maleic acid and fumaric acid are also extensively used in hydrolysis [46]. Both concentrated and diluted acids are used in acids pretreatment hydrolysis. Concentrated acids disturb the hydrogen

bonds in crystalline cellulose and convert crystalline cellulose into amorphous cellulose. Furthermore, these pretreatment methods decrease the degree of polymerization (DP) of cellulose and degrade the pentose. The advantage of using concentrated acid pretreatment is that it is not specific to biomass type. In addition, mild temperature condition and high monosaccharide yield (over 90%) are the characteristics that made this method appealing for decades [45]. This issue with concentrated acid pretreatment is that concentrated acids especially at higher temperatures (200-500ºC) lead to the formation of furfural or HMF, which reduces the sugars yield [47]. The other drawback for concentrated acid pretreatment is high corrosion of the equipments and high acid recycling cost. To combat these issues, diluted acid pretreatment became more favorable in the biofuels industry. There are typically two types of diluted acid pretreatments; high solid loading (10 to 40 percent) low temperature (less than 160ºC) batch pretreatment and low solid loading (5 to 10 percent) high temperature (more than 160ºC) continuous flow pretreatment. Besides reactions with cellulose, diluted acid pretreatment also cause hemicelluloses dissolution. This will release water soluble sugar monomers and oligomer from cell wall matrix, so that the porosity of the cell wall and enzyme digestibility are both increased. In addition, although diluted acid is not able to remove lignin, researchers suggested that the lignin in biomass will be modified [48]. As a result, the diluted acid pretreatment is less flexible in choosing feedstock. Biomass with lower lignin content is preferred. In addition, due to the mild condition, extension of time or increase of temperature is required [45].

2.6.1.3.2. Alkali pretreatment

Alkali pretreatment is a very commonly used method. During this alkali pretreatment, biomass is soaked in the dilute alkali solution (0.5 to 2 percent) and treated for varying of time periods and temperature. Neutralization and removal of lignin fragment and other inhibitor is usually involved at the end of the pretreatment. Generally alkali conditions are less severe than other pretreatments and prevent lignin condensation reactions. Due to the mild conditions, alkali pretreatments usually require longer pretreatment time, pressure or addition of oxygen which will facilitate some delignification [49, 50]. This milder condition allows alkali pretreatments to remove some lignin fraction without leading to severe carbohydrate lose or inhibitor formations. Under alkali condition, peeling reactions, which start from the reduced end-group of the polysaccharides, will happen and will reduce the overall sugar yield. At high temperature, random chain cleavage will break some of the 1-4-β-glycosidic bonds between glucose building blocks to lower the degree of polymerization and the crystallization of cellulose [51]. In this

process some lignin and hemicelluloses is removed. Ether linkages between carbohydrates and lignin are deconstructed by forming intermediate epoxide structure. This intermediate structure allows the nucleophilic substitution [52].

2.6.1.3.3. Oxidative pretreatment

Oxidative pretreatment is aimed at remove mainly lignin and hemicelluloses by using oxidants such as oxygen, ozone and hydrogen peroxide to increase cellulose digestibility. The mechanisms for degradation of lignin in oxidative pretreatment vary depending on oxidants used and reaction conditions. For oxygen delignification, the phenol ate ion, which is formed when a phenolic hydroxyl group in lignin reacts with alkali, reacts with oxygen to form reactive intermediate called hydro peroxide. This intermediate then undergoes fragmentation by several possible pathways to form lignin fragments that are mostly water soluble [53]. For ozone pretreatment, ozone is highly reactive towards compounds with conjugated double bonds and functional groups with high electron densities. Therefore, lignin which is enriched of unsaturated bonds is most likely to be oxidized by ozone to form soluble acidic compounds with less molecular weight including aliphatic, carboxylic and aromatic acids [53, 54]. Despite the high selectivity and positive environmental impact of ozone pretreatment, the cost of ozone and the mass transfer issues largely limit this pretreatment [54].

2.6.1.3.4. Organosolv pretreatment

The organosolvation method is a promising pretreatment strategy, and it has attracted much attention and demonstrated the potential for utilization in lignocellulosic pretreatment. In the organosolvation process, an organic or aqueous organic solvent mixture with inorganic acid catalysts (HCl or H_2SO_4) is used to break the internal lignin and hemicelluloses bonds. The solvents commonly used in the pretreatment are methanol, ethanol, acetone, ethylene glycol, triethylene glycol, and tetrahydrofurfuryl alcohol. Organic acids such as oxalic, acetylsalicylic and salicylic acids can also be used as catalysts in the organosolvation pretreatment. In essence, the organosolv pretreatment involves simultaneous prehydrolysis and delignification of lignocellulosic biomass supported by organic solvents and usually, dilutes aqueous acid solutions. A high yield of xylose can usually be obtained with the addition of acid [55]. This process uses a blend of ethanol and water in the ratio of 50:50 (w/w) at 200°C and 400 psi to extract most of the lignin from wood chips or other lignocellulosic biomass. Lignin is recovered as a fine precipitate by flashing the pulping liquor to atmospheric pressure, followed by rapid

dilution with water. Other coproduces such as hemicelluloses sugars and furfural are recovered from the water soluble stream. The largest component, cellulose, is partially hydrolyzed into smaller fragments that still remain insoluble in the liquor. The second largest component, hemicelluloses, is hydrolyzed mostly into soluble components, such as oligosaccharides, monosaccharide's and acetic acid [54, 55].

2.6.1.3.5. Autohydrolysis pretreatment

Autohydrolysis is a hydrothermal process in which lignocellulosic biomass is pretreated in a water only medium at elevated temperatures to solubilize mainly hemicelluloses and disrupt biomass structure for an improved enzymatic digestibility. This process has been described as LHW pretreatment, hydrothermal pretreatment, aqueous pretreatment, water prehydrolysis, hydrothermolysis and hot compressed water pretreatment as well in many other studies [55, 56]. It has long been studied as a prehydrolysis step to separate hemicelluloses in a modified Kraft pulping process. Nowadays autohydrolysis has drawn substantial attentions as a pretreatment technology for bioethanol production.

Autohydrolysis offers several attractive features as a pretreatment method including [56],

1. Water is only reaction medium and the whole process is environmental friendly
2. No chemical recovery units required
3. Less degradation products compared to acid process
4. Less equipment corrosion due to the mild pH of the reaction medium
5. Low technical risk owing to the use of proven equipment and technology
6. Low capital cost because of the process simplicity.

In an autohydrolysis process, acidic hydroniumions are generated by water autoionization at high temperature. They act as catalyst and attack the susceptible ether bonds of hemicelluloses, leading to both splitting of acetyl groups and generation of oligosaccharides [55]. The resulting acetic acid makes the solution more acidic and further catalyzes the depolymerization of biomass. It has been suggested that other organic acids such as uronic acid, formic acid and levulinic acid may also contribute to the generation of hydroniumions, but their effects are not well established [55,56]. Depending on the pretreatment severity, the dissolved sugars (pentose and hexoses) can be further degraded to form furfural and HMF, which combined with aromatic degradation products from lignin, may affect the yeast metabolism in the fermentation step [56].

2.6.1.4. Combination pretreatment

On the foundation of well established pretreatment methods, researchers become focused on combination pretreatment methods which combine at least two methods to maximize the utilization of biomass by overcoming the disadvantages of the single methods [57]. Several combination pretreatments have been summarized by Charles E. Wyman [42]. He listed five catalogs of examples including two or more physical pretreatments in sequence, two or more chemical pretreatment in sequence, physical pretreatment followed by chemical pretreatment, chemical pretreatment followed by physical pretreatment, chemical pretreatment followed by biological pretreatment [57]. Although these combination methods have better conversion rates compare to single methods, they might require higher expenses because of the complexity of the pretreatment equipments. Since the improvement is not dramatic and a large amount of additional expense is needed for large scale combination methods, the single methods are still considered to be the most economic feasible option for biomass pretreatment.

Table 2.6: Combination pretreatment used [57]

Combinations	Examples	Process	Concentrations
Physical pretreatments in sequences	Irradiation and mechanical crushing	Shorten reaction time, no need to neutralize, lower energy input	Higher cost
Physical pretreatment followed by chemical pretreatment	Ammonia fiber Explosion	No inhibitor to hydrolysis microbes, Completely lignin separation	Incompletely lignin separate, inhibitor to hydrolysis microbes, low yield, high cost
Chemical pretreatment followed by physical pretreatment	Acid Steam Explosion	Higher hemicelluloses Removal. Better yield than AFEX, no inhibitor to hydrolysis microbes	High cost
Chemical pretreatment followed by biological pretreatment	-	-	Inhibitor to hydrolysis microbes, low yield, high cost

Generally, an effective pretreatment should meet the following requirements [57],

1. Overcome lignocellulosic biomass recalcitrance, deconstructing the three dimensional structure of lignocelluloses and breaking down the semi-crystalline cellulose and hemicelluloses
2. Afford high yields to sugars or chemicals and/or give highly digestible pretreated solid
3. Avoid carbohydrates degradation and in particular preserve the utility of pentose (hemicellulose) fraction
4. Avoid the formation of inhibitory toxic byproducts
5. Allow lignin recovery and exploitation to give valuable co-products
6. Be cost effective, involving reasonable size reactors, low wastes amount and low energetic.

The most advanced methods of cellulosic ethanol production from lignocellulosic biomass are,

1. Separate Hydrolysis and Fermentation (SHF) which can be subdivided into;
   I. Enzymatic hydrolysis and fermentation
   II. Acid hydrolysis and fermentation
2. Simultaneous Saccharification and Fermentation(SSF)

2.6.2. Separate hydrolysis and fermentation (SHF)

Hydrolysis is the conversion of lignocellulose contents to reducing sugars. It is also known as Saccharification. The concentration of glucose in starch digests is 30 to 40 percent and highest glucose concentration in cellulose digest is 10 percent. Selby however obtained 47 percent glucose from cellulose. However, using specific techniques, 42 percent glucose can be obtained from cellulose [11]. The major problems in obtaining high concentrations of glucose from cellulose are [58];

1. Susceptibility of substrate
2. Activity of enzymes

Lignocellulosic biomass is a mixture of three basic components: lignin, cellulose and hemicelluloses. Wood contains 45 percent cellulose with softwood having 2 to 3 percent less than hardwood, 24 to 33 percent lignin and the remaining hemicelluloses (xylan: 8 to14 percent and glucomannan: 16 to 22 percent) [8]. Lignin serves as a sort of glue giving the biomass fibers its structural strength, while cellulose and hemicelluloses polymers are the basic building blocks or the fibers. In order to break down the lignocelluloses to reduced sugar, the basic structure of the biomass must be attacked. Once the structure of the biomass is disrupted, the lignocelluloses

can be converted to reducing sugars enzymatically. This can be done by the use of enzymes known as enzymatic hydrolysis or by acid known as acid hydrolysis [9, 58].

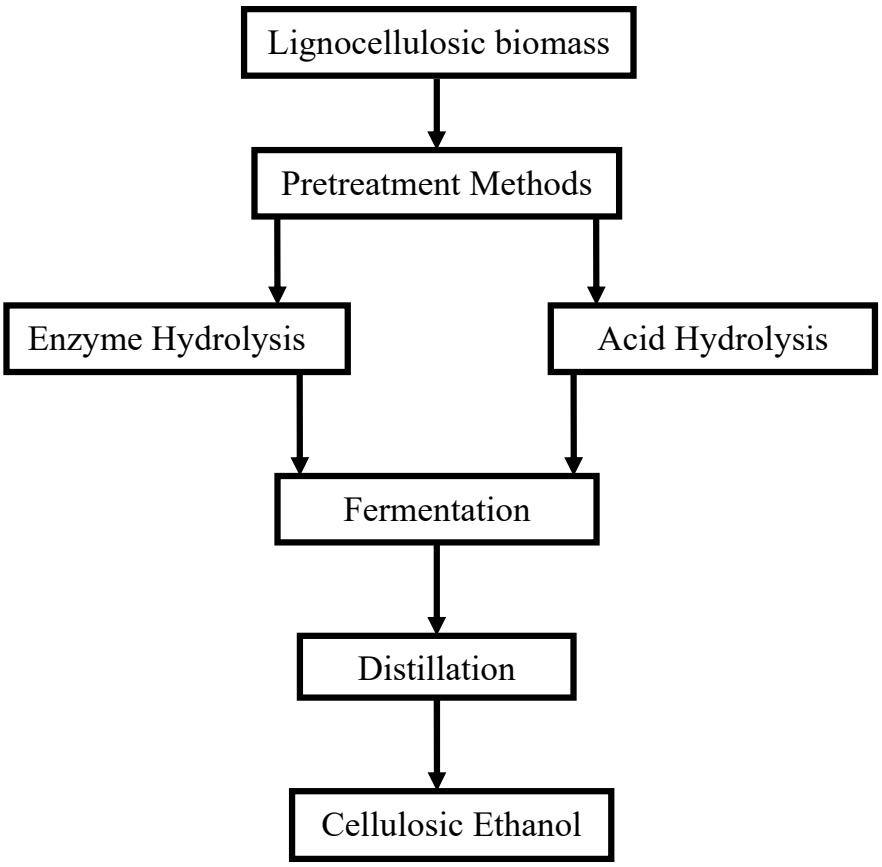


Figure 2.7: Hydrolyzed methods for cellulosic feedstocks [58]

2.6.2.1. Enzymatic hydrolysis

Enzymatic hydrolysis is a method in which lignocelluloses are utilized for the hydrolysis. This is a quite new approach compared to concentrate and dilutes acid hydrolysis. Cellulolytic enzymes were discovered during World War II when American scientists found the agent that was responsible for army clothing deterioration in the jungle of the South Pacific. The organism responsible for producing the cellulolytic enzymes was *Trichoderma reesei*, which today is used in the enzyme industry for producing a wide range of commercial enzymes [59]. The cellulases involved in the hydrolysis of lignocelluloses include endogluconase, which attack low-crystallinity regions of the cellulose fiber and generate free chain-ends and exogluconase, which remove cellobiose from the free chain ends. Then, β-glucosidase hydrolyses cellobiose to glucose [20]. However, a pretreatment of the lignocelluloses prior to the enzymatic hydrolysis is necessary to achieve feasible reaction rates. The aim of the pretreatment is to make the

lignocellulose more accessible to enzymatic attack due to weakening of the protecting lignin matrix or due to alteration of the pores in the material [20]. The advantages of enzymatic hydrolysis are high yields, due to the highly specific lignocellulose conversion and that the reaction is performed at moderate temperatures. Furthermore, the byproduct formation is low. The disadvantages are the slow reaction rate of the enzymes and the high enzyme cost [20, 60].

Ethanol production by enzymatic hydrolysis can be performed in a SHF modem or in a SSF mode. In the SHF process, hydrolysis is performed separately from fermentation, which means that the optimal temperatures for both the enzymatic hydrolysis and fermentation can be applied. A drawback with SHF is that the generated cellobiose functions as cellulase inhibitor. It has also been proved that β-glucosidase can be inhibited by glucose [59]. Another drawback is that SHF is a two step process. To reduce the risk for enzyme inhibition and reduce the number of process steps, SSF can be used. In SSF, hydrolysis and fermentation occur at the same time, which means that the glucose that is generated is immediately consumed by the fermenting microorganism and inhibition of β-glucosidase is therefore prevented. The disadvantage of the SSF process is that the optimal temperatures for the cellulases and the fermenting microorganism are not the same so the selected temperature is a compromise, which means that neither hydrolysis nor fermentation will be performed under optimal conditions [60]. Recently, efforts have been made to combine cellulase production, hydrolysis and fermentation in one single step. This concept is called consolidated bioprocess (CBP) and the aim is to create a microorganism that is able to perform these three steps simultaneously [60]. There are two different strategies to create a CBP microorganism, a naturally occurring cellulolytic microorganism can be modified by genetic engineering to gain important properties, such as the ability to give high ethanol yields, or alternatively a non-cellulolytic microorganism that gives high ethanol yields can be altered by genetic engineering to express heterogonous cellulases [60]. Today, most research efforts are focused on the enzymatic hydrolysis because of the high development potential. The dilute and concentrated acid hydrolysis is relatively mature techniques for which no major improvements are likely to happen. Nevertheless, the enzyme cost is still high for the enzymatic process. The cost of enzyme in the SSF and SHF processes has been calculated to be 10 to 20 percent of the total ethanol production cost [61].

$$(C_6H_{10}O_5)_n + H_2O \text{ (Alpha + Gluco Amylases)} \longrightarrow C_6H_{12}O_6 \qquad (2.1)$$

$$(C_5H_8O_4)_m + H_2O \text{ (Alpha + Gluco Amylases)} \longrightarrow C_5H_{10}O_5 \qquad (2.2)$$

2.6.2.2. Acid hydrolysis

Acids have been used to catalyze the hydrolysis of starch in "starch cookers" operating at temperatures of 50 to 150°C, a process referred to as acid hydrolysis. Acid pretreatment for ethanol production was developed in Germany in the 20th century. In the United States, the U.S. Forest Service, Forest Products Laboratory conducted extensive research using acid pretreatment for ethanol production from wood [51].There are two basic types of acid processes; dilute and concentrated acid each with variations. Dilute acid processes are conducted under high temperature and pressure and have reaction times in the range of seconds or minutes while concentrated acid process conducted under low temperatures and pressures employed allow the use of relatively low cost materials such as fiberglass tanks and piping [51].

Generally, the chemical reaction during the hydrolysis of lignocellulosic biomass using acid hydrolysis is described below.

$$[C_5H_8O_4]_m + mH_2O \xrightarrow{mH_2SO_4} C_5H_{10}O_5 \quad , \Delta H=79.0m \text{ kJ/mol} \qquad (2.3)$$

$$[C_6H_{10}O_5]_n + nH_2O \xrightarrow{nH_2SO_4} C_6H_{12}O_6 \quad , \Delta H=22.1n \text{ kJ/mol} \qquad (2.4)$$

2.6.2.2.1. Dilute acid processes

Most dilute acid (0.5-2.0%) processes are limited to a sugar recovery efficiency of around 40 to 65 percent. The reason for this is that at least two reactions are part of this process. The first reaction converts the cellulosic materials to sugar and the second reaction converts the sugars to other chemicals. Unfortunately, the conditions that cause the first reaction to occur also are the right conditions for the second to occur. Thus, once the cellulosic molecules are broken apart, the reaction proceeds rapidly to break down the sugars into other products most notably furfural, a chemical used in the plastics industry. Not only does sugar degradation reduce sugar yield, but the furfural and other degradation products can be poisonous to the fermentation microorganisms. The optimum results of ethanol yield from citrus peel waste were obtained at a temperature of 116^{o}C, 1% acid concentration and 12.5 minutes of retention time [11].

The biggest advantage of dilute acid processes is their fast rate of reaction, which facilitates continuous processing. The biggest disadvantage is their low sugar yield. For rapid continuous processes, in order to allow adequate acid penetration, feedstocks must also be reduced in size so that the maximum particle dimension is in the range of a few millimeters. Since 5-C sugars degrade more rapidly than 6-C sugars, one way to decrease sugar degradation is to have a two-

stage process. The first stage is conducted under mild process conditions to recover the 5-C sugars while the second stage is conducted under harsher conditions to recover the 6-C sugars. Unfortunately, sugar degradation is still a problem and the yields are limited [11].

2.6.2.1.2. Concentrated acid process

Concentrated acid (10-70%) process uses relatively mild temperatures and the only pressures involved are usually only those created by pumping materials from vessel to vessel. One concentrated acid process was first developed by united state of development agency (USDA) and further refined by Purdue University and the Tennessee Valley Authority (TVA). In the TVA concentrated acid process, corn stover is mixed with dilute (10%) sulfuric acid and heated to 100ºC for 2 to 6 hours in the first (or hemicelluloses) hydrolysis reactor. The low temperatures and pressures minimize the degradation of sugars. To recover the sugars, the hydrolyzed material in the first reactor is soaked in water and drained several times. The solid residue from the first stage is then dewatered and soaked in a 30 to 40 percent concentration of sulfuric acid for 1 to 4 hours as a precellulose hydrolysis step [11].

This material is then dewatered and dried with the effect that the acid concentration in the material is increased to about 70 percent. After reacting in another vessel for 1 to 4 hours at 100ºC, the reactor contents are filtered to remove solids and 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 sugars from the second stage hydrolysis are thus recovered in the liquid from the first stage hydrolysis [11].

The primary advantage of the concentrated process is the high sugar recovery efficiency, which can be on the order of over 90 percent of both hemicelluloses and cellulose sugars. The low temperatures and pressures employed allow the use of relatively low cost materials such as fiberglass tanks and piping. 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 [11].

2.6.2.3. Thermochemical process

There are two basic cellulosic ethanol production processes from lignocellulosic biomass that currently employ thermochemical reactions in their processes.

The first system is actually a hybrid thermochemical and biological system. Biomass materials are first thermochemically gasified and the synthesis gas (a mixture of hydrogen and carbon dioxide) bubbled through specially designed Fermenters. A microorganism that is capable of converting the synthesis gas is introduced into the Fermenter under specific process condition to cause fermentation to ethanol.

The second thermochemical cellulosic ethanol production process does not use any microorganisms. In this process, biomass materials are first thermochemically gasified and the synthesis gas passes through a reactor containing catalysts, which cause the gas to be converted into ethanol. An intensive effort was made to develop these processes for biofuels. Numerous efforts have been made since then to develop commercially viable thermochemical to ethanol processes.

Ethanol yields up to 50 percent have been obtained using synthesis gas to ethanol processes. Some processes that first produce methanol and then use catalytic shifts to produce ethanol have obtained ethanol yields in the range of 80 percent. Unfortunately, like the other processes, finding a cost effective all thermochemical process has been difficult [62].

2.6.3. Simultaneous Saccharification and fermentation (SSF)

SSF is thought to be the best process for enzymatic conversion of lignocellulose to cellulosic ethanol. The simultaneous Saccharification and fermentation process combines enzymatic hydrolysis of lignocellulose with simultaneous fermentation of its main derived reducing sugar (glucose and xylose) to ethanol. In SSF, enzymatic lignocellulose hydrolysis and reducing sugars fermentation to ethanol by yeast proceed simultaneously within one vessel. Compared with Saccharification in the absence of yeast, SSF using Trichoderma cellulose and *Saccharomyces cerevisiae* enhanced lignocellulose hydrolysis rates by 13 to 30 percent [10]. The optimum temperature for SSF was 35^{o}C. The requirement for βG in SSF was lower than for Saccharification. This is a very promising way of producing ethanol due to its ability to improve hydrolysis rates, yields and product concentration compared to SHF.

2.6.4. Fermentation

The fermenting of the biomass is conducted under standard fermenting conditions and will utilize all the major biomass. Yeast is the most commonly used microorganism in fermentation processes. Yeasts are minute, often unicellular fungi. The yeasts used are typically brewers' yeasts. Examples of yeast capable of fermenting the decaying biomass include, but are not limited to, *Saccharomyces cerevisiae and Saccharomyces uvarum*. Non-*Saccharomyces* yeasts, also known as non-conventional yeasts, are also used to make a number of commercial products. Some examples of non-conventional yeasts include *Kuyberomyces lactis, Yarrowia lipolytica, Hansenula polymorpha and Pichia pastoris* [11].

The fermentation reaction is caused by yeast or bacteria which feed on simple sugars. The reducing sugar produced from the hydrolysis described above is fermented with yeast to produce ethanol. Carbon dioxide is also produced as the reducing sugar is consumed. The simplified chemical reaction equation is;

$$3C_5H_{10}O_5 \xrightarrow{\text{Yeast}} 5C_2H_5OH + 5CO_2 \quad , \Delta H = -75\ kJ/mol \qquad (2.5)$$

$$C_6H_{12}O_6 \xrightarrow{\text{Yeast}} 2C_2H_5OH + 2CO_2 \quad , \Delta H = -84.4\ kJ/mol \qquad (2.6)$$

2.6.5. Distillation

Distillation is one of the purification steps. Distillation is the method used to separate two liquids based on their different boiling points. However, to achieve high purification, several distillations are required. This is because all materials have intermolecular interactions with each other and two materials will co-distill during distillation. This means that proportion between two materials, in this case ethanol and water can be changed and still, there are two materials in layers, the liquid and the vapor layers [63].

Whatever method of preparation is used, the ethanol is initially obtained in a mixture with water. The ethanol is then extracted from this solution by fractional distillation. Although the boiling point of ethanol (78.3^{o}C) is significantly lower than the boiling point of water (100^{o}C) these materials can't be separated completely by distillation. Instead, an azoetrope mixture (i.e. a mixture of 95% ethanol and 5% water) is obtained and the boiling point of the azoetrope is 78.15^{o}C. In a distillation, the most volatile material (i.e. the material that has the lowest boiling point) is the first material to distill from the distillation flask and this material is the azoetrope of 95% ethanol which has the lowest boiling point. If an efficient fractionating column is used,

95% alcohol could be obtained first and then a small intermediate fraction of lower concentration and then water. But no matter how efficient the fractionating column used, 95% alcohol can't be further concentrated by distillation because the vapor has exactly the same composition as the liquid; towards distillation, then 95% alcohol behaves exactly like a pure compound [64].

2.6.6. Dehydration

After distillation, about 5% of water remains in ethanol. Especially, this water is a big problem for fuel ethanol because the presence of this amount of water enhances the molecular polarity of ethanol when it is mixed with gasoline. Consequently, they separate into two phases, ethanol phase and gasoline phase. It is easy to imagine that this in homogeneous fuel is not acceptable. Thus, dehydration can be another issue [63]. For the ethanol to be usable as a fuel, water must be removed. Most of the water is removed by distillation, but the purity is limited to 95 to 96 percent due to the formation of a low boiling water ethanol azoetrope. For blending with gasoline, purity of 99.5 to 99.9 percent is required, depending on temperature, to avoid separation. Currently, the most widely used purification method is a physical absorption process using molecular sieves and another method is azeotropic distillation.

2.6.6.1. Molecular sieves

There is a lower bound on the fraction of ethanol entering the molecular sieve. Adsorption takes place at 95°C. Heat exchanger heats the inlet stream from the mixer up to 95°C. The molecular sieve is a bed of zeolite that operates in semi continuous mode. The bed is saturated with water after a period of time and is then regenerated. Hence, there are usually two sieves being operated in parallel one being saturated with water while the other is being regenerated (or dehydrated) using air under vacuum. Heat exchanger heats air with an assumed relative humidity of 70 percent at 20 to 95°C. The air at the outlet of the dehydrating molecular sieves cooled down to 25°C in heat exchanger and this stream leaves this exchanger saturated with water at 25°C [65].

2.7. Factors limiting for ethanol yield

The factors limiting for ethanol yield includes:-

- **pH of the substrate**: if the pH of the substrate must be within acceptable limits of (4.8-5.3) Fermentor (enzymes) otherwise it will destroy the enzymes or reduce ethanol that can be fermented.
- **Temperature of the substrate**: an acceptable limit of substrate is between 25-35^{o}C for optimum ethanol yield [62].
- **Substrate concentration**: the amount of substrate to be fermented is proportional to the ethanol that can be fermented from it, if all other condition is maintained.
- **Fermentation type**: SHF or SSF.

2.8. The benefits of fuel ethanol

A major benefit of a well functioned biofuels system is that it eliminates and converts organic waste into useful and valuable products. One of the main benefits with bioethanol is the replacement of expensive fossil fuel. Some advantages are connected to waste removal, like improving clean conditions. Protection of soil, water, air and woody vegetation can be mentioned as environmental advantages. If the actual conditions are satisfactorily, the bioethanol technology can contribute to conservation and development [65].

The fact that a bioethanol production can be building and operated locally creates opportunities to decrease the waste solid collection volume and land disposal costs. Also, the technology has also a potential to create job opportunities locally for several thousands of people [65].

Over the last 150 years, human activities have caused a dramatic increase in the emission of a number of greenhouse gases such as carbon dioxide, which has led to changes in the equilibrium of the earth's atmosphere. Fuel ethanol is suggested as a sustainable fuel which can be produced from renewable resources and led to maintain or even reduce the level of greenhouse gases. Finally, the standard of living can be enhanced which directly contributes to social and economical development of the country [65].

3. MATERIALS AND METHODS

The experiments of production of cellulosic ethanol from wood sawdust were carried out in the research center and Chemical Reaction Engineering laboratory of School of Chemical and Bio Engineering, Addis Ababa Institute of Technology, Addis Ababa University, Ethiopia.

3.1. Materials use for the experiment

3.1.1. Chemicals required; all were of analytical grade, used without further purification.

- ✓ Wood sawdust (Cheeped):- the raw material that was used as a source of cellulosic ethanol.
- ✓ Sulfuric acid (98%H_2SO_4):- used as a pretreatment and hydrolysis of wood sawdust.
- ✓ Sodium hydroxide (18M NaOH):- used to adjust the pH of hydrolysates solutions.
- ✓ Distilled water (pH 7.0):- used to hydrolyze and wash the acid and lignin mixture to separate the reduced sugars solution.
- ✓ Yeast Extracts Agar:- used in media preparation.
- ✓ Peptone:- used in media preparation.
- ✓ Dextrose:- used in media preparation.
- ✓ Urea:- used in media preparation.
- ✓ Magnesium sulfite (Mg SO_4.7 H_2O):- used in media preparation.
- ✓ Yeast (*Saccharomyces cerevisiae*).
- ✓ Phenol:- used in the analysis of total reduced sugar content, were the major materials and chemicals used in this study.

3.1.2. Equipments required

- ✓ Plastic bags:- used to collect and transport samples to the laboratory for analysis.
- ✓ Sieves:- used to sieve the crushed sample to a particle size of 0.5 to 3.5mm.
- ✓ Weight balance (ADAM, PW 124):- used to weigh samples and different chemicals used for culture media preparation and analyses.
- ✓ Vessels (Beakers):- used to hold samples and additives for hydrolysis, fermentation, and distillation experiments.
- ✓ Vertical Autoclave (ADOLF WOLF, La-MCS-204):- primarily used for hydrolysis and sterilization purpose.
- ✓ Centrifuge (INNOVA, U-584):- used to separate the sugar solution from non soluble part.

- ✓ Incubator Shaker (EDISON, Nj.USA 400):- used to shake samples and its additives after hydrolysis and before fermentation as well as for culture media and fermentation process.
- ✓ Digital pH meter (Jenway model 3510):- used to measure the pH of the culture media and hydrolysates samples before fermentation step.
- ✓ UV spectrophotometer (Lamabda 950, UV/VIS Spectrometer):- used to analyze the total sugar content (mainly concentration of glucose and xylose) of hydrolysates solutions.
- ✓ Graduated cylinders of different volumes:- used for volume measurement.
- ✓ Alcoholmeter and Pycnometer:- used for the measurement of ethanol concentration.
- ✓ Thermostats:- used to control temperature of the sample under experiment (fermentation and distillation) isothermally at the set point.
- ✓ Fermenters (Conical flasks) and distillation set ups:- used to ferment and distill the samples.

3.2. Experimental procedures

The study was aimed at the optimization of acid hydrolysis conditions during the extraction of reduced sugar from wood sawdust for the production of cellulosic ethanol. Wood sawdust was collected from Family Furniture's in Addis Ababa, Ethiopia. It was collected in plastic bags and transported to the laboratory of research center and chemical reaction engineering for the analysis of total reducing sugar content and ethanol yield. The following methods were investigated. This section describes about the methodologies and approaches of how experiments were carried out during this research; it includes all steps and procedures of the experiment.

The followings were the basic steps for the production of cellulosic ethanol. These were:-

- ✓ Sample collection and preparation for analysis.
- ✓ A pretreatment phase (size reduction and sieving) to make the wood sawdust agreeable to acid hydrolysis process.
- ✓ Hydrolysis to breakdown the molecules of lignocelluloses content into simple reducing sugars.
- ✓ Fermentation of the resulting sugar solutions, followed by distillation to obtain and analysis of cellulosic ethanol yield.

3.2.1. Sample preparation and analysis procedure

The sample that was acquired had to be prepared and conditioned for pretreatment, hydrolysis, fermentation and distillation.

3.2.1.1. Size reduction and sieve analysis of the wood sawdust

The sample is dried in an oven at 50°C for 24 hours to remove all the moisture content present in it. This dried sawdust is subjected to size reduction with a grinder, at a sieve size of 3.5mm. The powdered form is used in hydrolysis. The sample was sieved using vibrating shaker (Retsch, AS200 digit) with set of sieves arranged in descending order of size, 3.15mm, 3mm, 2.15mm, 2mm, 1.85mm, 1.15mm, 1mm, 0.85mm and 0.5mm to obtain particular sizes of 1mm, 2mm and 3mm. This is used to investigate the effect of sawdust particle size on the reducing sugars yield.

(a) (b)

Figure 3.1: (a) grinding machine and (b) vibrating shaker with a set of sieve sizes

(a) (b) (c)

Figure 3.2: Sample prepared in different particle sizes (a) 1mm, (b) 2mm and (c) 3mm

3.2.1.2. Analysis of moisture content of the sawdust

The sieved sample was weighed and dried in an oven at 105ºC and the weight was measured every 2 hours. The procedure was repeated until a constant weight was obtained. The percentage moisture content in the sawdust was calculated using the following formula as describing below,

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

Where;

W_1= Original weight of the sample before drying

W_2= Weight of the sample after drying.

3.2.2. Pretreatment and acid hydrolysis procedures

The lignocellulosic molecules which are composed of long chains are broken down to simple reducing sugars, before they are fermented for ethanol production. Even though there are many types of acid hydrolysis types; dilute and concentrated acid hydrolysis is an easy and productive process. Each sample had to pass through five primary experiments that were in series to get the final result of cellulosic ethanol, that is; size reduction, pretreatment, hydrolysis, fermentation and distillation. The four parameters were applied during the hydrolysis process step of the

experiment. The hydrolysis process experiments for reduced sugar production and optimization were conducted in a completely randomized design using Design-Expert® 7 software.

50g of wood sawdust were used for each experiment and the hydrolysis process variables were particle size (1-3mm), hydrolysis acid concentration (3-7%), hydrolysis temperature (90-110^{o}C) and hydrolysis time (120-240 minutes) for optimization.

Table 3.1: Minimum and maximum values of acid hydrolysis process variables

Factors	Name	Type	Minimum	Maximum
A	Sawdust particle sizes (mm)	Numeric	1.00	3.00
B	Hydrolysis acid concentration (%)	Numeric	3.00	7.00
C	Hydrolysis temperature (^{o}C)	Numeric	90.00	110.00
D	Hydrolysis time (minutes)	Numeric	120.00	240.00

Table 3.2: Experimental design formulated for acid hydrolysis process variables

Run No	Actual value				Run No	Actual value			
	A	B	C	D		A	B	C	D
1	1.00	3.00	110.00	240.00	16	3.00	3.00	90.00	240.00
2	2.00	5.00	110.00	180.00	17	2.00	7.00	100.00	180.00
3	2.00	5.00	100.00	240.00	18	1.00	3.00	90.00	120.00
4	2.00	5.00	100.00	180.00	19	1.00	7.00	90.00	240.00
5	3.00	3.00	110.00	240.00	20	2.00	5.00	100.00	120.00
6	3.00	3.00	110.00	120.00	21	3.00	7.00	90.00	120.00
7	1.00	7.00	110.00	240.00	22	3.00	7.00	110.00	120.00
8	2.00	3.00	110.00	180.00	23	1.00	7.00	110.00	120.00
9	1.00	3.00	110.00	120.00	24	2.00	5.00	100.00	180.00
10	3.00	7.00	90.00	240.00	25	3.00	3.00	90.00	120.00
11	1.00	7.00	90.00	120.00	26	2.00	5.00	100.00	180.00
12	1.00	3.00	90.00	240.00	27	3.00	7.00	110.00	240.00
13	2.00	5.00	100.00	180.00	28	3.00	5.00	100.00	180.00
14	2.00	5.00	100.00	180.00	29	2.00	5.00	100.00	180.00
15	1.00	5.00	100.00	180.00	30	2.00	5.00	90.00	180.00

During this experiment, new processes were investigated, which has the advantage of dilute and concentrated acid hydrolysis for the optimization of both the 5-C and 6-C sugars hydrolysis process with the minimum degradation of byproducts and formation of inhibitors.

In the first pretreatment step, 50g of the prepared sample (with the particle size described above) sawdust was weighed with a digital balance and soaked in 500 mL (with 10% of sawdust) of three different acid concentrations (3, 5 and 7%) of sulfuric acid (with density of 1.84 g/mL) for 24 hours in the 1000 mL conical flasks at room temperature.

After that, in the hydrolysis process step, the mixture of previous sample was subjected to heating in the vertical autoclave reactor to different hydrolysis temperatures (90, 100 and 110^{o}C) and for different hydrolysis times (120, 180 and 240 minutes).

(a) (b)

Figure 3.3: (a) sample ready for hydrolysis and (b) hydrolysis in autoclave reactor heater

During the second hydrolysis process step, 30 samples were obtained from the different particle size (1mm, 2mm and 3mm) for different hydrolysis acid concentrations (3%, 5% and 7%) with different hydrolysis temperatures (90, 100 and 110^{o}C) and for different hydrolysis times (120,180 and 240 minutes). Then, the resulted solution was separated (reduced sugar solution) from the solid part of the hydrolysates by centrifugation (to remove the non fermentable lignin portion). The total reduced sugar content (mainly glucose and xylose) of the

obtained reducing sugar solutions were analyzed using the phenol sulfuric acid method. After separated, the solid part of the sample was washed with distilled water two times in order to extract all soluble reducing sugar content from the solid material. This is to ensure that as much of the sugar/acidic solutions were recovered as possible and to decrease the hazardous content of the solid waste to affect the environment.

(a) (b) (c)

Figure 3.4: (a) sample ready for centrifugal separation, (b) filtrate solution and (c) lignin waste

3.2.2.1. Analysis of total reduced sugar content

After hydrolysis the liquid fraction of the hydrolysates samples were filtered, collected and their reduced sugar content (mainly glucose and xylose) was determined. These sugars can be individually determined by high performance liquid chromatography and gas chromatography.

One of the most all-around, relatively simple and low cost for reduced sugar concentration estimation is the calorimetric method. Among the colorimetric methods for total reduced sugar content analysis, the phenol sulfuric acid method is the easiest, most common and reliable method and has been extensively used in a broad range of fields. Phenol sulfuric acid method depends on dehydration of hydrolyzed Saccharides to furfural derivatives during reaction with concentrated sulfuric acid (reagent grade, 96% H_2SO_4) [66].

The sugar content was determined by using UV spectrophotometer at 490nm wavelengths of glucose absorbance and the quantification was made from calibration curve using glucose as standard and calculation was performed by equation of the linear regression obtained from calibration curve. The standard procedure of this method was as follows. A 1mL aliquot of a sample solution was mixed with 1mL of 5% aqueous solution of phenol in a test tube. Subsequently, 5mL of concentrated sulfuric acid (96% H_2SO_4) was added rapidly to the mixture. The test tubes were allowed to keep for 10 minutes at room temperature and placed in a water bath for 20 minutes for color development. Then, light absorption at 490nm was recorded on a spectrophotometer. Blank solutions were prepared in the same way as above, except that the 1mL aliquot of a sample solution was replaced by distilled water [66].

3.2.2.1.1. Standard and reagent solution preparation

Stock glucose solution was made by dissolving 1.5g of glucose in 100mL of distilled water. Various dilutions of the stock glucose solutions were made separately by pipetting a known volume of the stock solution (1, 2, 3, 4 and 5mL) into a 100mL five volumetric flask and filling the volume with distilled water up to the mark (diluted to 100mL). The concentrations made were; 0.15, 0.30, 0.45, 0.60 and 0.75mg/mL.

To determine the calibration curve for standard glucose, 1mL of each of the standard solutions were pipetted out and taken into a separate five test tubes. Then, 1mL of 5% aqueous solution of phenol reagent and immediately 5mL of 96% sulfuric acid were added. The test tubes were allowed to stand for 10 minutes at room temperature, vortexed for 30 seconds and placed in a water bath for 20 minutes at 25 to 30^{o}C for color development. Blank solutions were prepared in the same way as above, except that the 1mL aliquot of a sample solution was replaced by distilled water. Then, light absorption at 490nm was recorded on a spectrophotometer. Then, the amount of total reduced sugar content present in the sample solution was calculated using the standard graph and expressed as gram reduced sugar equivalents per 50g of sample.

The overall calculations were;

Absorbance corresponds to 1mL of the test = X mg of reduced sugar

$$\textbf{100 mL of the sample solution contains} = \frac{X}{1mL} * \textbf{100mL of reduced sugar} \quad (3.2)$$

$$= \textbf{\% of total reduced sugar present}$$

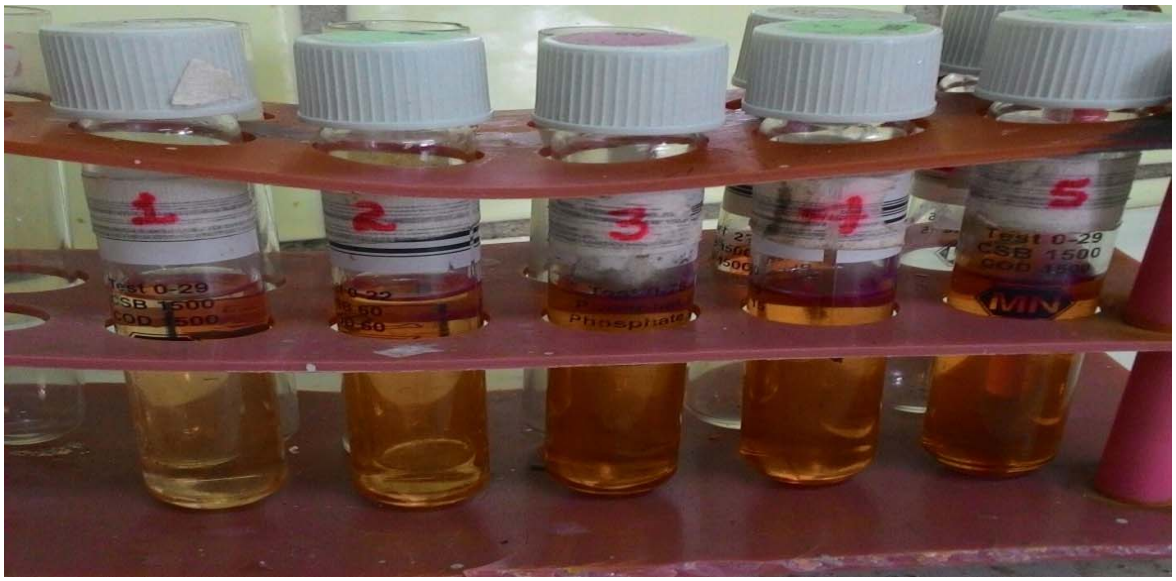


Figure 3.5: Standard glucose solution after addition of phenol and sulphuric acid

3.2.3. Neutralization

Before addition of any microorganism to the reduced sugar solutions, pH of these samples has to be adjusted. Otherwise the microorganism will die in hyper acidic or basic state. A pH of around 4.8 to 5.3 was maintained. This is the acceptable pH range for the growth of yeast (*Saccharomyces cerevisiae*). As samples are acid hydrolyzed, a highly basic solution is added to bring the pH in the range of 4.8 to 5.3. For this case, highly concentrated sodium hydroxide (18M NaOH) solution was prepared by dissolving sodium hydroxide pellets in distilled water which is exothermic. This sodium hydroxide solution was added drop wise to the prepared sample with constant stirring until the pH reaches to a range of 4.8 to 5.3. If suppose the pH is goes beyond 5.3, concentrated sulfuric acid (98% H_2SO_4) was added drop wise to maintain the pH in the required range.

The chemical reaction during the neutralization process between the reduced sugar solutions and concentrated sodium hydroxide to form water and salt which is not affected the fermentation process.

$$\mathbf{H_2SO_4 + 2NaOH \longrightarrow Na_2SO_4 + 2H_2O} \qquad \mathbf{(3.3)}$$

Figure 3.6: pH meter for neutralization of hydrolysates solutions

3.2.4. Sterilization

All the reactors and other measuring equipments that were used for pretreatment, hydrolysis, culture media preparation and fermentation purposes were sterilized using the vertical autoclave reactor heater at a temperature of 121^{o}C for 15 minutes.

Figure 3.7: Autoclave reactor heater used for sterilization

3.2.5. Fermentation

The aim of the experiment is to measure the cellulosic ethanol production by the fungus (*Saccharomyces cerevisiae*) using wood sawdust hydrolysates as energy and carbon source. After acid hydrolysis and filter, the reduced sugar solutions were prepared for fermentation process. The fermentation processes were carried out under anaerobic condition at a temperature of 30^{o}C, pH 4.8 to 5.3 with a 150 rpm stirring condition for 72 hours fermentation time using incubator shaker, is the effective medium of yeast growth [62, 66]. Before conducting the fermentation process, the culture media for the yeast growth was prepared.

3.2.5.1. Culture media preparation

The strain *Saccharomyces cerevisiae (saf-instant)* used in this work was obtained from local market. A fermentation broth of liquid medium which includes Yeast Extract Agar, Peptone and Dextrose (YEPD) containing; 1% Yeast Extract Agar, 2% Peptone and 2% Dextrose (glucose) was prepared in triplicates in 500mL sterilized conical flask. At first, the pH of the culture media was basic and adjusted to 4.8 to 5.3 using concentrated sulfuric acid (98% H_2SO_4) and stirred thoroughly, filled to the mark [67]. Each medium were inoculated under 0.5% dry yeast (*Saccharomyces cerevisiae*) [68]. The prepared broth were incubated at 30^{o}C for 24 hours and 150rpm using incubator shaker and used to inoculate the prepared samples.

For preparing 200mL of culture media, the following contents should be assorted.

- Distill water = 200mL
- Yeast Extract Agar = 2g
- Peptone water = 4g
- Dextrose = 4g
- Urea = 0.4g
- $MgSO_4.7H_2O$ = 0.2g

The above prepared medium were inoculated under dry yeast (*Saccharomyces cerevisiae*) of 1g were used. The conical flasks were properly covered with aluminum foil. The conical flask was then placed in an incubator shaker at a temperature of 30^{o}C for time of 24 hours and 150 rpm.

After the preparation of the culture media, the sample was conditioned to a temperature of 30^{o}C before initiation of the fermentation process step. All fermentation process experiments were

carried out at 30°C in an anaerobic condition. The prepared culture media with a 10% (v/v) concentration of the sample mix were subjected to shaking in the incubator shaker.

The fermentation process variables; yeast concentration, fermentation temperature and fermentation time were set to be 10% (with the proportion of 1:10 that was the prepared culture media and reduced sugar solutions by volume respectively), 30°C and 72 hours respectively [66]. After 72 hours of fermentation process, the fermented solutions were distilled and ethanol content was analyzed.

(a) (b)

Figure 3.8: (a) sample ready for fermentation process and (b) fermentation in incubator shaker

3.2.6. Distillation

Distillation is the last step during the production of cellulosic ethanol from wood sawdust. Distillation is the method used to separate two liquids based on their different boiling points. However, to achieve high purification, several distillations are required. In this experiment, separation was carried out in a rotary evaporator and all steps were carried out at a temperature of 78 to 85°C, for a time of 150 minutes.

Figure 3.9: Distillation for purification of ethanol

3.2.7. Analyses of ethanol content

The ethanol concentration of the samples was collected every 150 minutes intervals by rotary evaporator distillation of fermented solutions were measured through the following two methods.

1. Alcoholmeter:-

The Alcoholmeter measures the alcohol percentage (ethanol content) in alcohol water liquid (ethanol/water mixtures). The measuring was carried out in a flat bottomed jar of sufficient size, in which case the Alcoholmeter must float freely in the liquid.

It is of great importance that the Alcoholmeter is immersed in the liquid measured in an absolutely clean and dry condition, in order to achieve precise measuring results. In addition, the temperature of the liquid measured must correspond to the reference temperature of 20°C of the Alcoholmeter. Any significant deviation from this temperature will result in wrong indications of the Alcoholmeter which has to be offset by taking the correction values into account.

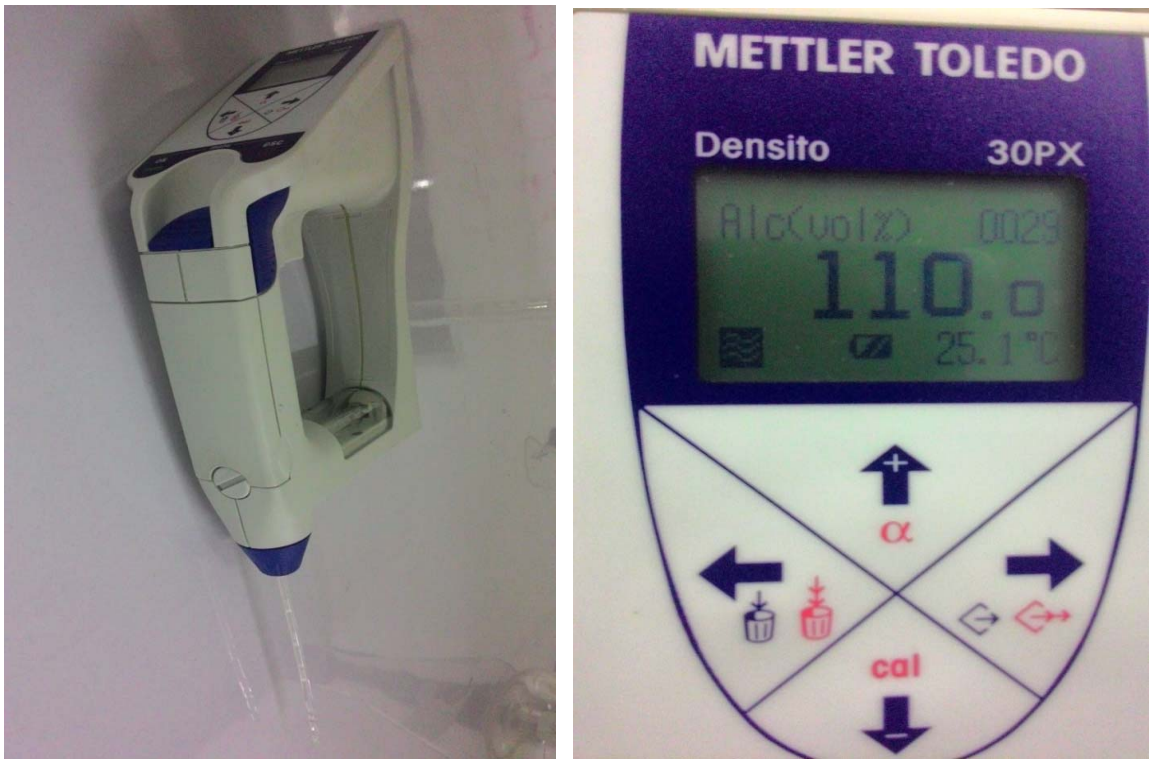


Figure 3.10: Alcoholmeter for ethanol content measurement

2. Pycnometer:-

The ethanol concentration of the distilled samples was measured following the technique of Geirwyr [69].

The specific gravity of the produced alcohol was determined and alcohol concentration was got from the relationship between the specific gravity and the proportion of ethanol in alcohol solution at 20^{o}C. The procedure of analysis was done as follows.

Weigh the Pycnometer (specific gravity of bottle) with stopper after cleaning, drying and note the weight as X_1 at 20^{o}C. Filled the Pycnometer with distilled water and take the weight of the water at 20^{o}C and note as X_3. Make the Pycnometer empty, clean, dry and then filled with sample (ethanol/water mixture) of the experimental result. Determine the weight of the sample at 20^{o}C and note as X_2. Calculate the net weight in grams of the alcoholic liquid in the Pycnometer by subtracting the weight of the empty specific gravity bottle or Pycnometer. Calculate the specific gravity of the sample according to the formula given below,

$$\textbf{Specific gravity of sample (sp.gr)} = \frac{X_2 - X_1}{X_3 - X_1} \quad \textbf{(3.4)}$$

Where;

X_1 = Weight (g) of empty Pycnometer

X_2 = Weight (g) of Pycnometer + sample

X_3 = Weight (g) of Pycnometer + water

Figure 3.11: Pycnometer for specific gravity measurements (50mL, 20^{o}C)

3.2.8. Analysis of FT-IR spectrum ethanol characterization

FT-IR stands for Fourier Transform Infrared, the preferred method of infrared spectroscopy. In infrared spectroscopy, infrared radiation is passed through a sample. Some of the infrared radiation is absorbed by the sample and some of it is passed through (transmitted). The resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample.

A Fourier Transform Infrared (FT-IR) Spectroscopy (Perkin Elmer and Shelton, Model 100) was used to characterize the presence of specific chemical groups of the ethanol and analyzed by FT-IR using transmittance mode. FT-IR spectrum was obtained in the range of wave number from 4000 to 400cm^{-1} during 64 scans, with 2cm^{-1} resolution. The FT-IR spectrum was normalized and major vibration bands were associated with the chemical groups of ethanol.

3.3. Data analysis procedure

Data analysis was performed by Design-Expert® 7 software using response surface method (RSM) to determine the effect of four operating parameters of the acid hydrolysis in the production of reduced sugars from wood sawdust for cellulosic ethanol production. These are sawdust particle size, hydrolysis acid concentration, hydrolysis temperature, and hydrolysis time with three levels. The response variable was total reduced sugar yield. Significance of the result was set from analysis of variance (ANOVA).

This design of the experiment helps us to differentiate the significance of the main and interaction factors. A mathematical model was developed to describe the effects of the main and interaction factors on the response variable.

4. RESULT AND DISCUSSION

4.1. Statistical analysis of the experimental results

4.1.1. Analysis of moisture content

The wood sawdust was collected, dried and sieved to three different particle sizes and by taking 55.75, 56.86 and 57.00g for 1mm, 2mm and 3mm respectively. The moisture content of the samples was obtained using equation 3.1 (Table 4.1).

Table 4.1: Moisture content analysis of sawdust

		Drying Time (hour)						Moisture Content (%)
	Particle Size (mm)	0	2	4	6	8	10	
Sample Weight (gm)	1	55.75	54.60	53.45	52.76	52.13	52.02	6.69
	2	56.86	53.79	53.62	52.35	52.05	52.05	8.46
	3	57.00	56.82	54.81	53.65	52.34	52.24	8.35

The moisture content of the sawdust sample with 55.75g of 1mm, 56.86g of 2mm and 57.00g of 3mm was 6.69, 8.46 and 8.35% respectively. Thus, the average moisture content of the three samples will be 7.83%; this is the acceptable moisture content of wood sawdust in the production of cellulosic ethanol using acid hydrolysis in the range of 6 to 13% [62].

4.1.2. Analysis of total reducing sugar content

In this study, the production of reduced sugar from wood sawdust was investigated through acid hydrolysis process. The wood sawdust through acid hydrolysis at different particle size, hydrolysis acid concentration, hydrolysis temperature, and hydrolysis time on the amount of total reduced sugar produced was investigated for optimization of process variables and the results are shown below.

The total reduced sugar content of hydrolysates samples were determined using phenol sulfuric acid method. From the phenol sulfuric acid method, the amount of reduced sugar content in the hydrolysates sample, when dehydrated by reaction with sulfuric acid they produce furfural derivatives. Further reaction between furfural derivatives and phenol develops detectible color

(Yellow-Orange).The reduced sugar content equivalent was calculated from the calibration curve of glucose standards.

The concentrations of unknown sugar content of samples were determined from the standard curve of glucose (Y = 0.263X + 0.001; R^2 = 0.998) (Figure 4.1). A correlation coefficient of 0.998 indicates that good linearity between the glucose concentrations and standards absorbance.

Table 4.2: Absorbance of standard solution

S. No	Concentrations (mg/mL)	Absorbance of standards
1	0.15	0.0371
2	0.30	0.0834
3	0.45	0.1224
4	0.60	0.1625
5	0.75	0.1954

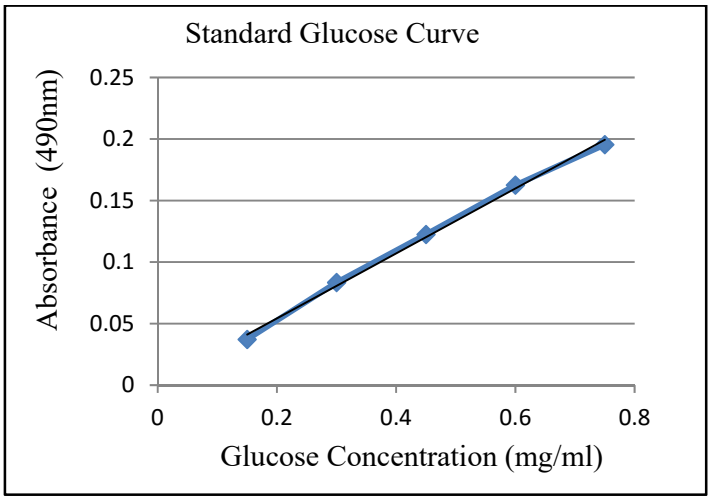


Figure 4.1: Calibration curve of glucose standard

Table 4.3: Absorbance of test samples

Run No	Absorbance of Test	Run No	Absorbance of Test	Run No	Absorbance of Test
1	0.128	11	0.153	21	0.119
2	0.175	12	0.158	22	0.116
3	0.174	13	0.192	23	0.136
4	0.189	14	0.183	24	0.179
5	0.125	15	0.150	25	0.126
6	0.140	16	0.139	26	0.186
7	0.131	17	0.169	27	0.134
8	0.169	18	0.109	28	0.171
9	0.156	19	0.127	29	0.181
10	0.109	20	0.159	30	0.168

The total reduced sugar content of hydrolysates samples were calculated using the regression equation obtained from the calibration curve of the glucose standard and using equation 3.2 (Table 4.4).

Table 4.4: Amount of reduced sugar content (%w/w) using Phenol-Sulfuric acid method

Run No	Actual factors of hydrolysis process variables				Amount of Sugar Content (%w/w)
	Particle Sizes (mm)	Hydrolysis Acid Concentration (%)	Hydrolysis Temperature (^oC)	Hydrolysis Time (minutes)	
1	1.00	3.00	110.00	240.00	48.22
2	2.00	5.00	110.00	180.00	66.11
3	2.00	5.00	100.00	240.00	65.74
4	2.00	5.00	100.00	180.00	71.69
5	3.00	3.00	110.00	240.00	47.07
6	3.00	3.00	110.00	120.00	52.83
7	1.00	7.00	110.00	240.00	49.61
8	2.00	3.00	110.00	180.00	63.74
9	1.00	3.00	110.00	120.00	58.86
10	3.00	7.00	90.00	240.00	42.03
11	1.00	7.00	90.00	120.00	57.65
12	1.00	3.00	90.00	240.00	59.83
13	2.00	5.00	100.00	180.00	72.51
14	2.00	5.00	100.00	180.00	69.05
15	1.00	5.00	100.00	180.00	56.76
16	3.00	3.00	90.00	240.00	52.38
17	2.00	7.00	100.00	180.00	63.78
18	1.00	3.00	90.00	120.00	41.24
19	1.00	7.00	90.00	240.00	48.04
20	2.00	5.00	100.00	120.00	60.11
21	3.00	7.00	90.00	120.00	45.03
22	3.00	7.00	110.00	120.00	43.52
23	1.00	7.00	110.00	120.00	51.34
24	2.00	5.00	100.00	180.00	67.67
25	3.00	3.00	90.00	120.00	47.32

Cont'd table 4.4

26	2.00	5.00	100.00	180.00	70.18
27	3.00	7.00	110.00	240.00	51.03
28	3.00	5.00	100.00	180.00	64.82
29	2.00	5.00	100.00	180.00	68.32
30	2.00	5.00	90.00	180.00	63.37

As shown in the above table 4.4, the total reduced sugar content produced in the hydrolysis process step using particle size (1, 2 and 3mm), hydrolysis acid concentrations (3, 5 and 7%), hydrolysis temperature (90,100 and 110^{o}C) and hydrolysis time (120,180 and 240 minutes).

Table 4.4 showed that, the highest total reduced sugar content 72.51% was achieved at 2mm particle size, 5% hydrolysis acid concentration, for 100^{o}C hydrolysis temperature and at hydrolysis time of 180 minutes which was run number 13 and the lowest reduced sugar content 41.24% was achieved at 1mm particle size, 3% hydrolysis acid concentration, for 90^{o}C hydrolysis temperature and at 120 minutes hydrolysis time which was run number 18.

Generally, to show the advantage of new pretreatment method for the production of reduced sugar for cellulosic ethanol, in compare to common dilute and concentrated acid hydrolysis, the data reported by other investigators are used in table 4.5 [62].

Table 4.5 shown that, the new pretreatment method not only has the mild operating conditions (100^{o}C, 1atm, 5% acid concentration and 180 minutes), but also has higher efficiency than the dilute acid process and lower duration time than the concentrated acid process.

Table 4.5: The comparison among different hydrolysis methods

Conditions	Concentrated Acid	Dilute Acid	This work
Hydrolysis Acid Concentration	(40 - 70)%	(1.0- 3.0)%	5.0%
Hydrolysis Pressure	1 atm	12 atm	1 atm
Hydrolysis Temperature	100^{o}C	(200-300)^{o}C	100^{o}C
Hydrolysis Time	(550-840) minute	(5-14) minute	180 minute
Efficiency	90%	50%	72.51%

In this study experimental design techniques were investigated to determine the effect of the particle size, hydrolysis acid concentration, hydrolysis temperature, and hydrolysis time on the efficiency of total reduced sugar yield. A total of 30 experiments were carried out for optimization purpose where the effect of each factor was analyzed by using lower and higher values from optimized conditions. The total reduced sugar yields obtained from experiments were used as a response variable for optimization.

The resulting data obtained from table 4.5, were analyzed using Design-Expert® 7 software to decide the effect of process variables of particle size, hydrolysis acid concentration, hydrolysis temperature, and hydrolysis time. The dependent variable used as a response variable was the total reduced sugar yield. All experiments were carried out in a randomized order to minimize the effect of unexpected variability in the observed response due to the extraneous factors.

Design Summary for the production of reduced sugar from sawdust with four factors and three levels. The design model of the experiments is quadratic polynomial, zero blocks and has six center points using Design-Expert® 7 software.

Table 4.6: Design summary of experimental designs

Design Summary of Design-Expert® 7 software	
Study type	Response Surface Methods
Initial design	Central Composite Design
Design model	Quadratic Polynomial
Runs	30
Blocks	No block

To determine whether or not the quadratic model is significant, it was crucial to perform the ANOVA (Table 4.7). The probability (P-values) values were used as a device to check the significance of each coefficient, which also showed the interaction strength of each variable. The smaller the P-values are, the bigger the significance of the corresponding coefficient.

Table 4.7: ANOVA for the Quadratic model

Source	Sum of square	Degree of freedom	Mean of square	F-value	P-value Prob>F
Model	2286.91	14	163.35	8.55	0.0011
A-Particle Size	39.11	1	39.11	1.33	0.0660
B-Hydro. Acid	23.23	1	23.23	0.79	0.0181
C-Hydro. Temp	8.96	1	8.96	0.30	0.0382
D-Hydro. Time	1.42	1	1.42	0.048	0.2188
AB	19.11	1	19.11	0.65	0.4327
AC	3.44	1	3.44	0.12	0.1371
AD	2.42	1	2.42	0.082	0.0783
BC	0.38	1	0.38	0.013	0.1309
BD	14.24	1	14.24	0.48	0.0971
CD	26.64	1	26.64	0.91	0.0563
A^2	130.62	1	130.62	4.44	0.0373
B^2	44.11	1	44.11	1.50	0.2395
C^2	25.66	1	25.66	0.87	0.0651
D^2	63.79	1	63.79	2.17	0.0615
Residual	441.10	15	29.41		
Lack of fit	422.83	10	42.28	0.27	0.473
Pure error	18.27	5	3.65		
Cor total	2728.02	29			

F-Value is a test for comparing model variance with residual (error) variance. If the variances are close to the same, the ratio will be close to one and it is less likely that any of the factors have a significant effect on the response. It is calculated by Model Mean of Square divided by Residual Mean of Square. Here the Model F-value of 8.55 implies the model is significant. There is only a 0.49% chance that a "Model F-Value" this large could occur due to personal error or disturbance. Probability values and/ or "Prob > F" values less than 0.0500 indicate model terms are significant. In this case B (hydrolysis acid concentration), C (hydrolysis temperature) and A^2 (Particle size) are significant model terms. Values greater than 0.1000 indicate the model terms are not significant.

The lack of fit F-value of 0.27 implies the lack of fit is not significant relative to the pure error. There is a 76.53% chance that a lack of fit F-value this large could occur due to noise. The non significant lack of fit is good. Co-efficient of Variation, the standard deviation expressed as a percentage of the mean; Predicted Residual Error Sum of Squares, which is a measure of how the model fits each point in the design; the R-Squared, measure of the amount of variation around the mean explained by the model; Adj R-Squared that is a measure of the amount of variation around the mean explained by the model; Pred R-Squared, a measure of the amount of variation in new data explained by the model and Adequate Precision, this is a signal to disturbance ratio due to random error, presented below in the table 4.8 are used to decide whether the model can be used or not.

Table 4.8: Model adequacy measures

Standard deviation	5.42	R-squared	0.8383
Mean	57.29	Adj R-squared	0.7874
C.V%	8.46	Pre R-squared	0.9055
PRESS	3196.89	Adeq Precision	16.538

The “Pred R-Squared” of 0.8383 is as close to the “Adj R-Squared” of 0.7874 in less than 0.2 difference as one might expect. “Adeq Precision” measures the signal to disturbance ratio due to random error. A ratio greater than 4 is desirable. Here ratio of 16.538 indicates an adequate signal. Therefore, this model can be used to navigate the design space. The regression coefficients and the corresponding 95% Confidence Interval (CI) High and Low were presented below in table 4.9. If zero was in the range High and Low 95% CI, 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 hydrolysis acid concentration, hydrolysis temperature and the second order of particle size highly significant effect in the reduced sugar yield.

Table 4.9: Regression coefficients and the corresponding 95% CI High and Low

Factors	Coefficient of Estimate	Standard Error	95% CI Low	95% CI High
Intercept	68.90	1.68	65.30	72.49
A-Particle Size	-1.47	1.28	-4.20	1.25
B-Hydro. Acid Con.	1.14	1.28	-3.86	-1.59
C-Hydro. Temp.	0.71	1.28	-2.02	-0.43

Cont'd table 4.9

D-Hydro. Time	0.28	1.28	-2.44	3.01
AB	-1.09	1.36	-3.98	1.80
AC	0.46	1.36	-2.43	3.35
AD	0.39	1.36	-2.50	3.28
BC	-0.15	1.36	-3.04	2.74
BD	-0.94	1.36	-3.83	1.95
CD	-1.29	1.36	-4.18	1.60
A^2	7.10	3.37	-14.28	-1.81
B^2	-4.13	3.37	-11.31	3.05
C^2	-3.15	3.37	-10.33	4.03
D^2	-4.96	3.37	-12.14	2.22

By the designed experimental data from table 4.9, the quadratic polynomial model to breakdown the sawdust into reducing sugars by acid hydrolysis was retreated as shown below.

- **Equation in terms of coded factors:**

Reducing Sugar Yield = +68.9 -1.47*A -1.14*B + 0.71*C + 0.28*D -1.09*AB + 0.46*AC
+0.39*AD - 0.15*BC - 0.94*BD -1.29*CD - 7.10*A^2 - 4.13*B^2
-3.15*C^2 - 4.96*D^2

- **Equation in terms of actual factors:-**

Reducing Sugar Yield = -390.2489+23.8583*Particle Size + 13.0276*Hydrolysis Acid Concentration+6.6971*Hydrolysis Temperature
+ 0.7423*Hydrolysis Time - 0.5465*(Particle Size*Hydrolysis Acid Concentration) + 0.0464* (Particle Size*Hydrolysis Temperature) +6.4760E-003*(Particle Size*Hydrolysis Time)
- 7.7156E-003*(Hydrolysis Acid Concentration*Hydrolysis Temperature) - 7.8630E-003*(Hydrolysis Acid Concentration *Hydrolysis Time) - 2.1505E-003*(Hydrolysis Temperature *Hydrolysis Time) -7.1003*(Particle Size)^2 - 1.0316 *(Hydrolysis Acid Concentration)^2 - 0.0314* (Hydrolysis Temperature)^2 - 1.3782E-003*(Hydrolysis Time)^2

The actual versus predicted values using model in the above equation (in terms of actual factors) are tabulated in table 4.10.

Table 4.10: Actual versus Model Predicted of total reduced sugar yield

Run No	Actual Value (%)	Predicted Value (%)	Residual	Leverage	Internally Studentized Residual	Externally Studentizd Residual	Influene on Fitted Value	Cooks Distance
1	45.03	43.83	1.2	0.659	0.379	0.368	0.511	0.018
2	48.22	51.02	-2.80	0.659	-0.883	-0.876	-1.216	0.100
3	57.65	50.66	6.99	0.659	2.206	2.592	3.60	0.626
4	48.04	51.14	-3.11	0659	-0.981	-0.979	-1.360	0.124
5	51.02	45.31	5.72	0.659	1.805	1.970	2.74	0.419
6	69.05	68.90	0.16	0.096	0.030	0.029	0.010	0.000
7	66.11	66.45	-0.35	0.485	-0.089	-0.086	-0.084	0.001
8	52.83	51.32	1.51	0.659	0.477	0.464	0.645	0.029
9	72.51	68.90	3.62	0.096	0.710	0.689	0.225	0.004
10	52.38	51.90	0.48	0.659	0.152	0.147	0.205	0.003
11	43.52	48.44	-4.92	0.659	-1.552	-1.637	-2.27	0.310
12	49.61	48.71	0.87	0.659	0.278	0.267	0.371	0.010
13	71.69	68.90	2.79	0.096	0.541	0.528	0.173	0.002
14	58.85	51.93	6.93	0.659	2.186	2.559	3.55	0.615
15	63.74	65.90	-2.17	0.485	-0.557	-0.544	-0.528	0.020
16	56.76	63.27	-6.51	0.485	-1.674	-1.793	-1.741	0.176
17	59.83	52.81	7.02	0.659	2.215	2.608	3.62	0.631
18	64.81	60.32	4.49	0.485	1.155	1.169	1.136	0.084
19	47.31	46.09	1.23	0.659	0.385	0.376	0.522	0.015
20	63.37	65.04	-1.67	0.485	-0.429	-0.405	0.012	0.000
21	67.67	68.90	-1.22	0.096	-0.237	-0.230	-0.075	0.000
22	68.32	68.90	-0.58	0.096	-0.112	-0.108	-0.035	0.000
23	47.07	51.96	-4.89	0.659	-1.543	-1.625	-2.26	0.306
24	51.34	53.42	-2.08	0.659	-0.656	-0.643	-0.893	0.055
25	70.18	68.90	1.29	0.096	0.250	0.242	0.079	0.000
26	63.78	63.63	0.15	0.485	0.039	0.037	0.036	0.000

Cont'd table 4.10

27	41.24	46.09	-7.31	0.659	-2.306	-2.777	-3.86	0.685
28	65.74	64.21	1.53	0.485	0.393	0.381	0.370	0.010
29	60.11	63.65	-3.54	0.485	-0.911	-0.906	-0.879	0.052
30	41.03	45.86	-4.83	0.659	-1.523	-1.601	-2.22	0.299
*Exceeds limits								

To see how well the model satisfies the assumptions of the ANOVA, the plots of residuals versus predicted (Table 4.10) were analyzed. Normal probability plot of the raw data used to check the assumption of normality when using t-test. In the ANOVA, it is usually more effective and straight forward to do this with the residuals. This shown below resembles a straight line. In visualizing the straight line, place more emphasis on the central values of the plot than on the extremes.

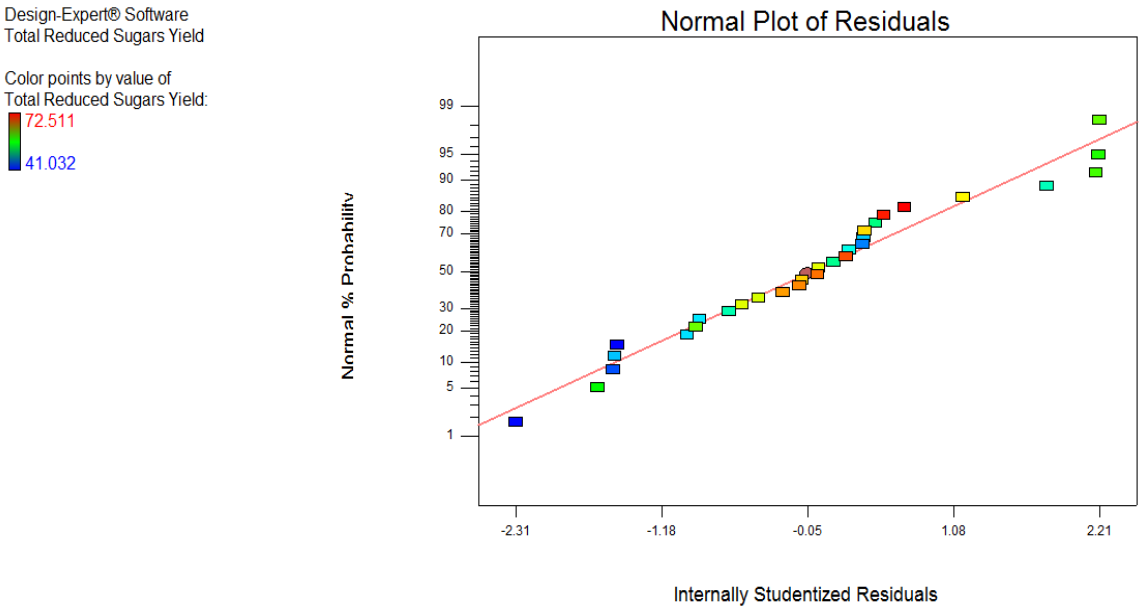


Figure 4.2: Normal plots of residuals

The normal probability plot (Figure 4.2) indicates the residuals following a normal distribution, in which case the points follow a straight line. This indicates the model satisfies the assumption of ANOVA, i.e. the error distribution are approximately normal.

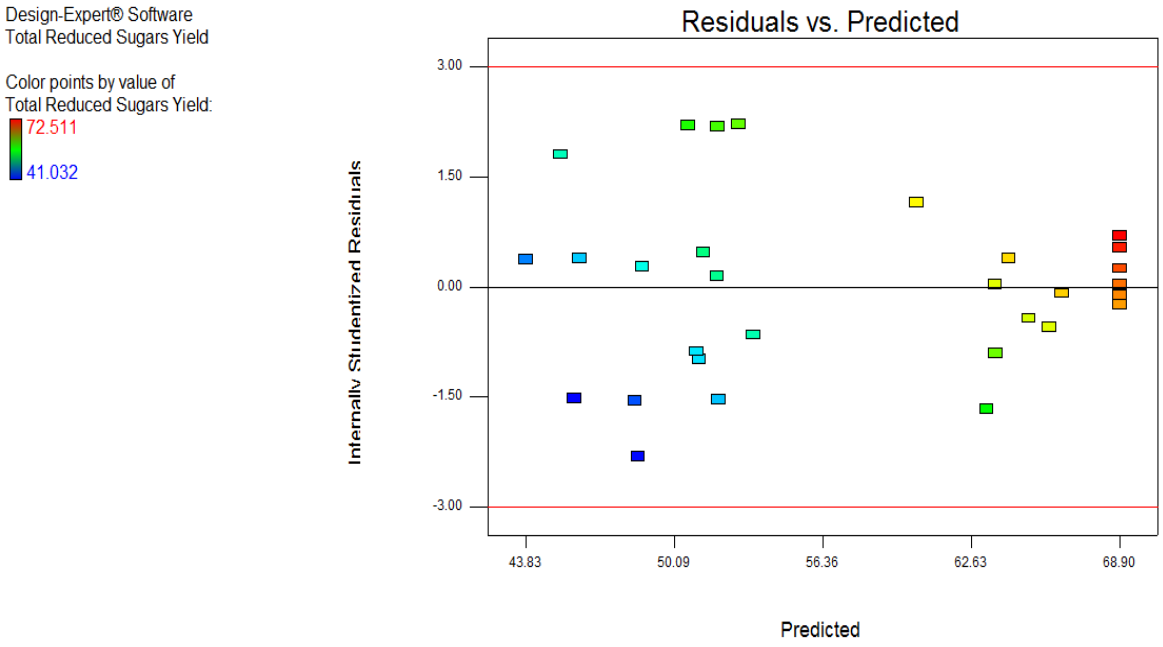


Figure 4.3: Residual versus predicted values

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.

4.1.3. Analysis of ethanol content

The production of cellulosic ethanol from wood sawdust consists of four parts; pretreatment (to remove lignin, reduce lignocelluloses crystallinity and increase the porosity of the materials), acid hydrolysis (convert the lignocelluloses content into simple sugars), fermentation (convert the fermentable sugar to ethanol) and distillation (to separate the ethanol-water mixture in order to purify the ethanol content) are the main steps.

After following the above key series procedures, the experimental outcomes of those particular results were measured using Alcoholmeter directly to know the ethanol yield and using Pycnometer for measuring their specific gravity (density) to know the ethanol yield using equation 3.4. The results are shown below table 4.11 (using Pycnometer) and table 4.12 (using Alcoholmeter).

Table 4.11: Ethanol yield using Pycnometer (50mL, 20°C)

Run No	Densities (g/mL)	Ethanol yield (%v/v)	Run No	Densities (g/mL)	Ethanol yield (%v/v)
1	0.9225	46.00	16	0.9318	41.70
2	0.9127	50.50	17	0.9156	49.31
3	0.9110	51.60	18	0.9465	33.20
4	0.9093	52.00	19	0.9402	37.72
5	0.9231	45.80	20	0.9212	46.93
6	0.9283	43.04	21	0.9272	43.48
7	0.9424	34.07	22	0.9404	37.81
8	0.9209	46.65	23	0.9342	40.57
9	0.9268	44.00	24	0.8809	64.22
10	0.9305	42.85	25	0.9313	41.99
11	0.9257	44.56	26	0.8924	59.76
12	0.9190	47.12	27	0.9180	48.07
13	0.8689	69.45	28	0.9146	49.78
14	0.8762	66.31	29	0.8894	60.33
15	0.9041	54.10	30	0.9175	48.64

Table 4.12: Ethanol yield using Alcoholmeter (20°C)

Run No	Ethanol yield (%v/v)	Run No	Ethanol yield (%v/v)	Run No	Ethanol yield (%v/v)
1	51.60	11	46.20	21	47.30
2	55.90	12	49.30	22	40.60
3	52.40	13	73.10	23	42.70
4	61.70	14	69.80	24	67.60
5	47.50	15	56.90	25	44.20
6	49.30	16	43.70	26	62.90
7	38.70	17	51.90	27	50.60
8	49.10	18	35.20	28	52.40
9	46.30	19	39.70	29	63.50
10	45.10	20	49.40	30	51.20

As shown in the above tables (Table 4.11, and 4.12) the concentration of ethanol (ethanol yield (%v/v)) are different since Pycnometer is measured using the sensitive balance to calculate rough estimation of ethanol content where as the Alcoholmeter is measure directly by inserting into the sample to read the ethanol content in volume percent. As shown in the above tables, the Alcoholmeter result was more accurately. Due to this reason, the Alcoholmeter measurement was more precise than that of Pycnometer calculations. Hence, the discussions were continued depend on the result of Alcoholmeter measurements.

Table 4.13: Result using Design-Expert® 7 software

Run No	Particle Size (mm)	Hydrolysis Acid Concentration (%)	Hydrolysis Temperature (°C)	Hydrolysis Time (minutes)	Ethanol Yield (%v/v)
1	1.00	3.00	110.00	240.00	51.60
2	2.00	5.00	110.00	180.00	55.90
3	2.00	5.00	100.00	240.00	52.40
4	2.00	5.00	100.00	180.00	61.70
5	3.00	3.00	110.00	240.00	47.50
6	3.00	3.00	110.00	120.00	49.30
7	1.00	7.00	110.00	240.00	38.70
8	2.00	3.00	110.00	180.00	49.10
9	1.00	3.00	110.00	120.00	46.30
10	3.00	7.00	90.00	240.00	45.10
11	1.00	7.00	90.00	120.00	46.20
12	1.00	3.00	90.00	240.00	49.30
13	2.00	5.00	100.00	180.00	73.10
14	2.00	5.00	100.00	180.00	69.80
15	1.00	5.00	100.00	180.00	56.90
16	3.00	3.00	90.00	240.00	43.70
17	2.00	7.00	100.00	180.00	51.90
18	1.00	3.00	90.00	120.00	35.20
19	1.00	7.00	90.00	240.00	39.70
20	2.00	5.00	100.00	120.00	49.40
21	3.00	7.00	90.00	120.00	47.30
22	3.00	7.00	110.00	120.00	40.60

Cont'd table 4.13

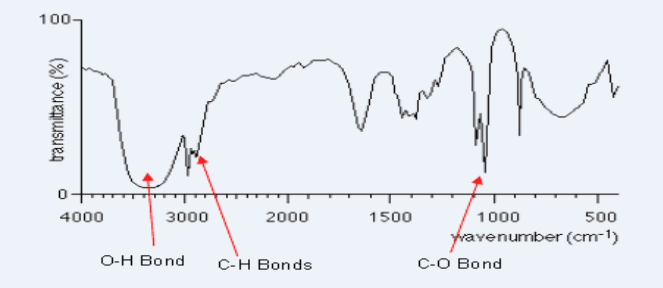
23	1.00	7.00	110.00	120.00	42.70
24	2.00	5.00	100.00	180.00	67.60
25	3.00	3.00	90.00	120.00	44.20
26	2.00	5.00	100.00	180.00	62.90
27	3.00	7.00	110.00	240.00	50.60
28	3.00	5.00	100.00	180.00	52.40
29	2.00	5.00	100.00	180.00	63.50
30	2.00	5.00	90.00	180.00	51.20

As showed in table 4.13, the maximum ethanol yields were achieved at run number 13, 14, 24, 29 and 26 descending order, whereas the minimum ethanol yields were obtained at run number 18, 7 and 19.

Table 4.13 shown that, the highest ethanol yield of 73.10% was achieved at 2mm sawdust particle size, 5% hydrolysis acid concentration, 100^{o}C hydrolysis temperatures and 180 minutes hydrolysis time from the highest sugar, whereas the lowest ethanol yield of 35.20% was achieved at 1mm, 3%, 90^{o}C and 120 minutes respectively. Generally, the higher reducing sugar yield gives, the maximum ethanol yield, and the lower reducing sugar yield gives the minimum ethanol yield at fixed fermentation and distillation process variables.

4.1.4. Analysis of FT-IR spectrum ethanol characterization

FT-IR test displays a number of functional groups indicating its complex nature. Its complex nature can be displayed as O-H, C-H and C-O stretching; Carboxylic acids, Alkanes and Aromatic hydrocarbons structure. The FT-IR spectrum of ethanol is shown below. The very broad strong band of the O-H stretch (3391), the C-H stretch (2981) and the C-O stretch (1102, 1055).



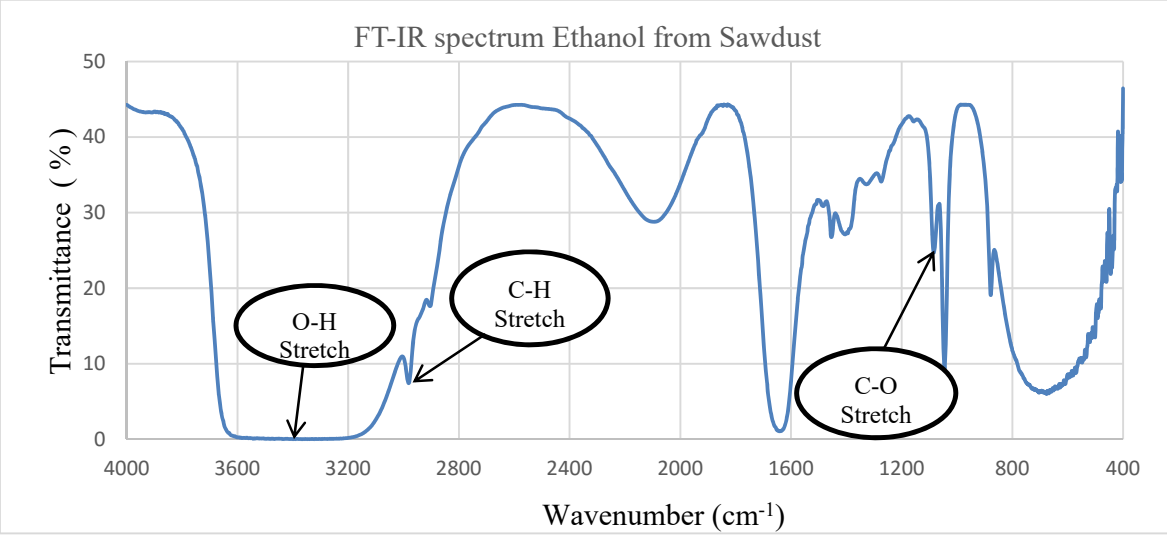


Figure 4.4: FT-IR spectrum ethanol characterization

4.2. Effect of hydrolysis process variables on reducing sugar conversion

The efficiency of acid hydrolysis process of lignocellulosic biomass is mainly affected by particle size, hydrolysis acid concentration, hydrolysis temperature, hydrolysis time and solid fraction to breakdown the lignocellulosic content into simple sugars or fermentable sugars. The total reduced sugar yield has a complex relationship with independent variables that contain first and second order polynomials and may have more than one maximum point.

The best way of expressing the effect of any parameter on the yield within the experimental space under investigation was to generate response surface plots of the equation. The three dimensional; interactions, contours, and response surfaces were plotted in figures 4.5-4.10 a, b, c and d, as a function of the interactions of any two of the factors by holding the other two at center point. In the interaction plots the black line represents low level of variables and the red line represents high level of variables.

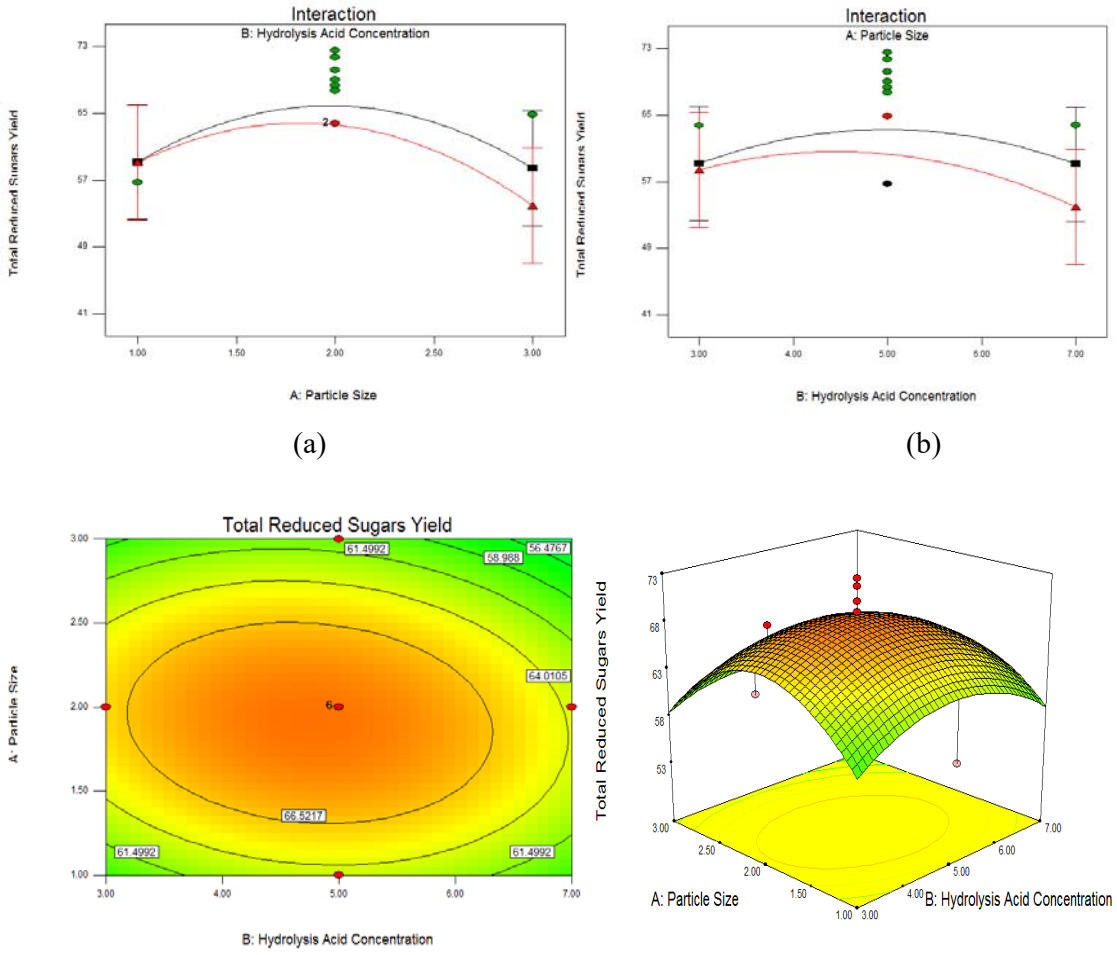


Figure 4.5: Interaction plot (a) and (b), Contour plot (c) and Response Surface plot (d) of total reduced sugar yield as a function of particle size and hydrolysis acid concentration

The effects of particle size and hydrolysis acid concentration on the yield of total reduced sugar, when hydrolysis temperature and hydrolysis time were selected at the center point, are shown in figure 4.5 (a) & (b). At the lower and higher levels of particle size and hydrolysis acid concentration, the total reduced sugar yield level decrease. At lower particle size and hydrolysis acid concentration the sawdust might not hydrolysis to simple monomeric sugars and at higher particle size and hydrolysis acid concentration there may be due to decomposition of the reduced sugars and the formation of some inhibitor such as furfural and HMF [70]. Hence, both particle size and hydrolysis acid concentration have strong relationship on the yield of reducing sugar.

Contour plot graph showing predicted response of reducing sugar yield as a function of particle size and hydrolysis acid concentration was shown in figure 4.5 (c). As particle size increases at

lower level of hydrolysis acid concentration and as hydrolysis acid concentration increases at low level of particle size gives a positive effect on the reduced sugar yield.

The response surface figure 4.5 (d), obtained from particle size and hydrolysis acid concentration was conical shape. Hence from the result, there were well defined optimums operating conditions. As particle size increases at lower level of hydrolysis acid concentration and as increase level of hydrolysis acid concentration and lower level of particle size gives a positive effect on the yield of reduced sugar. The response surface suggests that there were dominance interactions of these two factors.

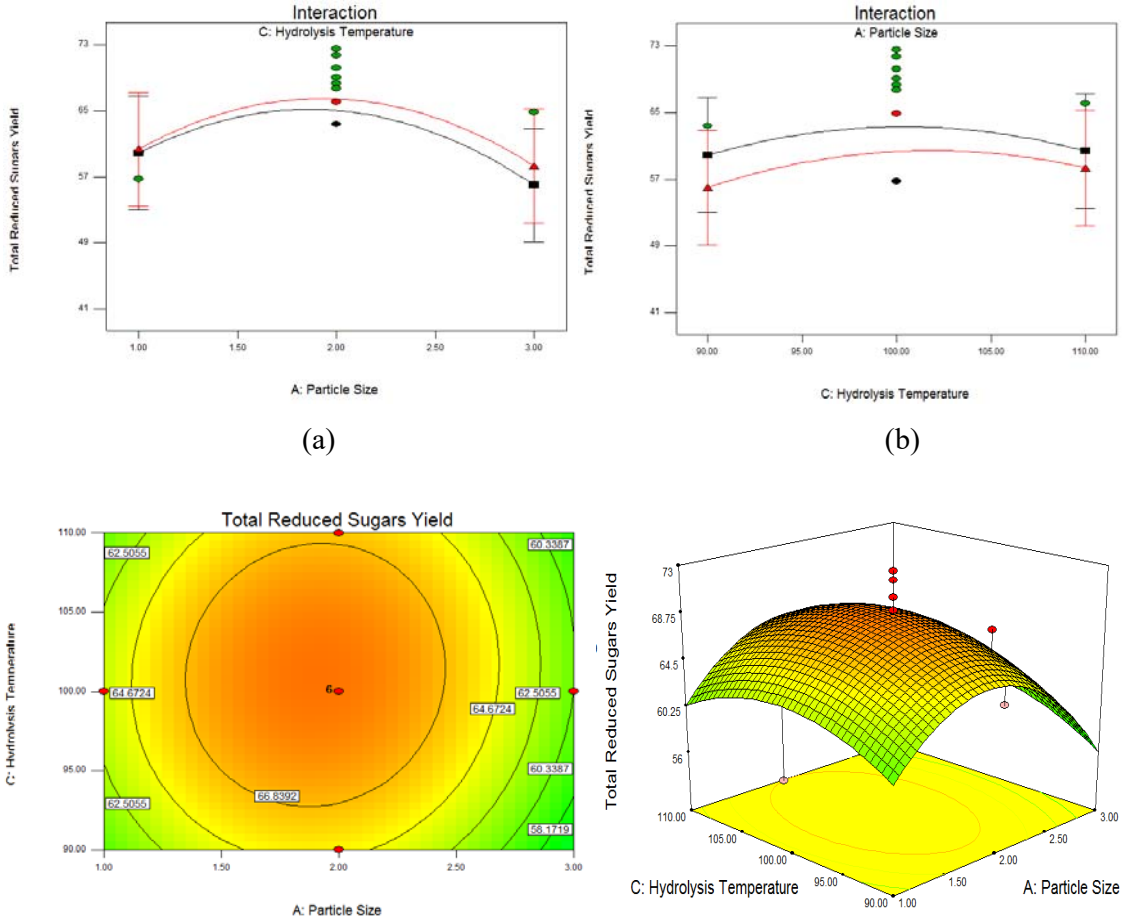


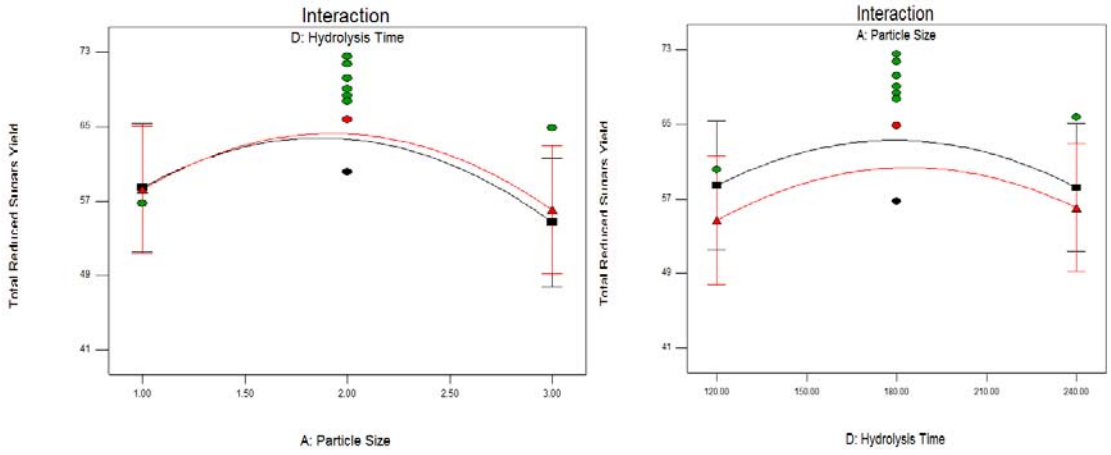
Figure 4.6: Interaction plot (a) and (b), Contour plot (c) and Response Surface plot (d) of total reduced sugar yield as a function of particle size and hydrolysis temperature

The effects of particle size and hydrolysis temperature on the yield of total reduced sugar, when hydrolysis acid concentration and hydrolysis time were selected at the center point, are shown in figure 4.6 (a) & (b). At the lower and higher levels of particle size and hydrolysis temperature,

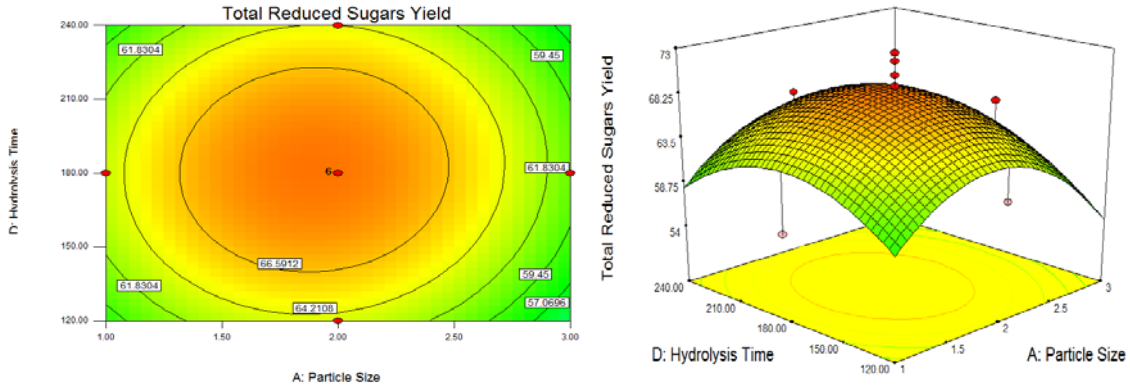
the total reduced sugar yield level decrease. At lower particle size and hydrolysis temperature the sawdust might not hydrolysis to simple monomeric sugars and at higher particle size and hydrolysis temperature the degradation of reduced sugar in to unwanted materials like HMF and furfural [71]. Hence, both particle size and hydrolysis temperature have strong relationship on the yield of fermentable sugar.

Contour plot graph showing predicted response of reduced sugar yield as a function of particle size and hydrolysis temperature was shown in figure 4.6 (c). As particle size increases at lower level of hydrolysis temperature and as hydrolysis temperature increases at low level of particle size gives a positive effect on the yield of reduced sugar.

The response surface figure 4.6 (d), obtained from particle size and hydrolysis temperature was conical shape. Hence from the result, there were well defined optimums operating conditions. As particle size increases at lower level of hydrolysis temperature and as increase level of hydrolysis temperature and lower level of particle size gives a positive effect on the yield of reduced sugar. The response surface suggests that there were dominance interactions of these factors.



(a) (b)



(c) (d)

Figure 4.7: Interaction plot (a) and (b), Contour plot (c) and Response Surface plot (d) of total reduced sugar yield as a function of particle size and hydrolysis time

The effects of particle size and hydrolysis time on the yield of total reduced sugar, when hydrolysis acid concentration and hydrolysis temperature were selected at the center point, are shown in figure 4.7 (a) & (b). At the lower and higher levels of particle size and hydrolysis time, the total reduced sugar yield level decrease. At lower particle size and hydrolysis time the sawdust might not hydrolysis to simple monomeric sugars and at higher particle size and hydrolysis time the sugars degraded to form inhibitors such as furfural and HMF [70]. Hence, both particle size and hydrolysis time have strong relationship for the yield of fermentable sugars production.

Contour plot graph showing predicted response of reduced sugar yield as a function of particle size and hydrolysis time was shown in figure 4.7 (c). As particle size increases at lower level of hydrolysis time and as hydrolysis time increases at low level of particle size gives a positive effect on the yield of reduced sugar.

The response surface figure 4.7 (d), obtained from particle size and hydrolysis time was conical shape. Hence from the result, there were well defined optimums operating conditions. As particle size increases at lower level of hydrolysis time and as increase level of hydrolysis time and lower level of particle size gives a positive effect on the yield of reduced sugar. The response surface suggests that there were dominance interactions of these factors.

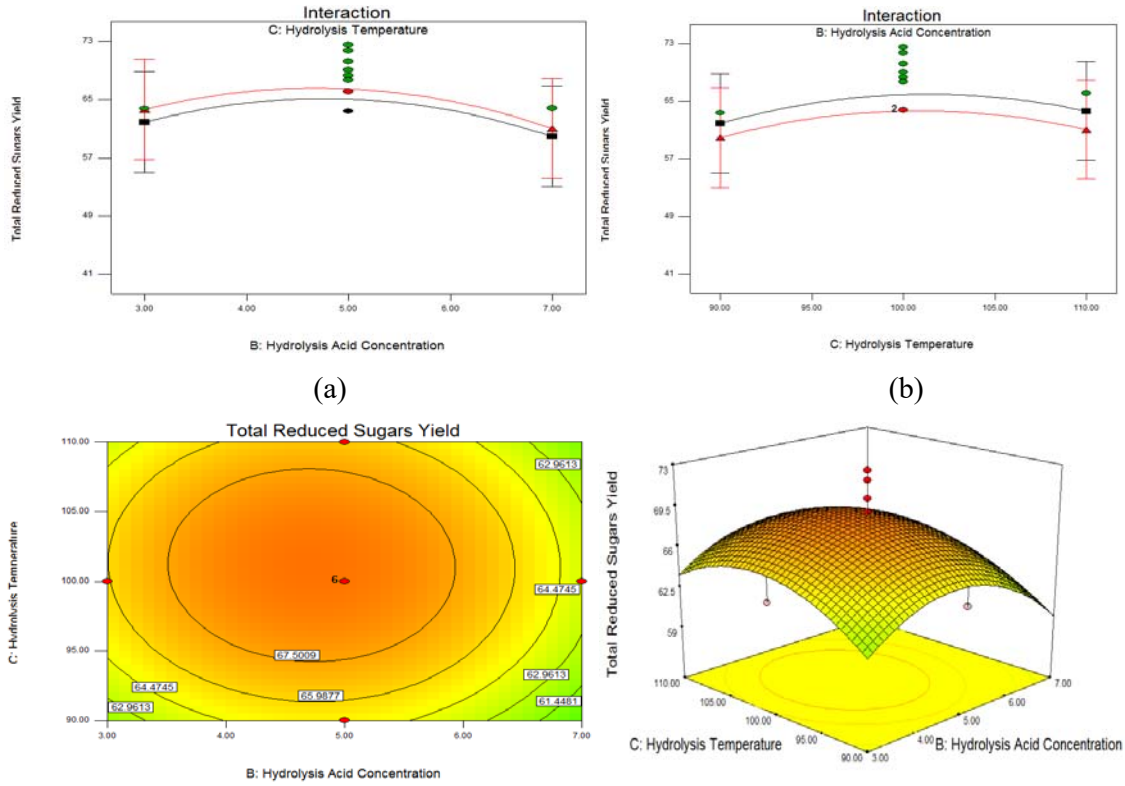


Figure 4.8: Interaction plot (a) and (b), Contour plot (c) and Response Surface plot (d) of total reduced sugar yield as a function of hydrolysis acid concentration and hydrolysis temperate

The effects of hydrolysis acid concentration and hydrolysis temperate on the yield of total reduced sugar, when particle size and hydrolysis time were selected at the center point, are shown in figure 4.8 (a) & (b). At the lower and higher levels of hydrolysis acid concentration and hydrolysis temperature the reduced sugar yields decreases. At lower hydrolysis acid concentration and hydrolysis temperature the sawdust might not hydrolysis to simple reduced sugar and at higher hydrolysis acid concentration and hydrolysis temperature, the reduced sugar may decompose to the formation of some inhibitor such as furfural and HMF. These substances are toxic substances for yeast and can inhibit the yeast growth [70]. Hence, both hydrolysis acid concentration and hydrolysis temperature have strong relationship for the yield of reduced sugar.

Contour plot graph showing predicted response of total reduced sugar yield as a function of hydrolysis acid concentration and hydrolysis temperature was shown in figure 4.8 (c). As hydrolysis acid concentration increases at lower level of hydrolysis temperature and as hydrolysis temperature increases at low level of hydrolysis acid concentration gives a positive effect on the yield of reduced sugar.

The response surface figure 4.8 (d), obtained from hydrolysis acid concentration and hydrolysis temperature was conical shape. Hence from the result, there were well defined optimums operating conditions. As hydrolysis acid concentration increases at lower level of hydrolysis temperature and as increase level of hydrolysis temperature and lower level of hydrolysis acid concentration gives a positive effect on the yield of reduced sugar. The response surface suggests that there were dominance interactions of these two factors.

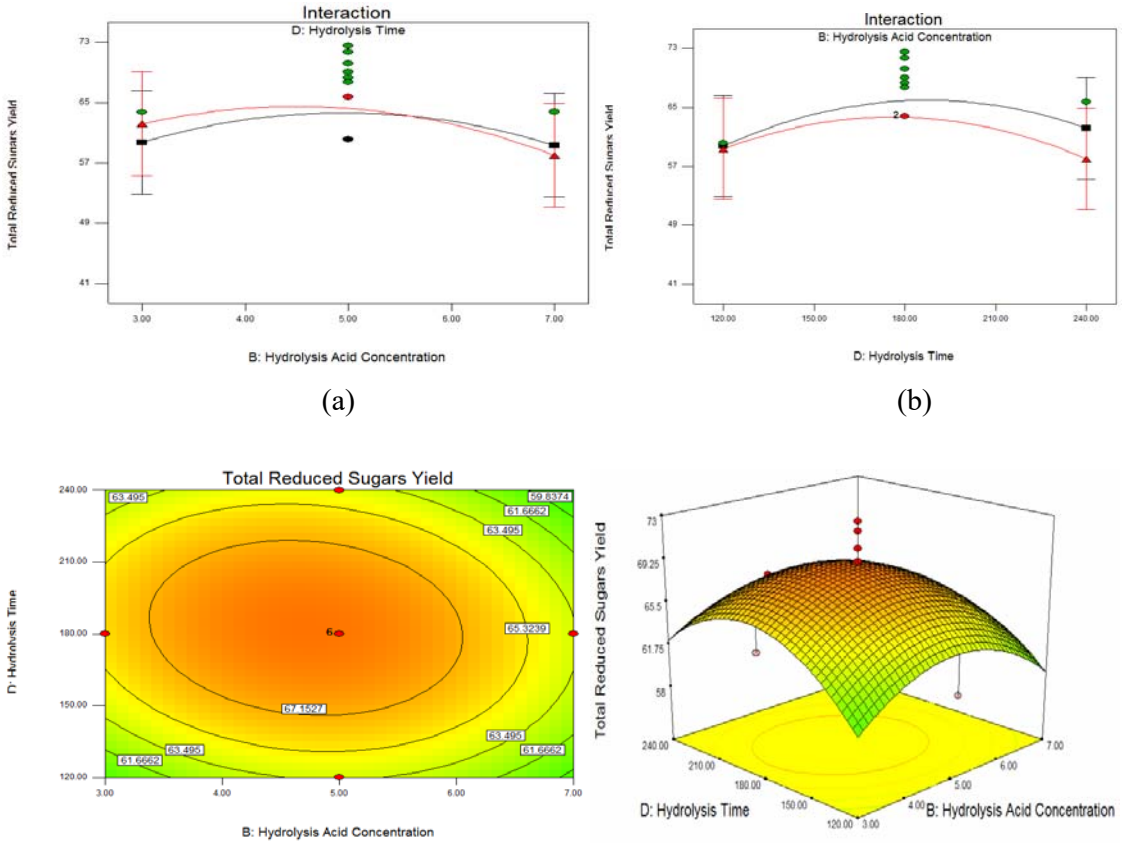


Figure 4.9: Interaction plot (a) and (b), Contour plot (c) and Response Surface plot (d) of total reduced sugar yield as a function of hydrolysis acid concentration and hydrolysis time

The effects of hydrolysis acid concentration and hydrolysis time on the yield of total reduced sugar, when particle size and hydrolysis temperature were selected at the center point, are shown in figure 4.9 (a) & (b). At the lower and higher levels of hydrolysis acid concentration and hydrolysis time, the total reduced sugar yield level decrease. At lower hydrolysis acid concentration and hydrolysis time the sawdust might not hydrolysis to simple monomeric sugars and at higher hydrolysis acid concentration and hydrolysis time the concentration of HMF and furfural became higher [70].

Hence, both acid concentration and time have strong relationship for the reduced sugar yield.

Contour plot graph showing predicted response of reduced sugar yield as a function of hydrolysis acid concentration and hydrolysis time was shown in figure 4.9 (c). As hydrolysis acid concentration increases at lower level of hydrolysis time and as hydrolysis time increases at low level of hydrolysis acid concentration gives a positive effect on the yield of reduced sugar.

The response surface figure 4.9 (d), obtained from hydrolysis acid concentration and hydrolysis time was conical shape. Hence from the result, there were well defined optimums operating conditions. As hydrolysis acid concentration increases at lower level of hydrolysis time and as increase level of hydrolysis time and lower level of hydrolysis acid concentration gives a positive effect on the yield of reduced sugar. The response surface suggests that there were dominance interactions of these factors.

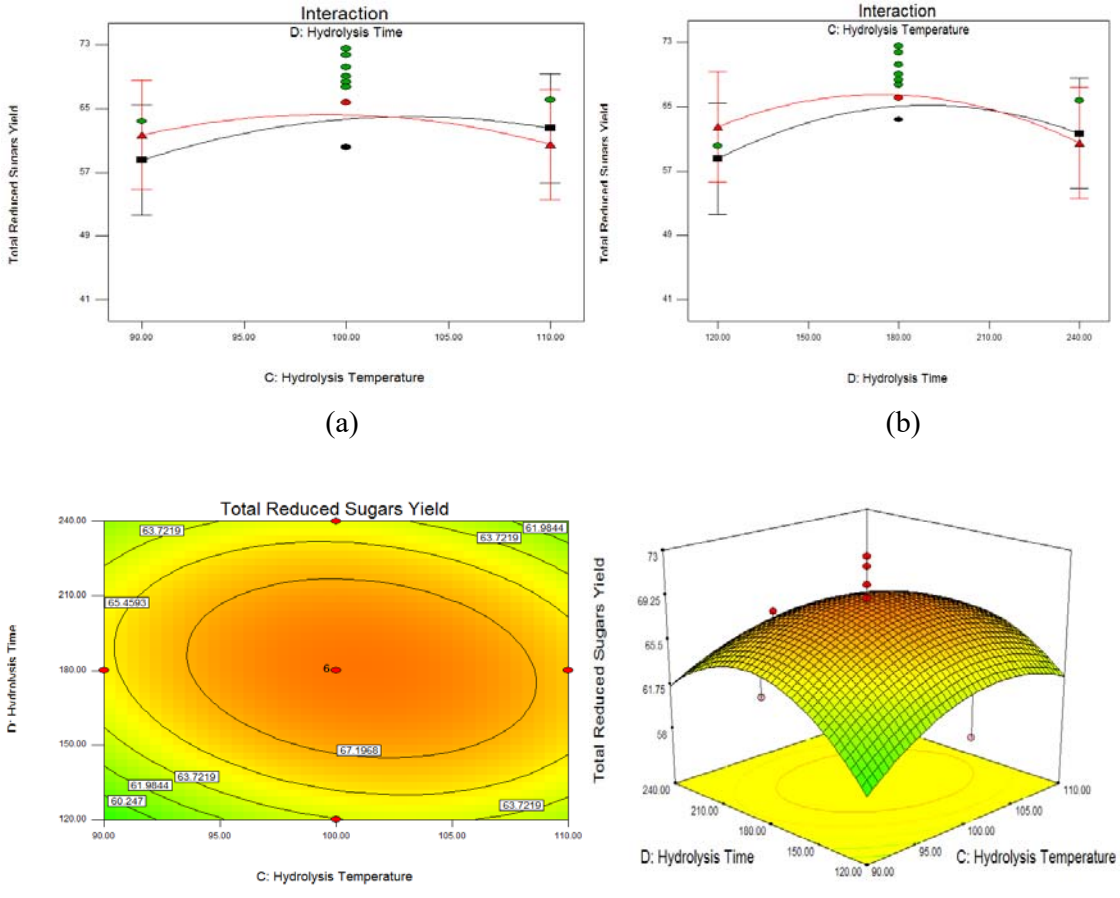


Figure 4.10: Interaction plot (a) and (b), Contour plot (c) and Response Surface plot (d) of total reduced sugar yield as a function of hydrolysis temperature and hydrolysis time

The effects of hydrolysis temperature and hydrolysis time on the total reduced sugar yield, when particle size and hydrolysis acid concentration were selected at the center point, are shown in figure 4.10 (a) and (b). When the levels of hydrolysis temperature and hydrolysis time increases, resulted in higher total reduced sugar yield. However, as shown that from the graph after some increments of hydrolysis temperature, the yield of total reduced sugar became decreases. This is due to degradation of reduced sugar in to unwanted materials like HMF and furfural that are toxic for *Saccharomyces cerevisiae* in the fermentation process [70, 71 & 72]. Similarly, at higher hydrolysis time the yield of total reduced sugar decrease. This may be due to the longer residence time, which makes the reduced sugar degraded to form inhibitors such as furfural and HMF [70].

From the contour plot graph showing predicted response of total reduced sugar yield as a function of hydrolysis temperature and hydrolysis time was shown in figure 4.10 (c). As hydrolysis temperature increases at lower level hydrolysis time gives positive effect on the yield of total reduced sugar and it decrease when the hydrolysis temperature and hydrolysis time became higher and higher.

The response surface figure 4.10 (d), obtained from hydrolysis temperature and hydrolysis time was conical shape. It suggests that there were well defined optimum operating conditions. The response optimized value for the production of total reduced sugar was based on both in hydrolysis temperature and hydrolysis time.

4.3. Optimization of hydrolysis process variables

Optimization is the act of obtaining the best result under given circumstances. In design, construction and maintenance of any engineering system, process engineers have to take many technological and managerial decisions at several stages. The ultimate goal of all such decisions is either to minimize the effort required or maximize the benefit desired. Since the effort required or the benefit desired in any practical situation can be expressed as a function of certain decision variables, optimization can be defined as the process of finding the conditions that give the maximum or minimum value of a function. The optimizations of hydrolysis process variables criteria for reducing sugars production from sawdust using acid hydrolysis are summarized below.

Table 4.14: Optimization criteria for optimum total reduced sugar yield

Parameters	Purpose	Minimum	Maximum
Particle Size (mm)	Minimize	1	3
Hydrolysis Acid Concentration (%)	Minimize	3	7
Hydrolysis Temperature (°C)	Minimize	90	110
Hydrolysis Time (minutes)	Minimize	120	240
Total reduced sugar yield (%)	Maximize	41.24	72.51

The optimum possible solutions in hydrolysis of different process variables for the total reduced sugar production and the corresponding contours and response surfaces plot are presented in table 4.15 and figures 4.11 and 4.12 respectively.

Table 4.15: Optimum possible solutions

Se. No	Particle Size (mm)	Hydrolysis Acid Concentration (%)	Hydrolysis Temperature (°C)	Hydrolysis Time (minutes)	Sugar Yield (%)	Desira-bility
1	**1.72**	**4.21**	**102.46**	**156.60**	**67.56**	**1 (Selected)**
2	2.57	4.12	91.22	177.59	62.27	1.00
3	2.58	3.43	92.36	191.45	62.10	1.00
4	2.77	5.40	109.78	222.89	57.88	1.00
5	2.35	6.45	92.25	145.94	59.71	1.00
6	1.04	5.96	95.38	226.67	59.07	1.00
7	1.19	5.69	102.75	156.47	64.25	1.00

Cont'd table 4.15

8	1.43	4.87	92.69	146.69	63.61	1.00
9	1.12	5.47	91.06	232.24	58.72	1.00
10	2.50	4.43	100.88	234.43	63.06	1.00
11	1.73	3.32	109.28	129.97	61.27	1.00
12	2.69	5.63	98.11	197.78	62.95	1.00
13	2.10	6.25	109.40	162.72	64.15	1.00
14	2.97	3.36	104.60	170.92	60.11	1.00
15	2.00	3.60	90.96	223.21	63.31	1.00
16	1.39	5.05	92.95	157.31	64.24	1.00
17	2.25	4.39	101.08	142.54	65.35	1.00
18	1.49	5.34	97.99	224.00	64.82	1.00
19	2.91	3.12	107.04	183.85	59.40	1.00
20	2.38	3.95	93.82	128.57	60.07	1.00
21	1.21	6.10	101.36	230.77	60.02	1.00
22	2.43	3.52	109.54	154.18	63.12	1.00
23	2.76	6.30	99.41	191.11	60.47	1.00
24	1.03	6.91	93.04	200.46	57.53	1.00
25	2.49	6.64	109.46	175.44	60.42	1.00
26	1.69	6.84	107.35	209.76	61.00	1.00
27	1.44	3.86	95.22	210.68	64.78	1.00
28	2.36	3.90	99.43	128.18	62.38	1.00
29	2.73	4.82	91.00	208.64	60.45	1.00
30	2.99	5.18	101.56	121.73	55.28	1.00

As table 4.15 shown that, the optimum values of particle size, hydrolysis acid concentration, hydrolysis temperature, and hydrolysis time for maximum reducing sugar yield of 67.56% are; 1.72mm, 4.21%, 102.46^{o}C and 156.60 minutes respectively.

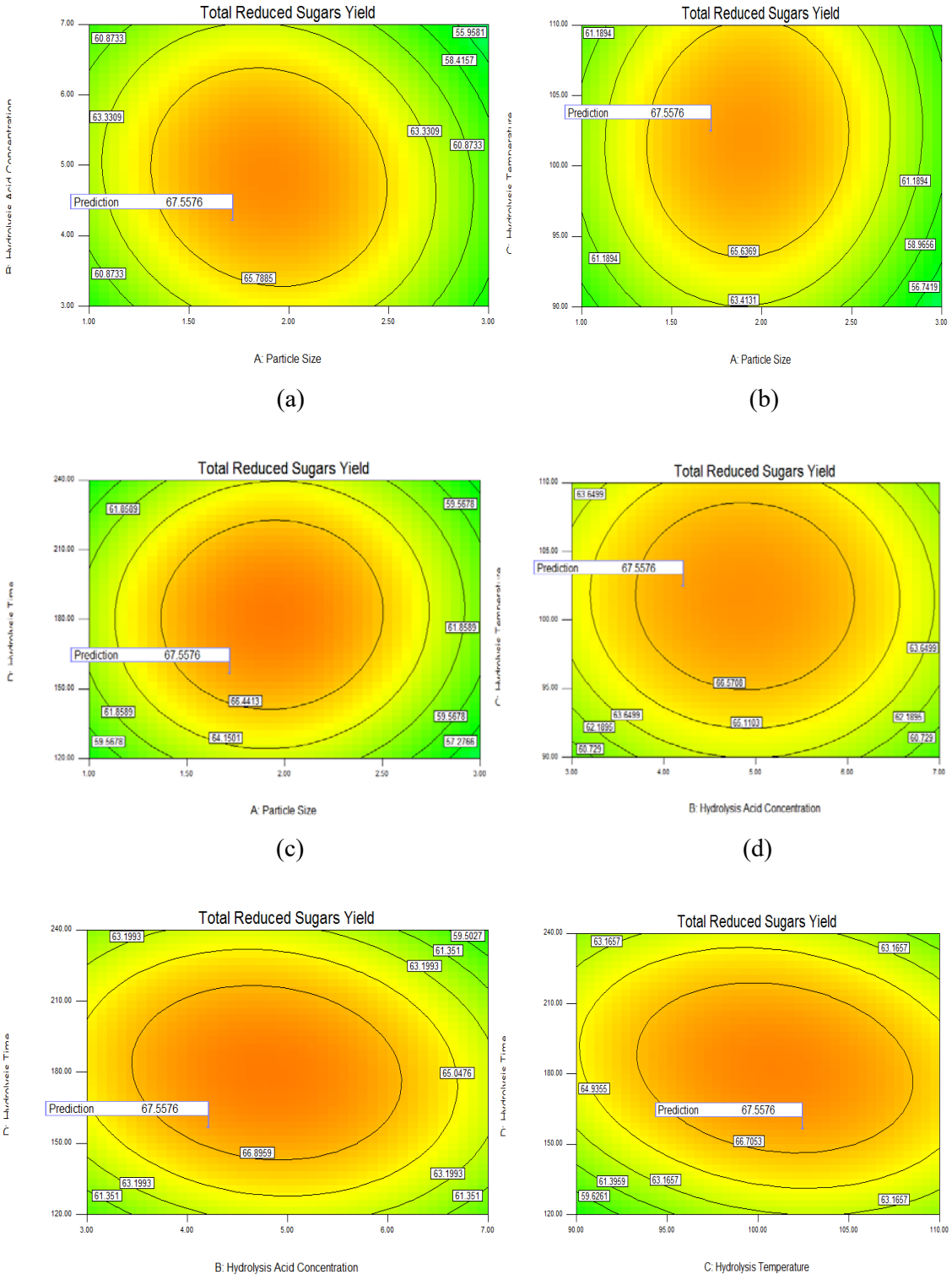


Figure 4.11: (a), (b), (c), (d), (e) and (f) Contours plot optimization in reducing sugar yield

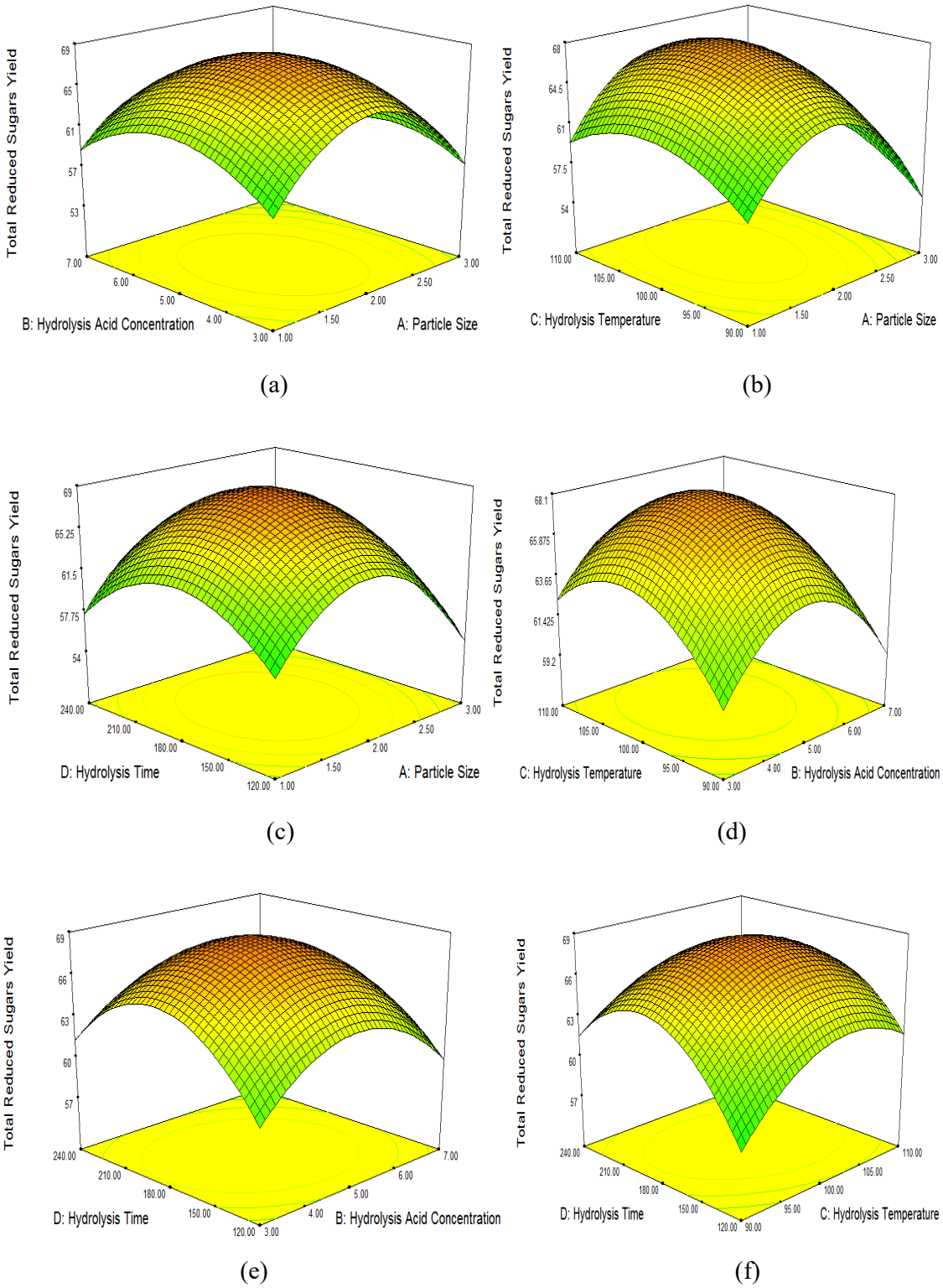


Figure 4.12: (a), (b), (c), (d), (e) and (f) Response Surface plot of possible optimum solutions

4.4. Model validation

As determined the result by response surface method using Design-Expert® 7 software, an experiment with particle size, hydrolysis acid concentration, hydrolysis temperature and hydrolysis time was conducted to carry out the effect of the design used. The experiment was carried out at the optimized conditions.

The above figures 4.11 and 4.12 (a), (b), (c), (d), (e) and (f) shown that, the total reduced sugar yield of average predicted value was 67.56% (w/w). As a result, the model was considered to be accurate and reliable for predicting the yield of reduced sugar from wood sawdust using acid hydrolysis. Based on the second order models, numerical optimizations were carried out to maximize the reduced sugar yield using the response surface method optimizer in Design-Expert® 7 software.

The optimal values of test factors were 1.72mm, 4.21%, 102.46°C and 156.60 minutes. The average total reduced sugar yield of 65.53% (w/w) obtained and was in good agreement with the predicted one. Therefore, the model is considered to be accurate and reliable for predicting the total reduced sugar yield.

5. PRELIMINARY ECONOMIC FEASIBILITY OF CELLULOSIC ETHANOL PRODUCTION FROM WOOD SAWDUST

5.1. Material and energy balances

Material quantities, as they pass through processing operations, can be described by material balances. Such balances are statements on the conservation of mass. Similarly, energy quantities can be described by energy balances, which are statements on the conservation of energy. If there is no accumulation, what goes into a process must come out. This is true for batch operation. It is equally true for continuous operation over any chosen time interval. Material and energy balances are very important in process industry.

5.1.1. Material balances

Material balances (also called mass balance) are essential of process design. A material balance taken over the complete process will determine the quantities of raw materials required and products produced. Balances over individual process units set the process stream flows and compositions. Material balances are also useful tools for the study of plant operation and troubleshooting. They can be used to check performance against design; to extend the often limited data available from the plant instrumentation; to check instrument calibrations; and to locate sources of material loss.

❖ **Basic data**:-

- One operation batch of 22 hours per day
- Production rate of $3*10^6$ Litter per year of ethanol (with the purity of 99.9%) throughout the plant operation of 310 days per year.

Anhydrous ethanol $\left(\frac{lit}{hr}\right) = \frac{total\ capacity}{22*310} = \frac{3*10^6 \text{lit}}{22\frac{\text{hr}}{\text{day}}*310\ \text{day}} = 440\frac{\text{lit}}{\text{hr}}$

$$\rho = \frac{\text{m}}{\text{v}},\ \text{Hence m} = \rho * \text{v}$$

Where;

ρ = density of Anhydrous ethanol $\left(0.789\frac{\text{kg}}{\text{lit}}\right)$

m = mass flow rate of Anhydrous ethanol

v = volumetric flow rate of Anhydrous ethanol

$$m = 440 \frac{lit}{hr} * 0.789 \frac{Kg}{lit} = \mathbf{347} \frac{\mathbf{kg}}{\mathbf{hr}}$$

$$m = 347 \frac{kg}{hr} * 22 \frac{hr}{day} * 310 \frac{day}{yr} * \frac{1\ ton}{1000\ kg}$$

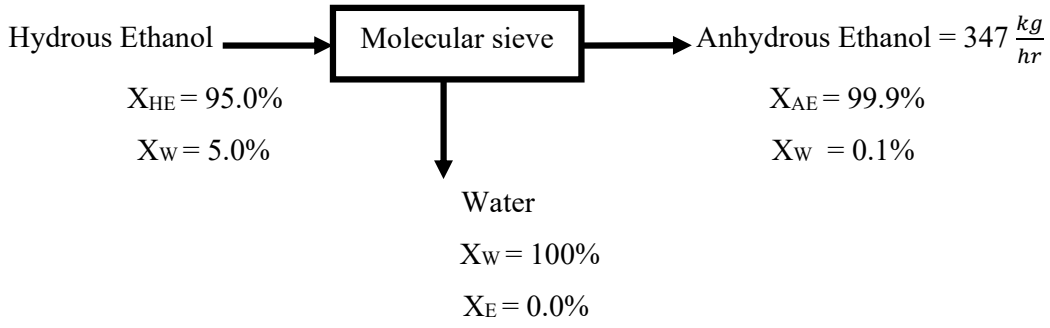
$$m = \mathbf{2,366.54} \frac{\mathbf{ton}}{\mathbf{yr}}$$

5.1.1.1. Detail material balance calculations

The detailed material balance calculations were been done from the starting raw material of the wood sawdust to the final product of cellulosic ethanol in each unit operations and processes.

1. Material balance on molecular sieve

It is the equipment which following the distillation column and bring the ethanol concentration or anhydrous ethanol from 95.0 to 99.9%.



Where;

X_{AE} = Percentage of Anhydrous Ethanol

X_{HE} = Percentage of Hydrous Ethanol

X_E = Percentage of Ethanol

X_W = Percentage of Water

Hydrous Ethanol's ($\frac{kg}{hr}$) enter into sieve = $\frac{\text{Anhydrous Ethanol} * 0.999}{\text{Hydrous Ethanol fraction}} = \frac{347 * 0.999 \frac{kg}{hr}}{0.95}$

$$HE = \mathbf{365.0} \frac{\mathbf{kg}}{\mathbf{hr}}$$

$$HE = 365.0 \frac{kg}{hr} * 22 \frac{hr}{day} * 310 \frac{day}{yr} * \frac{1\ ton}{1000\ kg}$$

$$HE = \mathbf{2,489.3} \frac{\mathbf{ton}}{\mathbf{yr}}$$

The water that is trapped by the molecular sieve is;

Mass flow rate of hydrous ethanol = Mass flow rate of water leave the molecular sieve

+ Mass flow rate of anhydrous ethanol leaves the sieve

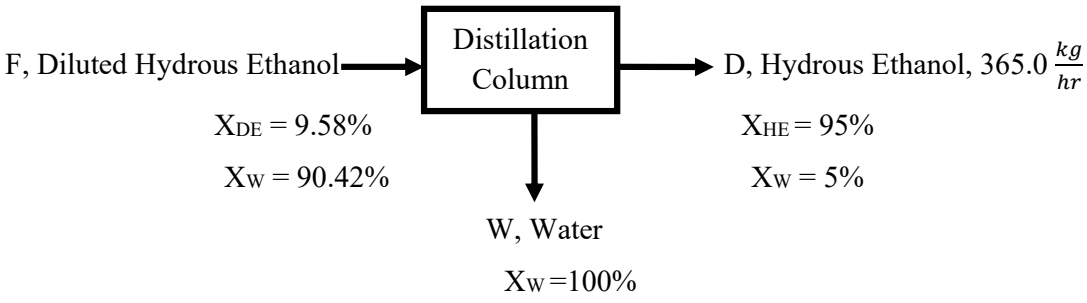
$m_{HE} = m_W + m_{AE}$

$m_W = m_{HE} - m_{AE} = 365 - 347 = \mathbf{18}\ \frac{\mathbf{kg}}{\mathbf{hr}}$

$m_W = \mathbf{122.76}\ \frac{\mathbf{ton}}{\mathbf{yr}}$

2. Material balance on distillation

It is the equipment used for the purification of ethanol from water-ethanol mixture. The mixture from fermentation enter in to the distillation with the concentration of 9.58% ethanol with water, then with the distillation column the purification of ethanol come to 95.0%.



Where;

X_{DE} = fraction of Diluted Hydrous Ethanol

X_{HE} = fraction of Hydrous Ethanol

X_W = fraction of Water

From the ethanol component balance;

$F*X_{DE} = W*X_E + D*X_{HE}$, Since $X_E = 0$

$F*X_{DE} = D*X_{HE} = 365*0.95 = \mathbf{346.75}\ \frac{\mathbf{kg}}{\mathbf{hr}}$

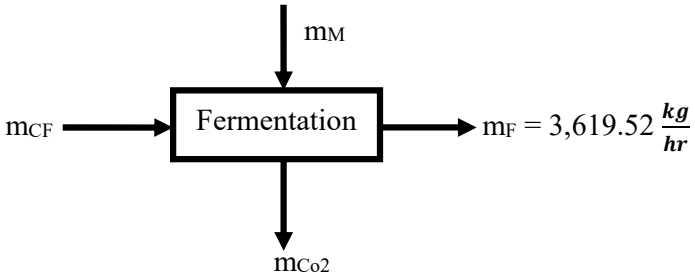
$F = \mathbf{3{,}619.52}\ \frac{\mathbf{kg}}{\mathbf{hr}} = \mathbf{24{,}685.13}\ \frac{\mathbf{ton}}{\mathbf{yr}}$

From which the amount of water as a bottom product is;

$W = F - D = 3619.52 - 365.0$

$W = \mathbf{3{,}254.52}\ \frac{\mathbf{kg}}{\mathbf{hr}} = \mathbf{22{,}195.83}\ \frac{\mathbf{ton}}{\mathbf{yr}}$

3. Material balance on fermentation



Where;

m_{CF} = mass flow rate of the centrifuge fluid

m_M = mass of media

m_F = mass of fermented mash.

m_{CO2} = mass of Co_2 released

From the decomposition of biological fermentation process;

$$\mathbf{C_6H_{12}O_6 \xrightarrow{Yeast} 2C_2H_5OH + 2CO_2} \quad \textbf{(5.1)}$$

$$\mathbf{3C_5H_{10}O_5 \xrightarrow{Yeast} 5C_2H_5OH + 5CO_2} \quad \textbf{(5.2)}$$

180 g/mol Glucose ⟶ 92 g/mol ethanol + 88 g/mol Co_2 for 100% conversion

450 g/mol Xylose ⟶ 229 g/mol ethanol + 221 g/mol Co_2 for 100% conversion.

But, 1 – 9% of glucose and xylose were not changed completely and take the value i.e. 90% efficiency.

$$\frac{m_{GF}}{450+180} = \frac{365.0}{229+92}$$

$$m_{GF} = \frac{365.0*630*0.90}{321} = \mathbf{644.72}\,\frac{\mathbf{kg}}{\mathbf{hr}}$$

$$m_{GF} = \mathbf{4,397.00}\,\frac{\mathbf{ton}}{\mathbf{yr}}$$

And the amount of carbon dioxide produced is;

$$\frac{644.72}{630} = \frac{m_{Co2}}{309}$$

$$m_{Co2} = \frac{644.72*309}{630} = \mathbf{316.22}\,\frac{\mathbf{kg}}{\mathbf{hr}}$$

$m_{Co2} = \mathbf{2,156.62}\,\frac{\mathbf{ton}}{\mathbf{yr}}$ of Carbon dioxide produced.

The mass of culture media was used 10% of the mass of filter mash, hence from the total mass balance;

$$m_M + m_{CF} = m_F + m_{Co2}$$

$$1.1m_{CF} = 3619.52 + 316.22 = 3{,}935.74\ \frac{kg}{hr}$$

$$m_{CF} = \mathbf{3{,}577.95}\ \frac{kg}{hr}$$

$$m_{CF} = \mathbf{24{,}401.60}\ \frac{ton}{yr}$$

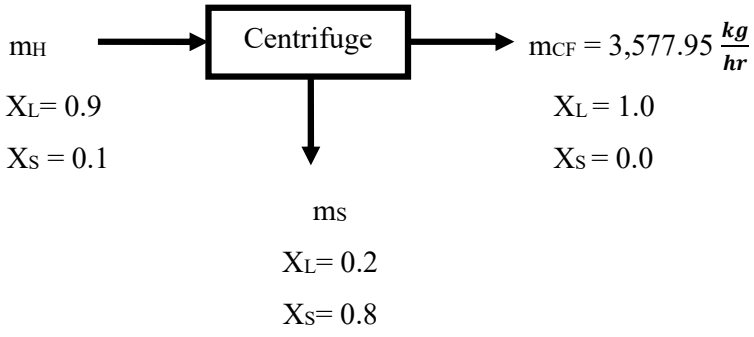
The amount of culture media required is;

$$m_M = 0.1 * m_{CF} = 0.1 * 3577.95 = \mathbf{357.80}\ \frac{kg}{hr}$$

$$m_M = \mathbf{2{,}440.16}\ \frac{ton}{yr}$$

4. Material balance on centrifugal separation

Centrifuge is used for separating insoluble solid from hydrolysates solutions. The moisture content of centrifuge solid is 20%.



Where;

m_H = mass flow rate of acid treated fluid

m_S = mass flow rate of bottom centrifuge solid.

From the solid component balance;

$$m_H * X_S = m_S * X_S$$

$$0.1m_H = 0.8m_S \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(a)}$$

From the liquid component balance;

$$m_H * X_L = m_{CF} * X_L + m_S * X_L$$

$$0.9m_H = m_{CF} + 0.2m_S$$

$$0.9m_H = 3577.95 + 0.2m_S \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(b)}$$

Substitute equation (a) in to equation (b) and solve for m_S and m_H

$$7.2m_S = 3577.95 + 0.2m_S$$

$$7m_S = 3577.95$$

$$m_S = \frac{3577.95}{7} = \mathbf{511.14}\,\frac{\mathbf{kg}}{\mathbf{hr}}$$

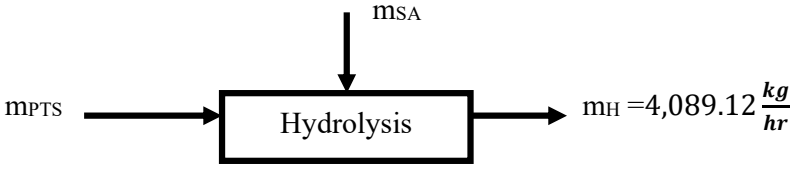
$$m_S = \mathbf{3,485.95\ ton/yr}$$

$$m_H = 8m_S = 8 * 511.14 = \mathbf{4,089.12}\,\frac{\mathbf{kg}}{\mathbf{hr}}$$

$$m_H = \mathbf{27{,}887.80}\,\frac{\boldsymbol{ton}}{\boldsymbol{yr}}$$

5. Mass balance on pretreatment (acid hydrolysis)

The pretreated sample was soaked and treated with 4.21% sulfuric acid is the optimum concentration to convert lignocelluloses content into reduced sugar and serve as catalyst (i.e. it has no any side reaction with lignocelluloses content).



Where;

m_{PTS} = Mass flow rate of sample (mixture of sawdust and water)

m_H = mass flow rate of acid treated fluid

m_{SA}= mass of acid used

The chemical reaction occurred in this reactor is;

$$\mathbf{C_6H_{10}O_5 + H_2O \xrightarrow{H_2SO_4} C_6H_{12}O_6} \qquad \mathbf{(5.3)}$$

$$\mathbf{C_5H_8O_4 + H_2O \xrightarrow{H_2SO_4} C_5H_{10}O_5} \qquad \mathbf{(5.4)}$$

162 g/mole Cellulose ⟶ 180 g/mole glucose for 100% conversion

132 g/mole Hemicelluloses ⟶ 150 g/mole xylose for 100% conversion

But, 1 – 8% of cellulose and hemicelluloses were not changed from literature and take the value i.e. 90% efficiency.

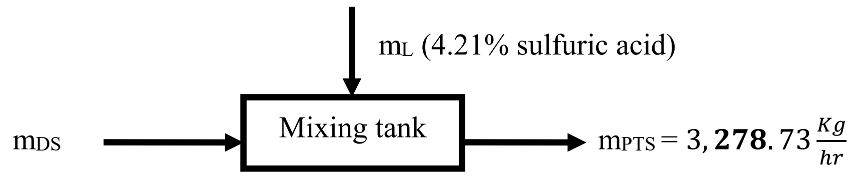
$$\frac{m_{PTS}}{162 + 132} = \frac{m_H}{180 + 150} = \frac{4089.12}{180 + 150}$$

$$m_{PTS} = \frac{294 * 4089.12 * 0.9}{330} = \mathbf{3,278.73}\ \frac{\mathbf{kg}}{\mathbf{hr}}$$

$$m_{PTS} = \mathbf{22{,}360.94}\ \frac{\mathbf{ton}}{\mathbf{yr}}$$

6. Material balance on mixing

It is the equipment used to make homogenous suspension of distilled water and dry sawdust powder.



Where;

m_{DS} = Mass flow rate of dry sawdust

m_L = Mass flow rate of liquid

$$m_{DS} + m_L = m_{PTS} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(*)$$

From the liquid to solid ratio, 10:1, i.e. $m_L = 10m_{DS}$, substitute in to equation (*)

$$m_{DS} + 10m_{DS} = m_{PTS}$$

$$11m_{DS} = m_{PTS} = 3278.73$$

The amount of dry sawdust;

$$m_{DS} = \frac{3278.73}{11} = \mathbf{298.07}\,\frac{\mathbf{kg}}{\mathbf{hr}}$$

$$m_{DS} = \mathbf{2,032.81}\,\frac{\mathbf{ton}}{\mathbf{yr}}$$

The amount of liquid used is;

$$m_L = 10 * 298.07 = \mathbf{2,980.70}\,\frac{\mathbf{kg}}{\mathbf{hr}}$$

$$m_L = \mathbf{20,328.37}\,\frac{\mathbf{ton}}{\mathbf{yr}}$$

From this, the amount of sulfuric acid needed is;

$= 0.0421*2980.7 =$ **125.50** $\frac{\mathbf{kg}}{\mathbf{hr}}$

$=$ **855.82** $\frac{\mathbf{ton}}{\mathbf{yr}}$ of sulfuric acid (98-99%) are needed.

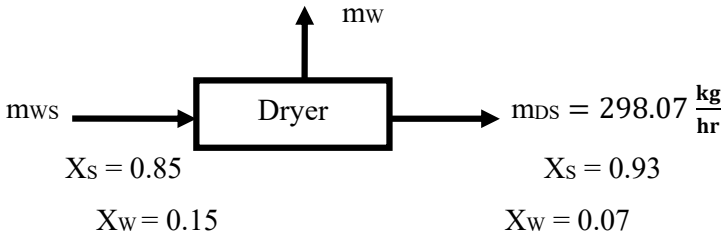
The amount of distilled water required is;

$= 0.9579*2980.7 =$ **2,855.00** $\frac{\mathbf{kg}}{\mathbf{hr}}$

$=$ **19,470.52** $\frac{\mathbf{ton}}{\mathbf{yr}}$ of distilled water are required.

7. Material balance on dryer

It is used to dry the wood sawdust i.e. to make agreeable for easily soluble and mixing with the distill water.



Where;

m_{WS} = mass flow rate of wet sawdust

X_W = fraction of water in the sawdust

X_S = fraction of solid in the sawdust

m_W = mass flow rate of water removed

The amount of wet sawdust required is;

$$m_{WS} * X_S = m_{DS} * X_S$$

$$0.85 m_{WS} = 0.93 m_{DS} = 0.93 * 298.07$$

$$m_{WS} = \mathbf{326.12\ \frac{kg}{hr}}$$

$$m_{WS} = \mathbf{2{,}224.14\ \frac{ton}{yr}}$$

The amount of water removed is;

$$m_{WS} = m_{DS} + m_W$$

$$m_W = m_{WS} - m_{DS} = 326.12 - 298.07$$

$$m_W = \mathbf{28.05\ \frac{kg}{hr}} = \mathbf{191.30\ \frac{ton}{yr}}$$

5.1.2. Energy balances

Energy balances are used in the examination of the various stages of a process, over the whole process and even extending over the total production system from the raw material to the finished product. Energy balances are also essential for process design to determine the energy requirements of the process; the heating, cooling and power required. In this plant operation, it is thought that an energy balances (energy audit) on the plant will show the pattern of energy usage and suggest areas for the energy conservation and savings.

Energy is an important and costly input in the production process of ethanol. After feedstock costs, it is the most expensive variable cost. This energy is utilized in two forms; thermal (steam) and electrical. Electrical energy is used to run machinery with moving parts or motors such as pumps, centrifuges and mills.

5.1.2.1. Detail energy balance calculations

- **Basic data:- one operation hour for all energy balance calculations**

1. Energy balance on dryer:- the dryer is dried the sawdust from 25^{o}C to 105^{o}C.

The amount of energy required is;

$$Q = mC_p\Delta T \text{…….. (*)}$$

Where;

m = mass of wet sawdust

C_p = specific heat of wood sawdust (3.85 kJ/kg.k)

$\Delta T = T_f - T_i$

Mass of wet sawdust from material balance (m) = **326.12** kg/hr

$$C_p = C_{ps}*X_s + C_{pw}*X_w$$

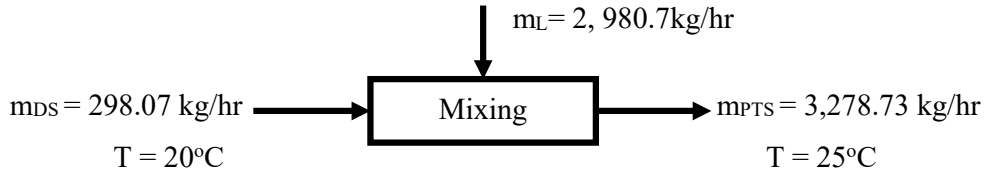
$$C_p = 3.85*0.85 + 4.18*0.15 = \mathbf{3.9\ kJ/kg.k}$$

The amount of energy required by substitute in equation (*);

$$Q = 326.12\ \frac{kg}{hr} * 3.9\ \frac{kJ}{kg.k} * (105\text{-}25)\ k$$

$$Q = \mathbf{101{,}749.44\ kJ/hr}$$

2. Energy Balance on Mixing:- the energy used for mix the grind sawdust powder with liquid to make suspension.



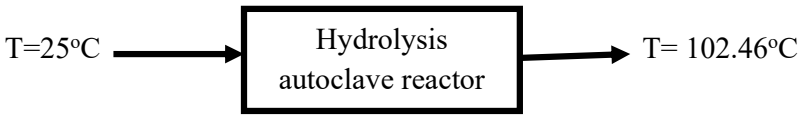
$$C_P = \frac{298.07 * 3.9 + 298.07 * 4.18}{298.07 + 298.07} = \mathbf{4.155}\ \frac{\mathbf{kJ}}{\mathbf{kg.k}}$$

The heat required for mixing is;

$$Q = m_{PTS}C_P\Delta T = 3278.73\frac{kg}{hr} * \frac{4.155kJ}{kg.k} * (25-20)k$$

Q = **68,035.65 kJ/hr**

3. Energy balance on Hydrolysis:- the energy used to hydrolyze the lignocellulose content into the reducing sugars. The optimum hydrolysis temperature from optimization is 102.46°C



The specific heat of the mixture is;

$$C_p = \frac{298.07*3.85+2980.7*4.18}{3278.73}$$

$$C_p = \mathbf{4.15\ \frac{kJ}{kg.k}}$$

The heat required for hydrolysis is;

$$Q = m_{WS}C_p\Delta T = 3278.73\frac{kg}{hr} * 4.15\frac{kJ}{kg.k} * 77.46\ k$$

Q = **1,020,504.71kJ/hr**

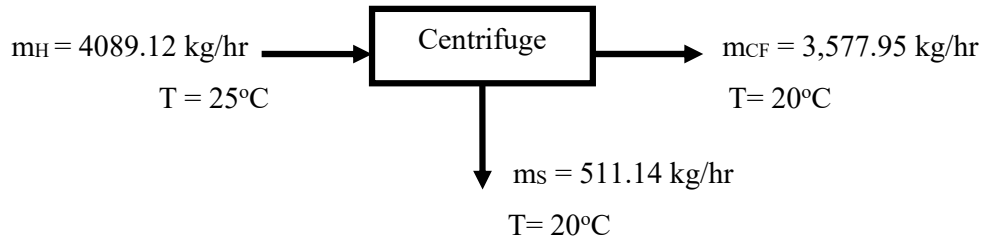
The amount of Steam consumption for this process is;

$$Q = M_S(C_p\Delta T + h_{lw}),\ h_{lw} = 2256.9\ kJ/kg$$

$$M_S = \frac{Q}{C_p\Delta T + h_{lw}} = \frac{10020504.71}{4.15*75+2256.9}$$

$$M_S = \mathbf{397.34\frac{kg}{hr}}$$

4. Energy balance on centrifugal separation:-



The specific heat at the filtrate residue is determined as follows;

$$C_{ps} = C_p*X_s+*X_L*C_{pw} = 0.8*4.15+0.2*4.18$$

C_{ps} = **4.16 kJ/kg.k**

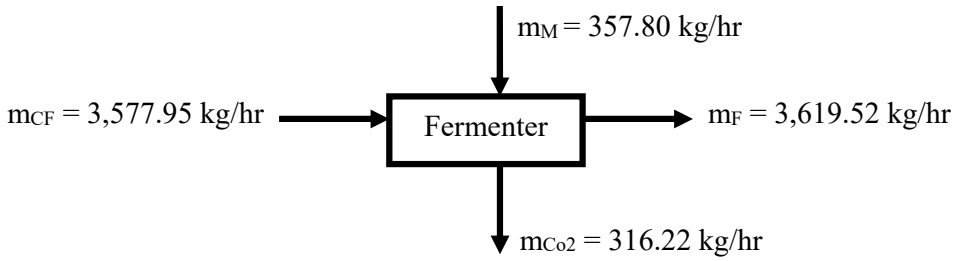
The heat released during the separation process is;

$$Q = m_S * C_p * \Delta T + m_{CF} * C_p * \Delta T$$

$$Q = 511.14 * 4.16 * (25-20) + 3577.95 * 4.18 * (25-20)$$

$$Q = \mathbf{77{,}334.86\ kJ/hr}$$

5. Energy balance on Fermenter:- the fermentation is an exothermic reaction heat will be generated inside it and the outlet temperature is 30°C. The energy balance in the Fermenter is at a 0°C reference temperature.



Basic data;

C_p of mix at 30°C = 4.124 kJ/kg.k

C_p of Co_2 at 30°C = 0.846 kJ/kg.k

The amount of energy generated during the fermentation is;

$$Q_{MIX} = Q_{Co2} + Q_F + Q$$

$$Q = Q_{MIX} - Q_{Co2} - Q_F$$

$$Q = m_{MIX}\, C_{pMIX}\, \Delta T - m_{Co2}\, C_{pCo2}\, \Delta T - m_F\, C_{PF}\, \Delta T$$

The specific heat of the mixture is;

$$C_{PF} = C_{PMIX}\, X_{MIX} + C_{PCo2}\, X_{Co2}$$

$$X_{Co2} = \frac{m_{Co2}}{m_{CF}+m_M} = \frac{317.51}{3597.14+359.71} = \mathbf{0.08}$$

$$X_{MIX} = 1 - X_{Co2} = 1 - 0.080 = \mathbf{0.92}$$

Now, the specific heat of the feed is;

$$C_{PF} = C_{PMIX}\, X_{MIX} + C_{PCo2}\, X_{Co2}$$

$$C_{PF} = 4.124 * 0.92 + 0.846 * 0.08$$

$$C_{PF} = \mathbf{3.862\ kJ/kg.k}$$

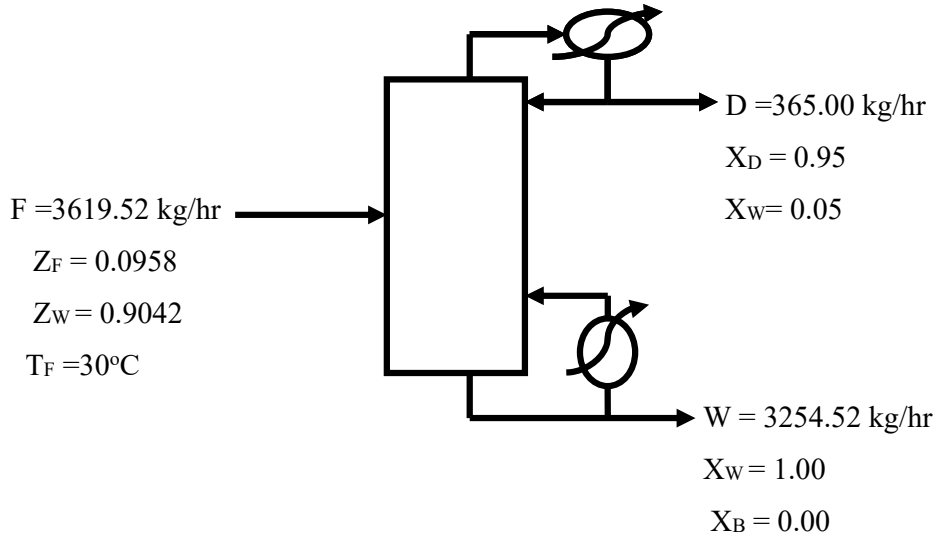
The amount of energy required is;

$$Q = m_{MIX}\, C_{pMIX}\, \Delta T - m_{Co2}\, mC_{pCo2}\, \Delta T - m_F C_{PF} \Delta T$$

$$Q = 3935.75*4.124*30 - 316.22*0.846*30 - 3619.52*3.862*30$$

$$Q = \mathbf{59,547.74\ kJ/hr}$$

6. Energy balance on distillation column



Basis temperature; 25°C

The specific heat capacity on top (distillate);

$$C_{PD} = 0.95*2.72 + 0.05*4.18$$

$$C_{PD} = \mathbf{2.793\ kJ/kg.k}$$

The specific heat capacity on the bottom is;

$$C_{PW} = \mathbf{4.18\ kJ/kg.k}$$

Energy balance on Condenser is;

$$\text{Reflux ratio (R)} = 2.5 = \frac{L}{D} = 2.5$$

$$L = 2.5*D = 2.5*365.00$$

$$L = \mathbf{912.50}\ \text{kg/hr}$$

$$V = L+D = 3.5D = 3.5*365.00$$

$$V = \mathbf{1,277.50}\ \text{kg/hr}$$

From the VE data;

$$\text{Boiling point of 95.0\% alcohol} = 78.3^{o}C$$

At steady state the input output calculation is given by;

$$\text{Input} = \text{Out put}$$

$$H_F = H_D + H_L + Q_C$$

$$Q_{Cv} = H_F - H_D - H_L$$

For the complete condensation process;

Enthalpy of Vapor = Latent heat + Sensible heat

$Q_C = H_V = m_v\lambda_v + m_vC_p\Delta T$

$Q_C = H_V$=1277.50 *789 + 1277.50 *2.72*(78.30 - 25)

$Q_C = H_V =$ **1,192,563.62 kJ/hr**

Q_B = is determined from the overall system;

Input = Out put

$Q_B + H_F = Q_C + H_D + H_W$

$Q_B = Q_C + H_D + H_W - H_F$

Where;

Q_B = Re-boiler heat input

Q_C = Condenser cooling system

Heat capacity of feed;

$H_F = mC_p\Delta T$=3619.52*4.04*(30-25)

$H_F =$ **146,228.61 kJ/hr**

Heat capacity of bottom;

$H_W = m_W.C_p.\ \Delta T$= 3254.52*4.18*(100-25)

$H_W =$ **1,020,292.02 kJ/hr**

Now, the heat capacity of Reboiler is;

$Q_B = Q_C + H_D + H_W - H_F$, where, H_D= 0

Q_B =1192563.62 +1020292.02 -146228.61

Q_B =**2,066,627.03 kJ/hr**

Q_B is supplied by condensing steam;

Latent heat of steam at 274KN/m^2

$\lambda_v =$ **2174 KJ/kg**

The amount of Steam required is;

$$m_S = \frac{Q_B}{\lambda_v} = \frac{20666627.03}{2174} = \mathbf{950.61}\ \frac{\mathbf{kg}}{\mathbf{hr}}$$

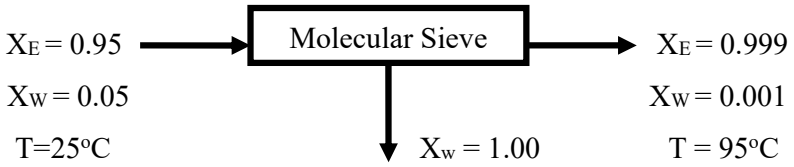
Q_C is removed by cooling water with a temperature rise to 30^{o}C;

$$Q_C = m_W C_P \Delta T$$

$$m_w = \frac{Q_C}{C_p \Delta T} = \frac{1192563.62}{4.18*30}$$

$$m_W = \mathbf{9,510.08}\,\frac{\mathbf{kg}}{\mathbf{hr}}$$

7. Energy balance on molecular sieve:-the adsorption takes place at 95.0%



The specific heat capacity of mixture;

$$C_P = C_{PE}X_E + C_{PW}X_W$$

$$C_P = 2.72*0.95+4.18*0.05$$

C_P = **2.37 kJ/kg.k**

The amount of energy required;

$$Q = mC_P\Delta T = 347 * 2.37 * (95 - 25)$$

Q = **61,679.25 kJ/hr**

5.2. Specification and design of major process equipments

Storage tank and pumps

Basic data:- operation on one hour basis for all calculations

1. Storage tanks for dried sawdust

- Solid to be handled — Dried sawdust (**298.07** $\frac{kg}{hr}$)
- Density of solids — 210 kg/m^3
- Temperature of solids — 25°C
- Materials of construction — Carbon steel

The capacity of storage tank;

$$\rho = \frac{m}{v}$$

$$v = \frac{m}{\rho} = \frac{298.07}{210} = \mathbf{1.42\ m^3}$$

The required storage volume including safety factor;

$$V_r = \frac{v}{0.75} = \frac{1.42}{0.75} = \mathbf{1.89 = 2.0\ m^3}$$

2. Storage tank for mixing of sawdust and water

- Slurry to be handled — water and sawdust powder mixture (**3,278.73** $\frac{kg}{hr}$)
- Density of liquid — 921 kg/m^3
- Temperature of liquid — 25^{o}C
- Materials of construction — Carbon steel

The capacity of storage tank;

$$\rho = \frac{m}{v}$$

$$v = \frac{m}{\rho} = \frac{3278.73}{921} = \mathbf{3.23\ m^3}$$

The required storage volume with safety factor;

$$V_r = \frac{v}{0.75} = \frac{3.23}{0.75} = \mathbf{4.3 = 4.5\ m^3}$$

3. Storage tank for hydrolyzed

- Slurry to be handled — Slurry of sawdust (**4,089.12** $\frac{kg}{hr}$)
- Density of liquid — 959 kg/m^3
- Temperature of slurry — 25^{o}C
- Materials of construction — Carbon steel

The capacity of storage tank;

$$\rho = \frac{m}{v}$$

$$v = \frac{m}{\rho} = \frac{4089.12}{959} = \mathbf{3.8\ m^3}$$

The required storage volume with safety factor;

$$V_r = \frac{v}{0.75} = \frac{3.8}{0.75} = \mathbf{4.2\ m^3}$$

4. Storage tank for fermentation

- Liquid to be handled — Hydrolysates treated (**3,619.52** $\frac{kg}{hr}$)
- Density of liquid — 925 kg/m^3
- Temperature of liquid — 25^{o}C
- Materials of construction — Carbon steel

The capacity of storage tank;

$$\rho = \frac{m}{v}$$

$$v = \frac{m}{\rho} = \frac{3619.52}{925} = 3.\mathbf{9\ m^3}$$

The required storage volume with safety factor;

$$V_r = \frac{v}{0.75} = \frac{3.9}{0.75} = \mathbf{5.0\ m^3}$$

5. Storage tank for ethanol

- Liquid to be handled — Ethanol ($\mathbf{347\ \frac{kg}{hr}}$)
- Density of liquid — 789 $\frac{kg}{m^3}$
- Temperature of liquid — 25°C
- Materials of construction — Carbon steel

The capacity of storage tank;

$$\rho = \frac{m}{v}$$

$$v = \frac{m}{\rho} = \frac{347}{789} = \mathbf{0.44\ m^3}$$

The required storage volume with safety factor;

$$V_r = \frac{v}{0.75} = \frac{0.44}{0.75} = \mathbf{0.6\ m^3}$$

6. Pump for delivering for mixing tank storage

- Type — Centrifugal pump
  - Operating condition
    - Head — 4m
    - Slurry to be handled — Mixture of sawdust and water
    - Density — 921 kg/m^3
    - Temperature — 25°C
    - Materials of construction — Carbon steel
    - Capacity — **1.43 m^3/hr**

7. Pump for delivering from hydrolyzed

- Type — Centrifugal pump
  - Operating condition
    - Head — 4m
    - Slurry to be handled — Acid treated hydrolysates solution
    - Density — 959 kg/m^3
    - Temperature — 25^oC
    - Materials of construction — Stainless steel
    - Capacity — **3.8 m^3/hr**

8. Pump for delivery from fermented tank

- Type — Centrifugal pump
  - Operation condition
    - Head — 4m
    - Liquid to be handled — Fermented of hydrolysates sugar solutions
    - Density — 925 kg/m^3
    - Temperature — 25^oC
    - Materials of construction — Carbon steel
    - Capacity — **3.9 m^3/hr**

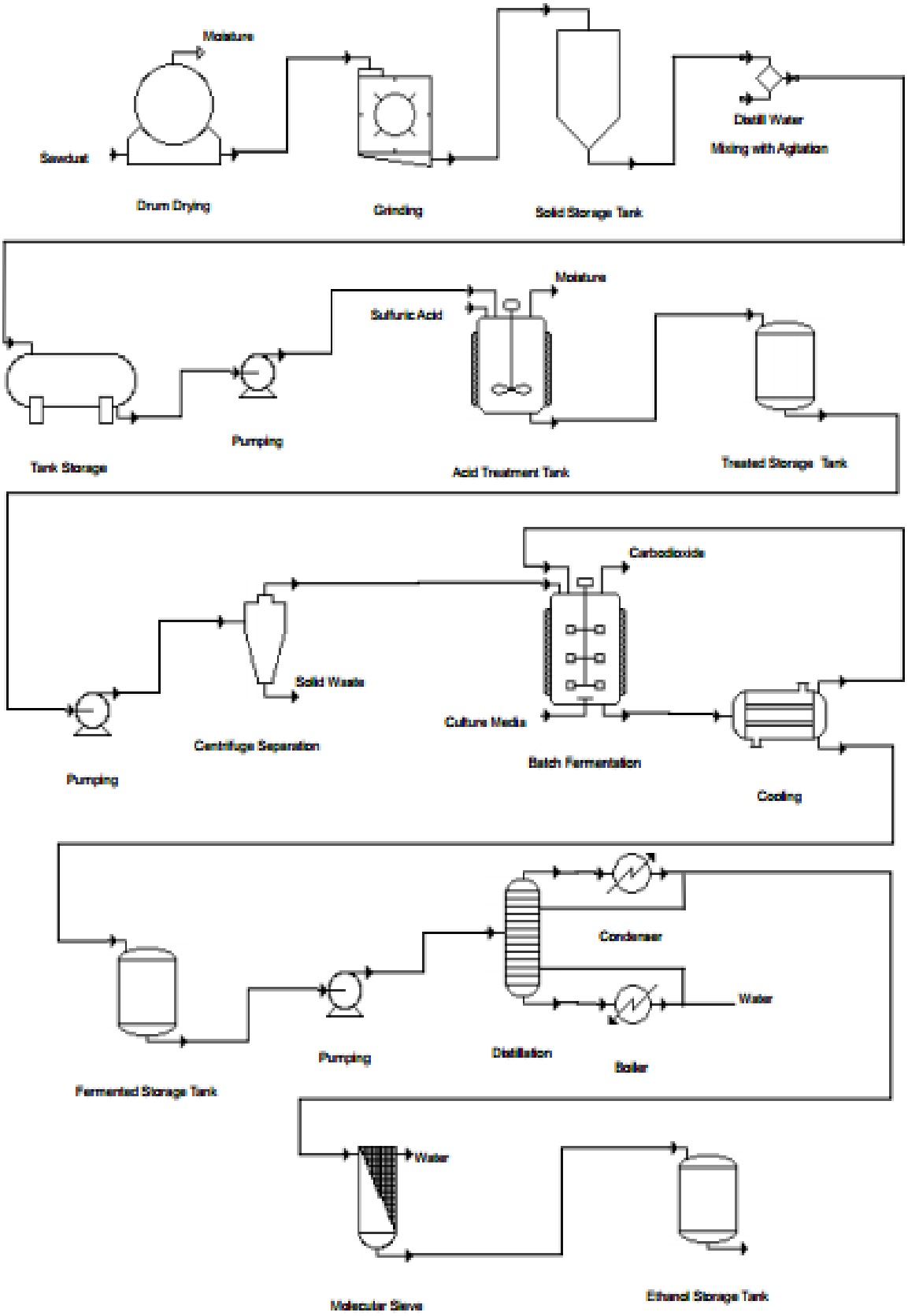


Figure 5.1: Process flow sheet for ethanol production from wood sawdust (SuperPro Design)

9. Design of distillation column

A continuous fractionating column is to design used to separate 3,619.52 kg/hr of ethanol-water mixture with 9.58% ethanol and 90.42% water so as to give 95.0% ethanol and the bottom product of waste of 100% water. A reflux ratio of 2.5 of product is assumed to be used. The column works at a vacuum of 0.4 bars with a vapor velocity of 0.73 m/s.

- ✓ Molecular weight of ethanol = C_2H_5OH

  = 2*12 + 6*1 + 16

  = **46 gram/mole**
- ✓ Molecular weight of water = H_2O

  = 2 + 16

  = **18 gram/mole**
- ✓ Boiling point of ethanol = 78.13 ^oC = 351.13 k
- ✓ Boiling point of water = 100 ^oC = 373 k
- ✓ Feed mean molecular weight = 0.0958*46 + 0.9042*18

  = **20.68gm/mole**
- ✓ Specific heat of feed (C_{pF}) = 0.0958*2.72 +0.9042*4.18

  = **4.04kJ/kg.k= 0.9292kJ/k.Kmol**
- ✓ Water mole fraction on feed (Y_F) = $\frac{\frac{90.42}{18}}{\frac{90.42}{18}+\frac{9.58}{46}} = \frac{5.02}{5.02+0.2}$

  $= \mathbf{0.961 = 96.1\%}$
- ✓ Water mole fraction in bottom(Y_B) = $\frac{\frac{100}{18}}{\frac{100}{18}}$

  $= \mathbf{1 = 100\%}$
- ✓ Ethanol mole fraction in feed (Z_F) = $\frac{\frac{9.58}{46}}{\frac{9.58}{46}+\frac{90.42}{18}} = \frac{0.20}{0.20+5.02}$

  $= \mathbf{0.0398 = 3.98\%}$
- ✓ Ethanol mole fraction in distillate (X_D) = $\frac{\frac{95}{46}}{\frac{95}{46}+\frac{5}{18}} = \frac{2.06}{2.06+0.27}$

  $= \mathbf{0.884 = 88.4\%}$
- ✓ Ethanol mole fraction in bottom (X_B) **= 0%**
- ✓ Reflux ratio = 2.5
- ✓ Molar latent heat of mixture in feed (λ) = 0.0958*789 + 0.9042*2376.7

  = **2224.69kJ/kg**

$$= 2224.69\text{kJ/kg} * \frac{1kg}{0.02kmol} = 105937.619 \frac{kJ}{Kmol}$$

$$= 105937.619\text{kJ/Kmol} * 2.39 * 1\text{kgcal/kJ}$$

$$= \mathbf{25319.09 kgcal/Kmol}$$

- ✓ Boiling point (average) of feed = $\Sigma X^i T^i = 0.0398*308 + (1- 0.0398)*308$

$$= \mathbf{332.52\ k = 59.5^oC}$$

- ✓ Heat capacity of feed (q)= $\frac{CpdT+\lambda}{\lambda} = \frac{0.9292(332.52-298)+25319.09}{25319.09}$

$$= \mathbf{1.00 \frac{kJ}{kg.k}}$$

- ✓ Intercept on y-axis = $\frac{X_D}{R_D+1} = \frac{0.95}{2.5+1} = \mathbf{0.27}$
- ✓ Feed in cold liquid:- Slope = $\frac{q}{q+1} = \frac{1.00}{1.00+1} = \mathbf{0.5}$, and $Z_F = 0.0398$, $X_D = 0.884$, $X_B = 0.00$
- ✓ Now, the operating line is, Y = 0.5X+0.27
- ✓ Vapor equation data of ethanol water solution given below

Table 5.1: VLE data for ethanol water solution at pressure of 0.4 bars

X (liquid)	0.0190	0.0721	0.0966	0.1238	0.1661	0.2337	0.2608	0.3273
Y (vapor)	0.1213	0.3891	0.4375	0.4704	0.5089	0.5445	0.5580	0.5826
X* (liquid)	0.3965	0.5079	0.5198	0.5732	0.6763	0.7472	0.8840	0.8895
Y* (vapor)	0.6122	0.6231	0.6564	0.6599	0.6841	0.7385	0.7815	0.7912

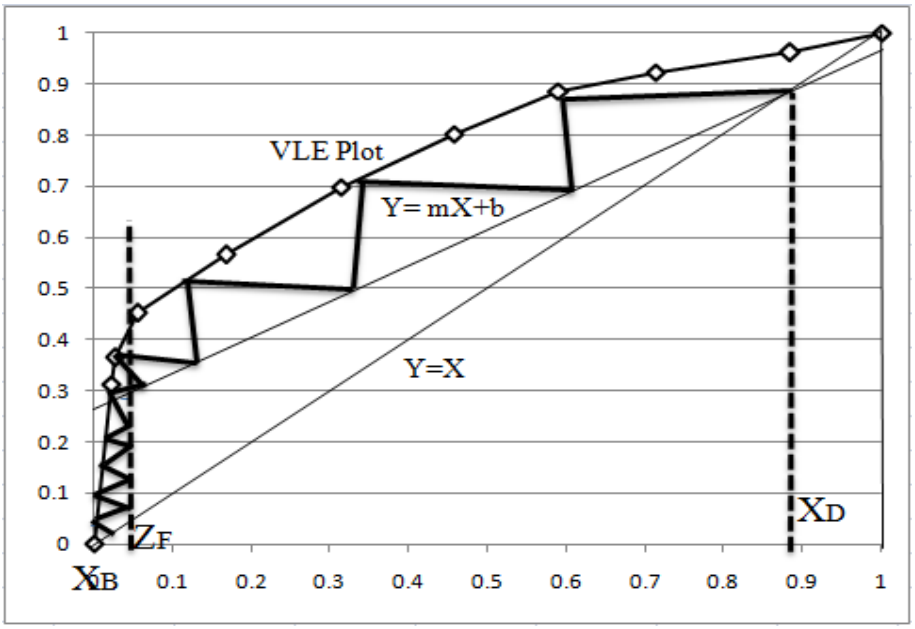


Figure 5.2: McCabe-Thiele diagrams to determine the number of stages

From the above the figure, number of theoretical stage is 9 and the feed is introduced at the 5.5 or 6 stages.

❖ **Diameter at the top:-** let be assumed, temperature at the top is 85°C (358 k)

✓ Assuming ideal gas behavior;

$$V=\frac{nRT}{p}, where\ n\ is\ taken\ the\ vapor\ load\ at$$

$$V = L + D = 1{,}277.50\ kg/hr$$

$$n=\frac{1284.50*0.95}{46}+\frac{1284.50*0.05}{18}=26.53+3.57=\mathbf{30.00}\ \frac{\mathbf{Kmol}}{\mathbf{hr}}$$

R= 0.082atm.m^3/ Kmol.k

P= 0.4atm, substitute the values in the above equation

$$\mathbf{V}=\frac{30.00*0.082*358}{0.4}=\mathbf{2,196.69\ m^3/hr}$$

✓ Cross sectional area;

$$A_s=\frac{V}{linear\ velocity}=\frac{2196.69}{0.73*3600}=\mathbf{0.84\ m^2}$$

✓ Diameter at the top;

$$D=\sqrt{\frac{4A}{\pi}}=\sqrt{\frac{4*0.84}{\pi}}=\mathbf{1.03\ m}$$

❖ **Bottom diameter of column:-**temperature of the bottom is 100°C (373k)

$$V=\frac{nRT}{p}, where\ n=\frac{M_B}{18}=\frac{3254.52}{18}=\mathbf{180.80}\ \frac{\mathbf{Kmol}}{\mathbf{hr}}$$

R = 0.082atm.m^3/ Kmol.k

P = 0.4atm, and T = 373k

$$V=\frac{180.80*0.082*373}{0.4}=\mathbf{13,825.38}\ m^3/hr$$

✓ Cross sectional area;

$$A_s=\frac{V}{linear\ velocity}=\frac{13825.38}{0.73*3600}=\mathbf{5.3\ m^2}$$

✓ Diameter at the top;

$$D=\sqrt{\frac{4A}{\pi}}=\sqrt{\frac{4*5.3}{\pi}}=\mathbf{2.60m}$$

❖ **Area of down flow:-**the weir length is assumed 0.75D = 0.75*2.6 = **1.95 m**

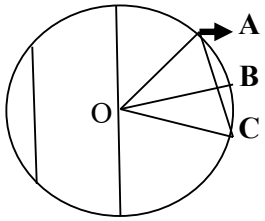


Figure 5.3: Plate diagram showing weir length and down flow section plate design trial

- ✓ Here is; a = 0.5*wire length = 0.5*1.95

  a = **0.97 m**

- ✓ Down flow area = area OABC – area OAC
- ✓ The angle subtended by the down flow area = 180*0.97 =**174.60 º**

$$\text{Area of OABC} = \frac{174.60}{360} * \frac{\pi D^2}{4} = \mathbf{0.38}D^2$$

$$\text{Area of OAC} = \frac{1}{2} * 0.75D * \frac{D}{2} * \cos\left(\frac{174.60}{2}\right) = \mathbf{0.009}\boldsymbol{D^2}$$

- ✓ Down flow area = $0.38D^2 - 0.009D^2 = \mathbf{0.371}\boldsymbol{D^2}$

A_d = 0.371*2.60 = **0.96 m²**

10. Plate Design

Provision,

- ❖ Column diameter, D_0 = 1m
- ❖ Column area, A_L = 0.97m²
- ❖ Down flow area, A_d = 0.26m²

- Net area, $A_n = A_L - A_d$ = 0.97-0.26

  = **0.71m²**

- Active area, $A_a = A_L - 2A_d$ = 0.97-2*0.26

  = **0.5 m²**

- Hole area, A_h taken 10% of A_a, trial

  A_h= 0.1*0.45 = **0.045m²**

❖ **Weir length = 0.75m**

Take with the below provisions;

- ✓ weir height = 52 mm
- ✓ Hole diameter = 5.5 mm
- ✓ Plate thickness = 5.0 mm

- Molecular weight of feed (M_{wF}) = % of ethanol*M_w of ethanol + % of water*M_w of water

  = 0.0958*46 + 0.9042*18

  = **20.6824 kg/kmol**

- Molar flow rate of Feed = $\frac{3619.52\frac{kg}{hr}}{20.6824\frac{Kg}{kmol}}$

$$= \mathbf{175.00}\,\frac{\mathbf{kmol}}{\mathbf{hr}}$$

- A mass balance of ethanol gives;
  - Top product, D =$175.00 * \frac{0.0958}{0.95} = \mathbf{17.65}\ \frac{\mathbf{kmol}}{\mathbf{hr}}$
  - Vapor rate, V = D (1+R) = 17.65 (1+2.5) = $\mathbf{61.77}\ \frac{\mathbf{kmol}}{\mathbf{hr}}$
  - Bottom product, B = 175.00 – 17.65 = $\mathbf{157.35}\ \frac{\mathbf{kmol}}{\mathbf{hr}}$
- Slope of the bottom operating lines;

$$\text{Slope} = \frac{L_{m'}}{V_{m'}} = 0.5$$

- Vapor flow below feed, $V_{m'} = L_{m'} + B$

$$V_{m'} = \frac{B}{0.5} = \frac{157.35}{0.5} = \mathbf{314.70}\ \frac{\mathbf{kmol}}{\mathbf{hr}}$$

$$L_{m'} = 0.5 * V_{m'} = 0.5 * 314.70 = \mathbf{157.35}\ \frac{\text{kmol}}{\text{hr}}$$

❖ **Check weeping :-**

- Maximum volumetric flow rate $(V) = \frac{157.35\ \frac{\text{kmol}}{\text{hr}} * 18\ \frac{\text{kg}}{\text{kmol}}}{3600\ \frac{\text{s}}{\text{hr}} * 0.97988\ \frac{\text{kg}}{\text{m}^3}}$

$$V = \mathbf{0.80}\ \frac{\mathbf{m^3}}{\mathbf{s}}$$

- Maximum liquid rate $(m) = \frac{157.35\ \frac{kmol}{hr} * 18\ \frac{kg}{kmol}}{3600\ \frac{s}{hr}}$

$$m = \mathbf{0.79}\ \frac{\mathbf{kg}}{\mathbf{s}}$$

- Maximum liquid rate at 80% turn down $(m_{mxm}) = 0.8 * .79$

$$m_{mxm} = \mathbf{0.63}\ \frac{\mathbf{kg}}{\mathbf{s}}$$

❖ **Weir liquid crest:-**the height of the liquid crest over the weir as per the farcies weir formula for the segmental down corner is;

$$\text{Maximum } h_{ow} = 750\left(\frac{L_w}{\rho * I_w}\right)^{2/3}$$

Where;

h_{Ow} = weir crest (mm liquid)

L_w = liquid flow rate

I_w = weir length = 0.75m

- Maximum $h_{ow} = 750\ (\frac{0.14}{979.88*0.75})^{\frac{2}{3}} = \mathbf{2.48\ mm\ liquid}$
- Minimum $h_{ow} = 750\ (\frac{0.098}{979.88*0.75})^{\frac{2}{3}} = \mathbf{1.96\ mm\ liquid}$
- At minimum rate; $h_w + h_{ow} = 50 + 1.96 = \mathbf{51.96\ mm}$

K_2 = 30.2, from weep point correlation graph [73]

- The minimum vapor velocity through the holes (weep point)

$$V_{h(mnm)} = \frac{K_2 - 0.9(25.4 - d_h)}{\rho_v{}^{\frac{1}{2}}} = \frac{30.2 - 0.9(25.4 - 5)}{979.88^{\frac{1}{2}}} = \mathbf{0.38}\,\frac{\mathbf{m}}{\mathbf{s}}$$

- Actual minimum vapor velocity $= \frac{mimimum\ vapor\ rate}{A_h}$

Where; minimum vapor rate $= \frac{314.70\,\frac{kmol}{hr} * 46\,\frac{kg}{kmol}}{0.72\,\frac{kg}{m3} * 3600\,\frac{s}{hr}} = \mathbf{5.60}\,\frac{\boldsymbol{m^3}}{\boldsymbol{s}}$

Now, the actual minimum vapor velocity $= \frac{5.60 * .8}{0.045} = \mathbf{99.29}\,\frac{\mathbf{m}}{\mathbf{s}}$

So that minimum operating rate will be well above the weep point.

❖ **Plate pressure drop:-**

- Maximum vapor velocity through holes $(V_h) = \frac{maximum\ volumetric\ flow\ rate}{hole\ area} = \frac{0.80}{0.045}$

$$= \mathbf{17.78}\,\frac{\mathbf{m}}{\mathbf{s}}$$

- The orffice coefficient from the graph showing discharge coefficient of sieve plate.

$$\frac{A_h}{A_a} = \frac{0.045}{0.5} = 0.09 = 9\%$$

$$\frac{\text{Plate thickness}}{\text{Hole diameter}} = \frac{5\text{ mm}}{5\text{ mm}} = \mathbf{1}$$

Hence, at 1 and 9% from the graph, C_0 = 0.83, orifice coefficient [73]

The pressure drop through the plate $(h_d) = 51(\frac{v_h}{c_o})^2 \frac{\rho_v}{\rho_l}$

$$\text{where, density of vapor}(\rho_v) = 0.95 * 790 + 0.05 * 1000$$

$$= \mathbf{800.5}\,\frac{\mathbf{kg}}{\mathbf{m^3}}$$

$$h_d = 51(\frac{17.78}{0.80})^2 \frac{0.8005}{979.88} = \mathbf{20.58\ mm}$$

The residual head can be calculated by the simple equation proposed by Hunt eta al [73]

$$\mathrm{h_r} = \frac{12.5 * 10^3}{\rho_L} = \frac{12.5 * 1000}{979.88} = \mathbf{12.76\ \boldsymbol{mm\ liquid}}$$

Therefore; total pressure drop of the plate (h_t) = h_d + (h_w + h_{ow}) + h_r = 20.58+51.96+12.76

= **85.30mm liquid**

❖ **Down comer pressure loss:-**

- The height of the bottom edges of the apron above the plate (h_{ap}) = h_w-10 = **40mm**

- The clearance area under the down corner given by (A_{ap}) = h_{ap}*I_{w} = 40 mm*0.75*10^3
  = **0.03m²**
- This is less than the down corner area; A_d = **0.26m²**

The main resistance to flow caused by the constriction at the down corner out let i.e. the head loss in the down comer is estimated by Cicalese et al [73].

$$h_{dc} = 166(\frac{L_{wd}}{\rho_l A_{ap}})^2 = 166(\frac{481}{979.88 * 0.03})^2 = \mathbf{231\ mm}$$

In terms of clear liquid the down comer back up (h_b) = (h_w + h_{ow}) + h_t +h_{dc}= 51.92+84.72+231
= **367.68 mm = 0.37m**

- Let us checking tray spacing $= 0.37 < \frac{1}{2}(\text{Plate spacing} + \text{weir length})$

$$= 0.37 < \frac{1}{2}(h_w + I_w)$$

$$= 0.37 < \frac{1}{2}(0.045 + 0.75)$$

$$= 0.37 < 0.3975, \text{tray spacing is acceptable}$$

Let us check also residence time as per the method proposed by Thomas and Shah [73].

$$t_r = \frac{A_d * h_{dc} * \rho_L}{L_{wd}} = \frac{0.26 * 0.37 * 979.88}{0.81} = \mathbf{3.36\ seconds}$$

The recommendation is greater than 3 seconds. So that, the residence time is satisfactory.

- Entrainment checking;

$$V_r = \frac{maximum\ volumetric\ flow\ rate}{net\ area} = \frac{0.80}{0.71} = \mathbf{1.13}\frac{\mathbf{m}}{\mathbf{s}}$$

Know that, $V_f = K_L\sqrt{\frac{\rho_L - \rho_v}{\rho_v}}, K_L = f(F_{LV}, Plate\ spacing)$

$Where\ F_{LV} = \frac{L_w}{V_w}\sqrt{\frac{\rho_v}{\rho_L}}, and\ \frac{L_w}{V_w} = slope = 1.13$

$\rho_v = 0.8005\ at\ 135\ and \rho_L = 979.88\frac{g}{ml}\ at\ 135^oC$

$$F_{LV} = 1.13\sqrt{\frac{0.8005}{979.88}} = \mathbf{0.032}$$

From the graph showing flood velocity Vs sieve plates, K_L= 0.09 [73].

$$V_f = 0.09\sqrt{\frac{979.88-0.8005}{0.8005}} = \mathbf{3.15}\,\frac{\mathbf{m}}{\mathbf{s}}$$

Hence the % flooding ($\frac{V_r}{V_f}$) $= \frac{1.13}{3.15} = \mathbf{0.36}$

From the entrainment correlation graph the entrainment correlation is

Ψ = 0.005, which is well below 0.1 [73]

Tray lay out use cartridge type construction. Allowing 50mm un-perforated strip round plate edge; 50 mm wide calming zone.

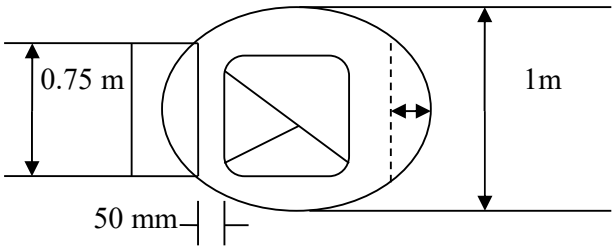


Figure 5.4: Tray layout of plate

❖ **Perforated area:-**from the above figure,

$$\frac{L_w}{D_c} = \frac{0.80}{1} = \mathbf{0.80}$$

Calculate, L_h = 1000mm – 750mm – 50mm = 200mm = **0.2 m**

$$\frac{L_h}{D_c} = \frac{0.2}{1} = \mathbf{0.2}$$

The angle subtended by chord, chord height and chord length the angle is 88^o [73].

- Angle subtended at plate edge by un-perforated strip = 180^0- 88^0 = $\mathbf{92^0}$
- Mean length of un-perforated edge strip $= (1 - 50 * 10^{-3})\frac{92}{180}\pi = \mathbf{1.52\ m}$
- Area of un-perforated edge strip = $50*10^{-3}*1.52$= **0.0765 m^2**
- Mean length of calming zone = $(1 - 50 * 10^{-3})\sin\frac{92}{2} = \mathbf{0.68\ m}$
- Area of calming zone = $2(0.68*50*10^{-3})$ = **0.068 m^2**
- Total area of perforation (A_p) = 0.5- 0.0765- 0.068 = **0.3555 m^2**
- Area ratio $(\frac{A_h}{A_p}) = \frac{0.045}{0.355} = \mathbf{0.126}$

From the hole and pitch relation graph [73].

$$\frac{I_p}{d_h} = f\left(\frac{A_h}{A_p}\right) = \mathbf{2.6}, \text{it is very satisfactory between } \mathbf{2.3 - 4.0}$$

- No of holes (Area of one hole) $= \frac{\pi d^2}{4} = \frac{\pi(5*10^{-3})}{4} = \mathbf{1.96 * 10^{-5}\ m^2}$

- Area of all holes, A_h = 10% of active area = 0.1*0.5 = **0.05m²**

From that number of holes = $\frac{A_h}{A_{single\ hole}} = \frac{0.05}{1.96*10^{-5}} = \mathbf{2,551\ holes}$

❖ **Plate spacing: -**

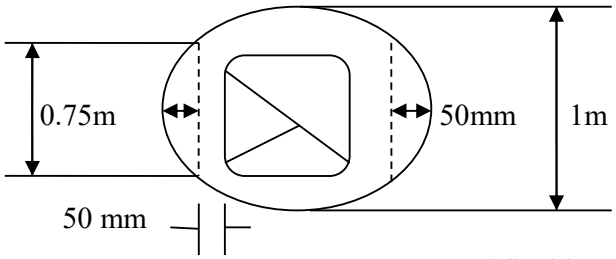


Figure 5.5: Proposed final layout of a plate

Summary the above calculations:-

- Plate no = 1
- Turn down = 80%
- Plate ID = 1
- Plate material = mild steel
- Hole size = 5mm
- Down comer = material mild steel
- Hole pitch = 12. 5mm triangle
- Plate spacing = 0.5m
- Active hole = 2551
- Plate thickness = 5mm

❖ **Plate pressure drop**:-for the height of 80mm of liquid

P = ϱgh = 979.88*9.81*0.08= **0.77kpa**

11. Design of condenser

The condenser to be used is a horizontal condenser design to condense 1,277.50 kg/hr of distilled ethanol at 25°C by exchange with water at 20°C and out let temperature of 30°C. Fluid allocation is given tube side to water and shell side to ethanol.

❖ Specification

✓ Permissible pressure drop on both sides is 0.8 bar

❖ Fouling factor

➢ Distilled ethanol = 0.0001m² °C/w

➢ Water = 0.0003 m² °C/w

- The mean temperature of ethanol= $\frac{85+30}{2} = 57.5\ ^oC = \mathbf{330.5\ k}$
- At this temperature the C_p of ethanol (C_p) = A + BT = 8.424 + 44.422*10^{-2}*330.5 k
  = 155.24kJ/kmol
  = 155.24kJ/kmol/46 kg/kmol
  = **3.375kJ/kg.k**

The heat duty = $\frac{\frac{347\ kg}{hr}}{\frac{3600s}{hr}} * 3.375\ (85-30) = \mathbf{17.89kw}$

As the first trial the mean temperature of water is equal to the inlet temperature, thus Specific heat capacity of water at this temperature is (C_{pw}) = **4.18 kJ/kg.k**

- Energy balance (M_w = 9064.54kg/hr)

$$17.89\text{kw} = \frac{\frac{9064.54\text{kg}}{\text{hr}}}{\frac{3600\text{s}}{\text{hr}}} * 4.18\frac{\text{kJ}}{\text{kg.k}}(t_2 - 20)$$

t_2 = **21.61°C**

- The mean temperature of water = $\frac{25+21.61}{2} = \mathbf{23.31^oC}$

The specific heat capacity of water remains the same up to 50°C, thus = 4.18 kJ/kg.k

Table 5.2: Thermodynamic properties for characterizing the ethanol and water

ETHANOL	Temperature (°C)	Specific Heat (kJ/kg.k)	Density (kg/m^3)
Inlet	85	3.37	790
Outlet	30	3.9	789
Mean	57.5	3.375	789.5
WATER	-	-	-
Inlet	21.61	4.18	1000
Outlet	25	4.18	1000
Mean	23.31	4.18	1000

❖ **Over all coefficients:-**

For the above specified condenser, the overall coefficients will be between 700-1000 w/m^2.°C. So, let assuming that with 700w/m^2.°C [73].

- **Condenser type and dimension**

Even number tube pass is selected to simplify the pipe work

Start with one shell pass and two tube pass,

$$\Delta T_{lm} = \frac{(85-23.31)-(30-20)}{\ln\left[\frac{85-23.31}{30-20}\right]} = \mathbf{28.21^oC}$$

Hence,

$$R = \frac{85-30}{23.31-20} = 16.62$$

$$S = \frac{23.31-20}{85-20} = 0.051$$

From the graph of temperature fouling factor, $F_t = 0.98$ [73]

The design temperature should be;

$$\Delta T_{lm} = 0.98*13.1 = \mathbf{12.84^oC}$$

The cooling heat transfer area;

$$A_u = \frac{Q}{U\Delta T_{lm}} = \frac{17.89*1000}{700*12.84} = \mathbf{2m^2}$$

- **Lay out**

Use a splitting floating head heat exchanger for efficiency and ease of cleaning. Carbon steel is used, since both fluids are not corrosive.

Assumed the following dimensions

- ✓ Outside diameter = 19.05 mm
- ✓ Inside diameter = 14.83 mm
- ✓ Tube length = 4 m since it is popular size
- ✓ Pitch triangular = 23.81mm
- ✓ $\frac{Pitch}{Diameter}$ = 1.25

- **Number of tubes**

Area of one tube (neglecting thickness of tube sheets) = πDL= 3.14*19.05*10^{-3}*4= **0.2393 m^2**

$$N_t = \frac{A_u}{\pi DL} = \frac{2}{0.2393} = 8.35 = \mathbf{9\ tubes}$$

For 2 passes, the tube per pass is, $\frac{9}{2} = 4.5 = \mathbf{5\ tubes}$

Tube cross sectional area= $\frac{\pi D^2}{4} = \frac{3.14*0.01485*0.01485}{4} = \mathbf{0.000173\ m^2}$

And the area per pass (A_P) = 5*0.00173 = **0.000865 m^2**

The volumetric flow rates (V_F) = $\frac{m_{H2o}}{\rho_{H2o}} = \frac{9064.54}{3600*1000} = \mathbf{0.0025\ \frac{m^3}{s}}$

Tube side velocity (V_T) = $\frac{A_P}{V_F} = \frac{0.0025}{0.000865} = \mathbf{2.89}\frac{\boldsymbol{m}}{\boldsymbol{s}}$, the velocity is satisfactory up to 4 $\frac{\boldsymbol{m}}{\boldsymbol{s}}$ to prevent fouling.

❖ **Bundle and shell diameter**

One shell and two tubes per pass

For two tubes pass of the triangular pitch K_1 and n_1 from the constant table

$K_1 = 0.249$ and $n_1 = 2.207$ [73]

$$\boldsymbol{D} = d_o\left(\frac{N_t}{K_1}\right)^{\frac{1}{n_1}} = 19.05\left(\frac{10}{0.249}\right)^{\frac{1}{2.207}} = 101.53 = \mathbf{102mm}$$

For a split ring floating, the typical shell clearance is 50 mm

$$D_{b\ clearance} = D_s - D_l$$

$$D_s = D_l + D_{b\ clearance} = 50+102 = \mathbf{152\ mm}$$

Tube side heat transfer coefficient;

$$R_e = \frac{\rho v d_i}{\mu} = \frac{1000*2.9*14.8*10^{-3}}{77*10^{-5}} = \mathbf{55,853}$$

$$P_r = \frac{C_p \mu}{K_f} = \frac{4.18*10^3*77*10^{-5}}{0.6} = \mathbf{5.36}$$

$$\frac{L}{d_i} = \frac{4000}{14.83} = \mathbf{269.72}$$

From the graph showing the tube side heat transfer factor J_h versus R_e, $J_h = 3.3*10^{-3}$ [73]

$$\mathbf{N}_u = J_h * R_e * P_r^{0.33} = 3.3 * 10^{-3} * 55853 * 5.36^{0.33} = \mathbf{320.76}$$

$$\mathbf{N}_u = \frac{h_i * d_i}{K_f} = \mathbf{320.76}$$

$$h_i = \mathbf{12,977.62\ W/m^2.^oC}$$

❖ **Shell side heat transfer coefficient**

- ✓ Condensing vapor side
- ✓ Kern's method was used

Baffle spacing;

$$I_B = \frac{D_s}{5} = \frac{152}{5} = \mathbf{30.4 = 31\ mm}$$

The area of the cross flow A_s, for the row of tubes at the shell equation

$$A_S = \frac{P_t - d_o}{P_t} * D_s * I_B$$

Where;

P_t = Pitch triangular (tube pitch)

D_o = outside tube diameter

D_s = shell inside diameter

I_B = baffle spacing

$$A_S = \frac{23.81-19.05}{23.81} * 152 * 31 \ = \mathbf{942\ mm^2}$$

For the equilateral triangle pitch arrangement, the shell side equivalent diameter

$$D_e = \frac{1.1}{d_o}\left(P_t^2 - 0.917 d_o^2\right) = \frac{1.1}{19.05}(23.81^2 - 0.917 * 19.05^2) \ = \mathbf{13.52mm}$$

Volumetric flow rate on the shell side (ethanol side) $(V) = \frac{m}{\rho}$

$$where\ \rho = \frac{P}{RT} = \frac{0.4*10^2}{8.314*358} = \mathbf{0.014\ kg/m^3}$$

$$V = \frac{1{,}277.50\frac{kg}{hr}}{3600\frac{s}{hr}*0.014\frac{kg}{m^3}} = \mathbf{25.35\frac{m^3}{s}}$$

Shell side velocity = $\frac{volumetric\ flow\ rate}{area} = \frac{25.35}{0.000942} = \mathbf{0.11\frac{m}{s}}$

$$R_e = \frac{\rho v d_i}{\mu} = \frac{790 * 0.11 * 13.52 * 10^{-3}}{1.2 * 10^{-3}} = \mathbf{979.1}$$

$$P_r = \frac{C_p \mu}{K_f} = \frac{4 * 10^{-3} * 1.2 * 10^{-3}}{0.6} = \mathbf{8 * 10^{-6}}$$

Use baffle cut 25 %, from the plot showing shell side heat transfer factor, $J_f = 1.8*10^{-2}$

$$\mathbf{N}_u = J_f * R_e * P_r^{0.33} = 1.8*10^{-2}*979.1*(8*10^{-6})^{0.33} = \mathbf{0.37}$$

$$\mathbf{N}_u = \frac{h_s * d_e}{K_f} = \mathbf{0.37}$$

Where;

$d_e = 13.52*10^{-3}$

$K_f = 0.6$

$$h_s = \mathbf{16.42\ W/m^2.^oC}$$

The overall coefficient;

$$\frac{1}{U_o} = \frac{1}{h_o} + \frac{1}{h_{od}} + \frac{d_o \ln\frac{d_o}{d_i}}{2k_w} + \frac{d_o}{d_i} * \frac{1}{h_{id}} + \frac{d_o}{d_i} * \frac{1}{h_{od}}$$

Where;

$h_0 = 16.42\ w/m^2\ .^o$

$h_i = 12{,}977.48\ w/m^2\ .^oC$

h_{0d} = 0.0001 w/m^2.^{o}C (outside dirt coefficient)

h_{id} = 0.0003 w/m^2.^{o}C

k_w = thermal conductivity of tube wall = 55 w/m^2.^{o}C

$$U_o = \frac{1}{16.42} + \frac{1}{0.0001} + \frac{19.05\ln(\frac{19.05}{14.83})}{2 * 55} + \frac{19.05}{14.83} * \frac{1}{0.0003} + \frac{19.05}{14.83} * \frac{1}{12977.48}$$

$$= \mathbf{14,281.96\ w/m^2.^oC}$$

❖ **Pressure drop**

- Number of tubes = 10
- Number of pass = 2
- Tubes inside diameter = 14.83mm
- Tube side velocity, V_t = 2.9 m/s
- R_e = 55853
- J_f = 3.3*10^{-3}

Hence, neglect viscosity correction factor;

$$\Delta P_t = N_p \left[8J_f\left(\frac{L}{d_i}\right)\left(\frac{\mu}{\mu_w}\right)^{-m} + 2.5\right]\frac{\rho {V_t}^2}{2}, \text{neglect viscosity correction factor}$$

Where;

ΔP_t = tube side pressure drop, N/m^2 (pa)

N_p = number of tube side passes.

U_t = tube side velocity, m/s

L = length of one tube

$\Delta P_t = 2\left[8 * 3.3 * 10^{-3}\left(\frac{4000}{14.83}\right) + 2.5\right]\frac{1000*2.9^2}{2}$ = **80910N/m^2** = **0.8** bars, It is satisfactory since within the specification.

❖ **Shell side**

- ✓ Shell diameter, D_s = 152mm
- ✓ Equivalent diameter, D_e = 13.52mm
- ✓ Tube length, L = 4m
- ✓ Baffle spacing, I_B = 942mm
- ✓ Reynolds number, R_e = 979.1
- ✓ Shell side velocity, U_s = 0.11m/s
- ✓ J_f = 1.8*10^{-2}

$$\Delta P_t = 8J_f\left(\frac{D_s}{D_e}\right)\left(\frac{L}{I_B}\right)\frac{\rho {V_s}^2}{2}\left(\frac{\mu}{\mu_w}\right)^{-m} = 8 * 1.8 * 10^{-2}\left(\frac{152}{13.52}\right)\left(\frac{4}{942 * 10^{-6}}\right)\frac{800.5 * 0.11^2}{2}$$

= **33293 N/m²** = **0.34bar**, satisfactory, since it is below 0.8 bar (allowable pressure).

❖ **The proposed design;**

- ✓ Split ring, floating head, 1sheel pass, 2 tube pass
- ✓ 9 tubes, 4m long 19.05mm outside diameter, 14.83mm inside diameter, pitch triangular 23.81mm.
- ✓ Heat transfer area = 2.393m^2 (based on outside diameter)
- ✓ Shell inside diameter 152mm, baffle spacing = 942 mm^2, 25% baffle cut
- ✓ Tube side coefficient = 12,977.48 w/m^2.^{o}C
- ✓ Shell side coefficient =16.42 w/m^2.^{o}C
- ✓ Over all coefficient estimated = 700 w/m^2.^{o}C
- ✓ Over all coefficient calculated = 14,281.96 w/m^2.^{o}C
- ✓ Dirt/fouling factor
- ✓ Tube (water) = 0.0003 w/m^2.^{o}C
- ✓ Shell side (ethanol-water mixture) = 0.0001 w/m^2.^{o}C
- ✓ Pressure drop
- ✓ Tube side, calculated = 0.8 bar,
- ✓ Shell side, calculated = 0.34 bar

5.3. Plant cost estimation and preliminary feasibility analysis

Cost estimation is a specialized subject and a profession in its own right. The design engineer, however, needs to be able to make quick and rough cost estimates to decide between alternative designs and for project evaluation. Chemical plants are built to make a profit and an estimate of the investment required and the cost of production are needed before the profitability of a project can be assessed. Simple costing methods and some cost data are given, which can be used to make preliminary estimates of capital and operating costs at the flow sheet stage.

The evaluation determines whether one should undertake the project, abandon it, continue with it (but with further research) or take it to the pilot plant stage. Even if insufficient technical information is available to design a plant completely, it must still make and economic evaluation to determine if it is economically and financially feasible. For a project economically feasible when it is more profitable than other competing project, and financially feasible when management can raise the capital for its implementation.

The economic evaluation of any chemical process proceeds in following steps. These are,

1. Preparing the process flow diagram
2. Calculating mass and energy balance
3. Specifying & sizing of major equipment
4. Estimating the production cost
5. Forecast the product sales price
6. Estimating the feasibility criteria's.

The ultimate purpose for developing such a detailed process design and cost estimate is to determine the economics of cellulosic ethanol production from sawdust using acid hydrolysis.

The total capital investment is first computed from the purchased equipment cost. Next to that variable and fixed operating costs are determined. With these costs, the use of discounted cash flow analysis to determine the net present value with a finite internal rate of return. This section describes the assumptions made in completing the discounted cash flow analysis.

The purchased equipment cost for a given component reflects a baseline equipment size. As changes are made to the process, the equipment size required may be different than what was originally designed. Instead of re-costing in detail, an exponential scaling expression was used. The equation described below is used to calculate the cost of new equipment.

$$\mathbf{C_2 = C_1(\frac{S_2}{S_1})^n} \quad \textbf{(5.5)}$$

Where;

C_2 = Equipment cost of new equipment with capacity S_2

C_1 = Equipment cost of baseline equipment with capacity S_1

n = a characteristic scaling exponent (typically in the range of 0.6 to 0.7) based upon some characteristic of the equipment related to production capacity, such as flow or heat duty. Such scaled costs are easier to calculate and generally give nearly the same result as resizing the equipment for each scenario. The scaling exponent can be inferred from vendor quotes if multiple quotes are given for different sizes, obtained from a standard reference [73-75].

5.3.1. Cost year indices

A cost index is a number that indicates the value of a piece of equipment or a plant at a given time, compared to that of the same unit or plant at a reference time. It is generally not recommended to use cost indices when the time interval is longer than ten years.

$$\text{Present Cost} = \text{Original Cost} * \frac{\text{Index value at the present time}}{\text{Index value at the original time}} \quad \textbf{(5.6)}$$

The cost estimation of the equipments of this plant design exchange rate is assumed to be with $1= 21.55 ETB. The cost indices obtained from literatures are as follows. The Marshal and Swift installed equipment index is obtained (Table 5.3).

Table 5.3: Marshal and Swift equipment index

Year	Process Industry Index
2004	1,468
2010	1,665
2016	1,862
2016 were extrapolated from the earlier data	

5.3.2. Total capital investment (TCI)

The next step is to determine the installed cost of that equipment. The installation cost can be determined by performing a detailed study of everything required to install the necessary equipment and make it operational.

- **Estimation of purchased equipment cost:-** shown below is to show the calculation for present cost of equipments based on the Marshall and Swift cost index of 1000.

1. Equipment cost for fluid pumps:- calculation for estimation of pump cost,

- Identification number P101
- Unit power, given = 10 hp [74]
- Unit power, actual = 0.042
- Exponent, n = 0.3 [74]
- Cost in $ = 4000

The basic calculation is using equation 5.5;

$$C_2 = C_1(\frac{S_2}{S_1})^n = 4000(\frac{0.042}{10})^{0.3} = \mathbf{\$774.52}$$

And also from the equation 5.6;

$$\text{Present cost} = \text{Original cost} * \frac{present\ cost\ index}{base\ year\ index} = 774.52 * \frac{1862}{1000} = \$\ \mathbf{1,442.2}$$

The estimation of the remaining pumps cost based on the above method was calculated and tabulated below (Table 5.4).

Table 5.4: Estimation of equipment cost for fluid pumps

Identification No	Quantity	Exponent	Unit (hp)	Source	Unit cost ($)	Unit cost (ETB)
Pump 101	1	0.3	0.042	[74]	1,442.20	31,079.41
Pump 102	1	0.3	0.026	[74]	1,132.77	24,411.20
Pump 103	1	0.3	0.034	[74]	1,167.50	25,159.63
Total Cost of Fluid Pumps						**80,650.24**

2. Estimation of equipment cost for storage tanks:-calculation for estimation storage tank,

- For VE-101, storage tank for sawdust powder
- Theoretical capacity = 3.8 m^3/hr
- Actual capacity = 1.5 m^3/hr
- Quantity = 1
- Exponent =0.57
- Cost in $ = 5000

The basic calculations;

$$C_2 = C_1(\frac{S_2}{S_1})^n = 5000*(\frac{1.5}{3.8})^{0.57} = \mathbf{\$\ 2{,}843.5}$$

From the above equation 5.6;

$$\text{Present cost} = \text{Original cost}* \frac{present\ cost\ index}{base\ year\ index} = 2843.5*\frac{1862}{1000} = \mathbf{\$\ 5{,}294.60}$$

Table 5.5: Estimation equipment cost of storage tanks

Equipment Name	Quantity	Exponent	Actual capacity (m^3)	Source	Unit cost ($)	Unit cost (ETB)
Sawdust powder storage tank	1	0.57	1.5	[74]	5,294.60	114,098.57
soaking mixture storage tank	1	0.57	3.6	[74]	13,060.01	231,443.22
Acid treated tank	1	0.57	3.8	[74]	13,413.00	289,050.15
Fermented storage	1	0.57	3.9	[74]	12,354.07	266,230.21
Ethanol storage tank	1	0.57	0.4	[74]	1,411.89	30,426.23
Total Cost of Storage Tanks						**931,248.38**

3. Estimation of unit operation equipment costs

Table 5.6: Estimation equipment costs unit operation

Equipment Name	Quantity	Capacity	Unit cost ($)	Unit cost (ETB)
Drum drying	1	1700 kg/hr	5,010	107,965.5
Grinding	1	1650 kg/hr	2,100	45,255
Mixer	1	4 m^3	3,090	66,589.5
Acid Treatment Tank	1	4 m^3	6,570	141,583.5
Centrifuge Separation	1	1000-2000rpm	8,930	192,441.5
Fermentation Tank	1	3.5m^3	2,570	55,383.5
Heat Exchanger	1	3 m^3	3,500	75,425.0
Distillation Tank	1	1 m^3	6,985	150,526.8
Condenser	1	2 m^3	7,650	164,857.5
Boiler	1	-	7,850	169,167.5
Molecular Sieve	1	-	6,190	133,394.5
Total Cost of Process Equipment				**1,302,589.0**
Total Equipment Cost (with10% transportation)				**2,545936.4**

Once the total equipment cost has been determined in the year of interest, in addition to that must added several other direct and indirect costs to determine the TCI. Project contingency and construction activities and other costs related to construction are computed relative to the total direct cost and give the fixed capital investment when summed. The project sum of fixed capital investment and the working capital is the TCI.

Table 5.7: Estimation costs;- direct, indirect and TCI

Direct cost (DC)	Factor	Cost (ETB)
Purchased equipment cost	1	2,545936.4
Purchased equipment installation	0.45	1,145,671.4
Instrumentation and control	0.17	432,809.2
Piping (installed)	0.64	1,629,400
Electrical(installed)	0.11	280,053
Building	0.16	407,350
Yard improvement	0.09	229,134.3

Cont'd table 5.7

Service facilities	0.25	636,484
Total Plant Direct Cost (TPDC)		7,306,837.5
Indirect cost (IDC)	Factors	Cost (ETB)
Engineering and supervision	0.33	840,159
Construction and expense	0.40	1,018,374.4
Total Indirect Cost (IDC)		**1,858,533.4**
Total Indirect and Direct Cost (TIDC)		**9,165,371**
Contractors fee (CF)	0.18	458,268.5
Contingency (C)	0.37	941,996.3
Fixed Capital Investment (FCI)		**10,400,264.8**
Working capital (WC)	0.85	2,164,046
Total Capital Investment (TCI) = FCI +WC		**12,564,310**

5.3.3. Variable operating costs

Variable operating costs, which include raw materials, waste handling charges and by-product credits are incurred only when the process is operating. Quantities of raw materials used and wastes produced were calculated in material balance.

Table 5.8: Direct production costs (Variable costs)

No	Item	Unit cost (ETB)	Total cost (ETB)
1	Raw Materials (RM)	0.29FCI	3,016,077
2	Operating Labor (OL)	0.26FCI	2,704,068.8
3	Direct supervisors and clerical labor	0.1OL	270,407
4	Utilities (electric & water cost)	0.12OL	324,488.3
5	Maintenance and repair	0.06FCI	624,016
6	Operating supplies	0.01FCI	104,002.6
Total Direct Production Cost			**7,043,060**

5.3.4. Fixed operating costs

Fixed operating costs are generally incurred in full whether or not the plant is producing at full capacity. These costs include labor and various overhead items. The number of employees was

estimated by considering the likely degree of automation for each area and adding a reasonable number of management and support employees. Salaries were estimated by using commercially available salary. A 90% labor burden is applied to the salary total and covers items such as safety, general engineering, general plant maintenance, payroll overhead (including benefits), plant security, janitorial and similar services, phone, light, heat, and plant communications.

Table 5.9: Fixed operating costs

No	Item	Unit cost	Total Cost(ETB)
1	Deprecation	0.1FCI	1,040,026.5
2	Local taxes	0.02FCI	208,005.3
3	Insurance	0.005FCI	52,001.3
Total Fixed Charges			**1,300,033**

Table 5.10: Plant overhead cost

No	Item	Unit Cost (ETB)	Total Cost (ETB)
1	Plant over head cost	0.003FCI	**31,201**

Manufacturing cost (MC) = total direct production cost + total fixed charges + plant overhead

Manufacturing cost (MC) = 7043060+ 1300033+ 31201 = **8,374,294 Birr**

Table 5.11: General expense

No	Item	Unit Cost (ETB)	Total Cost (ETB)
1	Administrative cost	0.5OL = 0.5*2704069	1,352,034.4
3	Distribution and sell cost	0.2OL = 0.2*2704069	540,814
Total General Expense			**1,892,848.4**

Total Production Cost = Manufacturing cost + General expense

Total Production Cost = 8374294.0 + 1892848.4 = **10,267,142.4 Birr**

Table 5.12: Financial source of TCI

S/N	Source	Percentage	Amount (ETB)
1	Shareholders' Equity	40%	5,025,724
2	Bank loan	60%	7,538,586
Total		**100%**	**12,564,310**

Table 5.13: Summary item costs

No	Item	Item Cost (ETB)
1	Total capital investment	12,564,310
2	Total production cost	10,267,142.4
3	Variable costs	7,043,060
4	Fixed costs (except depreciation)	260,006.3
5	Depreciation cost	1,040,026.5
6	General expense	1,892,848.4

5.3.5. Feasibility analysis

A feasibility analysis is prepared for the purpose of determining that a proposed investment meets the minimum requirements established by the management.

- **Gross Earn Cost:-** current price 99.9% Ethanol = 8-11 $\frac{Birr}{lit}$, based on current price set by Ethiopian Sugar Corporation. Lets took the cost of Ethanol = 6.8 $\frac{Birr}{lit}$
  - Annual revenue = 6.8*3000000 = **20,400,000 Birr**
  - Total production cost = **10,267,142.4 Birr**
  - Gross annual profit = Annual revenue - Total production cost
    = 20400000 - 10267142.4**= 10,132,858 Birr**
  - Income tax on gross profit (35%) = 0.35*10132858 = **3,546,500 Birr**
  - Net income = Gross annual profit - Income tax on gross profit
    = 10132858 -3546500 **= 6,586,358 Birr**
- **Percent Profit;**

$$\% \text{ Profit} = \frac{Net\ Income}{Total\ Production\ Cost} = \frac{6586358}{10267142.4} = \mathbf{0.64.2 = 64.2\%.}$$

- **Percent Rate Of Return On Investment (ROROI)**:- the discount rate that gives profit with the total capital investment and assume that the net profit is constant throughout the service life of the plant.
  - Net income = 6586358 Birr, and
  - Total capital investment = 12564310 Birr

$\%$ ROROI $= \frac{Average\ Annual\ Net\ Profit}{Total\ Capital\ Investment} = \frac{6586358}{12564310} = \mathbf{52.4\%}$.Therefore, the project will be returned the initial investment cost on the production rate of 52.4%.

❖ Discounted Cash Flow Return (DCFR)

The discount flow rate of return is the return obtained from an investment in which all investment and cash flows are discounted. It was assumed the plant would cease production after ten years of operation, the working capital would be sold at its estimated value and the plant equipment would have a salvage value of hundred thousand Birr. The DCFR of the project was calculated and values at individual years, considering the plant capacity starting with 75% capacity at the first year and 85% capacity in the second year and with 100% capacity the remaining project shelf life.

From the total capital investment, 60% will be borrowed from bank; the rest is from the shareholders' equity and will be returned in a five year with 10% of annual interest.

The income statement and the other indicators of profitability show that the project is viable, including the income tax of 35%.

1. **Net Present Value (NPV)**:- analysis at discount cash flow (DCF, r =12%)

Table 5.14: Cash flow chart

"000 Birr"

Y e a r	0	1	2	3	4	5	6	7	8	9	10
Capac. (%)	-	75	85	100	100	100	100	100	100	100	100
1. Cash in	-	15300	17340	20400	20400	20400	20400	20400	20400	20400	20500
Revenue Salvage V.	- -	15300 -	17340 -	20400 -	20400 -	20400 -	20400 -	20400	20400 -	20400 -	20400 100
2.Cash out	12564	9834	10194	10809	10560	10508	8849	8849	8849	8849	8849
Investment	12564	-	-	-	-	-	-	-	-	-	-
Raw Mater.	-	2262	2564	3016	3016	3016	3016	3016	3016	3016	3016
Utilities	-	243	276	325	325	325	325	325	325	325	325
Labor Sala.	-	2704	2704	2704	2704	2704	2704	2704	2704	2704	2704
Deprecatio.	-	1040	1040	1040	1040	1040	1040	1040	1040	1040	1040
Facto. O.h	-	1323	1499	1764	1764	1764	1764	1764	1764	1764	1764
Capital char	-	1508	1508	1508	1508	1508	-	-	-	-	-
Interest	-	754	603	452	302	151	-	-	-	-	-
Gr. P (1-2)	-12564	5466	8997	9591	9840	9892	11551	11551	11551	11551	11651
Net profit		3553	5848	6234	6396	6430	7508	7508	7508	7508	7573
DCF	1	0.893	0.797	0.712	0.636	0.567	0.506	0.452	0.404	0.351	0.322
PV	-12564	3173	4661	4439	4068	3646	3800	3394	3032	2635	2440

I. **NPV=** $\sum_{0}^{10} PV$ **= 22.73 million Birr,** which is positive, so that the plant should be constructed.

II. **NPVR** $= 1 + \frac{\text{NPV}}{\text{Investment}} = 1 + \frac{2273}{12564} = \mathbf{1.18}$ **,** which is acceptable.

2. Payback period (PBP)

Payback period is the time required after the start of the project to pay off the initial investment from income. It is a useful criterion for judging projects that have a short life, or when the capital is only available for a short time.

It is often used to judge small improvement projects on operating plant. Typically, a payback period of 2 to 5 years would be expected from such projects. Payback period as a criterion of investment performance does not, by definition, consider the performance of the project after the payback period. The investment cost and income statement projection are used to project the payback period.

Table 5.15: Net and Continuous cash flow analysis

Year	0	1	2	3	4	5	6	7	8	9	10
NCF	-12564	3553	5848	6234	6396	6430	7508	7508	7508	7508	7573
CCF	-12564	-9011	-3163	3071	9467	15897	23405	30913	38421	45929	53502

Where;

NCF = net cash flow

CCF = continuous cash flow

PBP = 2 years + $\frac{3163}{6234}$ = **2.5** years or 2 years and 6 months, this is around 3 years. Therefore, the project's initial capital investment will be fully recovered within 3 years.

3. Breakeven point (BEP)

Breakeven point is the production volume the project that will make no profit and no loss at the third year of the plant operation.

BEP = $\frac{\text{Fixed cost}}{\text{Sales}-\text{Variable costs}} = \frac{2600*100}{20400-7043} = \mathbf{19.46}$ %, this is to the required standard (acceptable).

6. CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

This work was intended to study the influence of different hydrolysis process variables (Particle size, hydrolysis acid concentration, hydrolysis temperature, and hydrolysis time) on the quality and quantity of total reduced sugar yield. These hydrolysis process variables are the main factors for the quality and quantity of the fermentable sugars for the cellulosic ethanol production.

This study, a two step pretreatment method was used for the extraction of reducing sugars, for the production of ethanol from sawdust. This method has the advantage of dilute and concentrated acid hydrolyses. In the first step, the sawdust with different particle sizes, 1, 2 and 3mm was soaked in sulfuric acid with different hydrolysis acid concentration such as 3, 5 and 7% at room temperature for 24 hours. In the second step, the mixtures were heated using vertical autoclave reactor for different hydrolysis temperature of 90, 100 and 110^{o}C, and for 120,180 and 240 minutes hydrolysis time. The results after analytical analysis showed that the maximum amount of total reduced sugar yield of 72.51% was achieved at 2mm particle size, 5% acid concentration, 100^{o}C and 180 minutes, whereas the minimum 41.24% total reduced sugar yield was achieved at 1mm, 3%, 90^{o}C and 120 minute.

In this study, response surface method employed the central composite design was used for the optimization of acid hydrolysis process conditions. The effects of hydrolysis process variables, namely particle size, hydrolysis acid concentration, hydrolysis temperature, and hydrolysis time on the total reduced sugar yield were investigated. Total reduced sugar yield of 67.56% was obtained when optimum conditions were particle size of 1.72mm, 4.21% sulfuric acid concentration, 102.46^{o}C and 156.60 minutes, which indicates that at this condition no inhibitors (furfural and HMF) are produced that inhibit the fermentation process. Validation experiments verified the availability and the accuracy of the model with desirability 100%. The predicted value was in agreement with the experimental value (65.53%). Based on this study, it is evident that the chosen method of optimization was efficient and reliable.

In this research, rough economic feasibility analysis of cellulosic ethanol production from sawdust using acid hydrolysis was carried out. The total capital investment required is estimated as 12.56 million Birr. The project is economically feasible with 52.4% rate of return on investment. The payback period is 3 years and the net present value is 22.73 million Birr.

6.2. Recommendations and future studies

Producing cellulosic ethanol from renewable resources is becoming an important issue for the world. Therefore, the work needs to be continued for further process development of cellulosic ethanol production from wood sawdust using acid hydrolysis. From the aforementioned output of the study on the optimization of reducing sugar extraction, for the production of cellulosic ethanol, the following points are recommended,

- ✓ Solid fraction (liquid to solid ratio) is one of the main factors that affect the efficiency of pretreatment or hydrolysis process which wasn't considered during the optimization of hydrolysis process variables. Future studies should include this hydrolysis process variable for maximizing the reducing sugar yield.
- ✓ This study focuses on optimizing the hydrolysis process variables during the extraction of reduced sugar. The production of ethanol is also affected by fermentation process variables. The main factors that affect ethanol yields during fermentation are fermentation temperature, fermentation time and yeast loadings. These weren't optimized in this study. Hence, future studies should focus on the optimization of fermentation process and distillation process variables for maximizing the ethanol yield.
- ✓ Additionally, it is recommended that preliminary design of pilot plant, process development and scale up has to be performed.

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Appendices

Appendix A: Properties of Ethanol

Molecular formula	C_2H_5OH
Molar mass	46.07g/mole
Appearance	Colorless liquids
Density	0.789 g/cm^3 (20^{o}C)
Melting point	-114^{o}C (-173^{o}F,159 K)
Boiling point	78.37^{o}C (173.07^{o}F,351.52 K)
log P	-0.18
Vapor pressure	5.95 kPa (20^{o}C)
Acidity (pk_a)	15.9 (H_2O)
Basicity (pK_b)	-1.9
Refractive index (n_D)	1.361
Viscosity	1.2cp (at 20^{o}C),1.074cp(at 25^{o}C)
Dipole moment	1.69 D (gas)
Latent heat of Vaporization	
Btu/gal at 60^{o}C	2378
Btu/Ib at 60^{o}F	396
Specific heat, Btu/Ib ^{o}F	0.57
Specific gravity,60^{o}F/60^{o}F	0.794
Solubility in water	Fully miscible

Appendix B: Density of Ethanol (Specific gravity versus Ethanol concentration at a given temperature)

%	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	0.90965	0.90580	0.90168	0.89750
1	785	725	636	520	379	217	034	51	0.91943	555	160	760	353	0.89940	519
2	692	542	453	336	194	031	0.98846	52	723	333	0.90936	534	125	710	288
3	426	365	275	157	014	0.98849	663	53	502	110	711	307	0.89896	479	056
4	258	195	103	0.98984	0.98830	672	485	54	279	0.90885	485	079	667	248	0.88823
5	098	032	0.98938	817	670	501	311	55	055	659	258	0.89850	437	016	589
6	0.98946	0.98877	780	656	507	335	142	56	0.90831	433	031	621	206	0.88784	356
7	801	729	627	500	347	172	0.97975	57	607	207	0.89803	392	0.89075	552	122
8	660	584	478	346	189	009	808	58	381	0.89980	574	162	744	319	0.87888
9	524	442	331	193	031	0.97846	641	59	154	752	344	0.89931	512	085	653
10	393	304	187	043	0.97875	685	475	60	0.90027	523	113	680	278	0.87851	417
11	267	171	047	0.97897	723	527	312	61	698	293	0.88882	446	044	615	180
12	145	041	0.97910	753	573	371	150	62	468	062	650	233	0.87809	379	0.86943
13	026	0.97914	775	611	424	216	0.96989	63	237	0.88830	417	0.87998	574	142	705
14	0.97911	790	643	472	278	063	829	64	006	597	183	763	337	0.86905	466
15	800	669	514	334	133	0.96911	670	65	0.88774	364	0.87948	527	100	667	227
16	692	552	387	199	0.96990	760	512	66	541	130	713	291	0.86963	429	0.85087
17	583	433	259	062	844	607	352	67	308	0.87895	477	054	625	190	747
18	473	313	129	0.96923	697	452	189	68	074	660	241	0.86817	387	0.85050	407
19	363	191	0.96997	782	547	294	023	69	0.87839	424	004	579	148	710	266
20	252	068	864	639	395	134	0.95856	70	602	187	0.86766	340	0.85908	470	025
21	139	0.96944	729	495	242	0.95973	687	71	365	0.86949	527	100	667	228	0.84783
22	024	818	592	348	087	809	516	72	127	710	287	0.85850	426	0.84986	540
23	0.96907	689	453	199	0.95929	643	343	73	0.86888	470	047	618	184	743	207
24	787	558	312	048	769	476	168	74	648	229	0.85806	376	0.84041	500	053
25	665	424	168	0.95895	607	306	0.94991	75	408	0.85588	564	134	698	257	0.83809
26	539	287	020	738	442	133	810	76	168	747	322	0.84501	455	013	564
27	406	144	0.95867	576	272	0.94955	625	77	0.85927	505	079	647	211	0.83768	319
28	268	0.95996	710	410	098	774	438	78	685	262	0.84835	403	0.83966	523	074
29	125	844	548	241	0.94922	590	248	79	442	018	590	158	720	277	0.82827
30	0.95977	686	382	067	741	403	055	80	197	0.84772	344	0.83911	473	029	578
31	823	524	212	0.94890	557	214	0.93860	81	0.84950	525	096	664	224	0.82780	329
32	665	357	038	709	370	021	662	82	702	277	0.83848	415	0.82974	530	079
33	502	186	0.94860	525	180	0.93825	461	83	453	028	599	164	724	279	0.81828
34	334	011	679	337	0.93986	626	257	84	203	0.83777	348	0.82913	473	027	576
35	162	0.94832	494	146	790	425	051	85	0.83951	525	095	660	220	0.81774	322
36	0.94986	650	306	0.93952	591	221	0.92843	86	697	271	0.82840	405	0.81965	519	067
37	805	464	114	756	390	016	634	87	441	014	583	148	708	262	0.80811
38	620	273	0.93919	556	186	0.92808	422	88	181	0.82754	323	0.81888	448	003	552
39	431	079	720	353	0.92979	597	208	89	0.82919	492	062	626	186	0.80742	291
40	238	0.93882	518	148	770	385	0.91992	90	654	227	0.81707	362	0.80922	478	028
41	042	682	314	0.92940	558	170	774	91	386	0.81959	529	094	655	211	0.79761
42	0.93842	478	107	729	344	0.91952	554	92	114	688	257	0.80823	384	0.79041	491
43	639	271	0.92897	516	128	733	332	93	0.81839	413	0.80983	549	111	669	220
44	433	062	685	301	0.91910	513	108	94	561	134	705	272	0.79835	393	0.78947
45	226	0.92852	472	085	692	291	0.90884	95	278	0.80852	424	0.79991	535	114	670
46	017	640	257	0.91868	472	069	660	96	0.80991	566	138	706	271	0.78831	388
47	0.92806	426	041	649	250	0.90845	434	97	698	274	0.79846	415	0.78081	542	100
48	593	211	0.91823	429	028	621	207	98	399	0.79975	547	117	684	247	0.77806
49	379	0.91995	604	208	0.90805	396	0.89979	99	094	670	243	0.78814	382	0.77946	507
								100	0.79784	360	0.78934	506	075	641	203

*For data from −78° to 76°C, see p. 2-142, Table 2N-5, *American Institute of Physics Handbook*, McGraw-Hill, New York, 1957. See Tables 2-214 and 2-305 for pure component densities.

Appendix C: Energy content of some fuels compared with ethanol

Fuel type	MJ/L	MJ/kg	Research Octane No
Dry wood (20% Moisture)	-	19.5	-
Methanol	17.9	19.9	108.7
Ethanol	21.2	26.8	108.6
E85 (85% Ethanol, 15% Gasoline)	25.2	33.2	105
Liquefied Natural Gas	25.3	55	-
Autogas LPG (60% Propane + 40% Butane)	26.8	50	-
Aviation gasoline (High-Octane Gasoline)	33.5	46.8	100/130(Lean/Rich)
Gasohol (90% Gasoline + 10% Ethanol)	33.7	47.1	93/94
Regular Gasoline/Petrol	34.8	44.4	Minimum 91
Premium Gasoline/Petrol	-	-	Maximum 104
Diesel	38.6	45.4	25
Charcoal, Extruded	50	23	-

Appendix D: The flash points of ethanol weight concentrations (%)

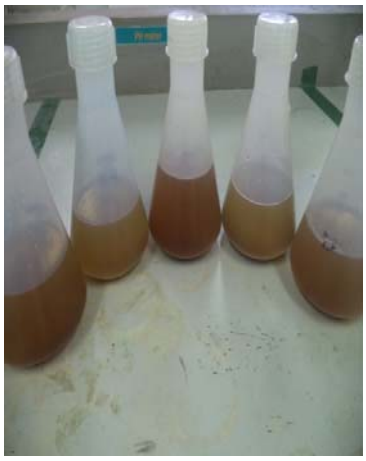
Weight Concentrations (%)	10	20	30	40	50	60	70	80	90	96
Temperature (^{o}C)	49	36	29	26	24	22	21	20	17	17

Appendix E: Laboratory equipments and samples photo

E1: Oven for moisture content

E2: Prepared wood sawdust

E3: Centrifuge



E4: Filtrate sugar solutions E5: Sample products

E6: Total reducing sugar analysis using UV Spectrophotometer