ADDIS ABABA UNIVERSITY

ADDIS ABABA INSTITUTE OF TECHNOLOGY (AAIT)

SCHOOL OF CHEMICAL AND BIOENGINEERING

BIOETHANOL PRODUCTION FROM WASTE PAPER (USED OFFICE PAPER)

By

Kiros Berhane

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

Advisor: Dr. Eng. Abubeker Yimam

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I hereby declare that the thesis is based on my original work except for flotation's and citations which have been duly acknowledged. I also declare that it has not been previously or currently submitted for any other schools at Addis Ababa University or other institutes.

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List of Acronyms

AFEX	Ammonia Fiber Explosion
C_2H_5OH	Ethanol
CO_2	Carbon Dioxide
E10	10 percent Ethanol 90 percent gasoline
E20	20 percent Ethanol 80 percent gasoline
E25	25 percent Ethanol 75 percent gasoline
E5	5 percent Ethanol 95 percent gasoline
E7	7 percent Ethanol 93 percent gasoline
E8	85 percent Ethanol 15 percent gasoline
EU	European Union
FCI	Fixed Capital Investment
FFV	Flexible Fuel Vehicles
FTIR	Fourier Transform Infrared Spectroscopy
GHG	Green House Gas
H_2SO_4	Sulfuric acid
HMF	Hydroxyl Methyl Furfural
IDC	Indirect Costs
ILs	Ionic Liquids
LHW	Liquid Hot Water
MSW	Municipal Solid Waste
PBP	Pay Back Period
pH	power of Hydrogen
ROR	Rate of Return
SO_2	Sulfur Dioxide
TCI	Total Capital Investment
TDC	Total Direct Cost
TRS	Total Reducing Sugar
USA	United States of America
USDA	United States Development Agenda

Abstract

There is a vested interest in developing alternate sources of fuel to fossil fuel due to lowering stocks, increasing prices and the need for environmentally sustainable energy sources. One major alternative to fossil fuels is bio-ethanol (ethanol from biomass) and waste paper (used office paper) represents a significant source for bioethanol production because of its high cellulose and hemicellulose contents.

This study involved ethanol production from waste paper (used office paper) and investigation of process variables (temperature and time) on the yield of bioethanol. The conversion of waste paper to ethanol can be achieved mainly by four process steps: pretreatment of waste paper to remove lignin and hemicellulose, acid hydrolysis of pretreated waste paper to convert cellulose into reducing sugar (glucose), fermentation of the sugars to ethanol using Saccharomyces cerevisiae and finally distillation of the fermented sugar into final product.

A three level full factorial design with two factors (time and temperature) was applied to optimize the acid hydrolysis process and study the interaction effects of the factors. The experimental results were analyzed by using Design Expert 7 software, to investigate the effects of hydrolysis parameters on yield of ethanol. High yield of bioethanol 14.5ml/50g (0.23g/g) was obtained at the optimum parameters, temperature of 140^0C and time 60 min. ANOVA (statistical analysis) showed that an ethanol yield of 14.2426ml/50ml (0.22g/g) was obtained at a temperature of 140^0C and time 63.24 min. Investigation on the preliminary economic analysis of the ethanol production was performed and results from the feasibility study indicated that the proposed work was feasible with rate of return (ROR) of 26% and the payback period of 1.8 year.

Keywords: Bioethanol, waste paper, fermentation, *Saccharomyces cerevisiae*

1 INTRODUCTION

1.1 Background

Ethanol has widespread use as a solvent of substances intended for human contact or consumption, including scents, flavorings, colorings, and medicines. In chemistry, it is both an essential solvent and a feedstock for the synthesis of other products. It has a long history as a fuel for heat and light and also as a fuel for internal combustion engines.

It is widely known that the Germans utilized ethanol as a neat (100 percent pure mixture) fuel in their combat vehicles during World War II, when they were suffering from a shortage of petroleum. So, even at this early date, ethanol had proved to be a viable fuel source. It performed at levels roughly equivalent to those reached by conventional gasoline. This is very surprising, considering that these vehicles were specifically designed to run on conventional gasoline. This is a tribute to the viability of ethanol. Imagine what could be accomplished if engines were designed for ethanol usage in particular. So, in 1978, in response to the oil price shocks that plagued that period, President Jimmy Carter committed the US to a gasohol (10 percent ethanol fuel) production program as part of his administration's emphasis on the utilization of domestic renewable fuel sources. In the 1850s, ethanol was a major fuel for lighting homes and businesses (Young *et al*., 2007).

In 1908, Henry Ford designed the Model T to run on a mixture of gasoline and alcohol, calling it the ''fuel of the future''. When Prohibition began in 1919, ethanol was banned because it was considered as liquor. It could only be sold when it was mixed with petroleum. With the end of Prohibition in 1933, ethanol was used as a fuel again. Ethanol use increased temporarily during World War II when oil and other resources were scarce.

Global depletion of fossil fuels, rising fuel prices, environmental concerns, and pressures for oil independence are creating a strong market for biofuels (USDA ERS, 2009). Biofuels have the potential to be domestically and globally available for energy security, with most being carbon neutral (introducing no additional carbon to the global carbon cycle) or potentially carbon negative (if coupled with carbon sequestration) and supportable within the current agricultural infrastructure (DOE, 2005). Presently, one of the most promising alternatives for petro-fuels is bioethanol. Ethanol is a simple alkyl alcohol that can be used as a transport fuel in spark ignition

engines. It has high octane levels and can be either blended into petrol or used in unmodified vehicles, or run as 100 percent ethanol in a converted engine (Rudkin, 2002).

As a transportation fuel, ethanol can be used as a total or partial replacement of gasoline. All vehicles that run on gasoline can use a blend of 10 percent ethanol and 90 percent gasoline (i.e., E10) without making modifications to their engines. Over 90 percent of the ethanol produced in the United States is mixed with gasoline to make E10. E85 is an alternative fuel that is 85 percent ethanol and 15 percent gasoline. Vehicles are not modified to run on E85; they are specially manufactured as flexible fuel vehicles. A number of flex fuel vehicles are on the road today, including vehicles from Ford, General Motors, Daimler Chrysler, Mercedes, Mercury, Nissan, Mazda and Isuzu.

Unlike fossil fuels, ethanol is a renewable energy source produced through fermentation of sugars. Ethanol is widely used as a partial gasoline replacement in the US. Fuel ethanol that is produced from corn has been used in gasohol or oxygenated fuels since the 1980s. These gasoline fuels contain up to 10 percent ethanol by volume. As a result, the US transportation sector now consumes about 4540 million liters of ethanol annually, about 1 percent of the total consumption of gasoline. Recently, US automobile manufacturers have announced plans to produce significant numbers of flexible-fueled vehicles that can use an ethanol blend E85 (85 percent ethanol and 15 percent gasoline by volume) alone or in combination with gasoline. Using ethanol blended fuel for automobiles can significantly reduce petroleum use and exhaust greenhouse gas emission.

According to European standards, ethanol can be used as a petroleum fuel additive in amounts up to 5 percent for un-modified engines and up to 85 in modified engines (Demirbas et al., 2007). In Brazil all cars run on at least a 25 percent ethanol/petroleum mix, with 60 percent of cars being referred to as "fuel flexible", i.e. these could run on up to 100 percent ethanol (Robson, 2007). In Ethiopia, the blending of Ethanol with Benzene was started in September 2008 with 5 percent Ethanol and 95 percent benzene (MoFED, 2010).

1.2 Statement of the problem

Fossil fuel is depleting day by day throughout the world. This limitation along with the problem of Green House Gas (GHG) emissions leads findings for alternative energy that are environmentally and commercially feasible. Every day, we are dumping lot of cellulosic waste

materials such as waste papers into the environment from which we can produce valuable product such as ethanol to trim down the energy demand on fossil fuel. In this thesis treated waste paper (used office paper) was used to produce bioethanol using dilute acid hydrolysis process.

As a fuel for transport, ethanol is in a fast-growing market, with the EU having set goals of 20 percent of transport fuels being provided by renewables by 2020. The fuels that are competitive today are biodiesel, biogas and ethanol. Ethanol is predominantly made from sugar cane in Brazil, corn in the US and grains in Sweden. All these raw materials result in what is known as first-generation ethanol. Over the last few years, there has been a growing debate on whether ethanol from crops is justified from a resource point of view, with many millions of people starving. The ongoing debate in the EU on a renewable energy supply of 20 percent in electricity production has also made wood-based ethanol production a hot topic.

In general, this study is important as waste paper (used office paper) is a widely available and cheap which is best alternative feedstock for ethanol production and overcomes problems related to energy security, promotes rural development through job creation, promotes environmental conservation and decreases greenhouse gas emission.

1.3 Objectives

1.3.1 General objective

The main objective of this study was to produce bioethanol from waste paper (used office paper) using dilute acid hydrolysis.

1.3.2 Specific objectives

The specific objectives of this study were:

- ✓ To investigate the effect of process variables (hydrolysis temperature and reaction time) on the yield of bioethanol.
- ✓ To specify the optimum operating conditions for maximizing the yield of bioethanol
- ✓ To undertake preliminary economic analysis for bioethanol production from waste paper.
- ✓ To characterize bioethanol by FTIR.

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 bio-fuel, solar, wind, geothermal, and hydrogen.

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 ways to produce fuel. Bioethanol production from lignocellulosic materials will substitute the petroleum derived fuel and minimize the greenhouse gas emissions.

2 LITERATURE REVIEW

2.1 Bioethanol

Ethanol or ethyl alcohol (C_2H_5OH) is an important organic chemical because of its unique properties, and therefore can be used widely for various purposes. Under ordinary conditions, ethanol is a volatile, flammable, clear, colorless liquid, miscible in both water and non-polar solvents.

Bioethanol is generally produced by fermentation of different plant based materials. As the original plant grows, carbon dioxide is absorbed and processed during photosynthesis and later used to synthesize organic compounds such as cellulose. When these plant tissues are used for bioethanol production the carbon fixed during photosynthesis is re-released as carbon dioxide during ethanol fermentation and successive bioethanol combustion in the engines of transport vehicles. The amount of carbon dioxide absorbed and then re-released is equal, making bioethanol a carbon-neutral fuel. Fossil fuels have a positive net carbon dioxide emission and are considered the biggest contribution by humans to climate change.

Bioethanol also has low toxicity, volatility and photochemical reactivity, resulting in less smog production as compared with conventional fossil fuels.

Bioethanol is produced by the fermentation of sugars such as glucose which abound in all plant life. Many of these sugars are readily fermentable into ethanol e.g. glucose. However, a significant portion of these sugars, including glucose, is locked in lignocellulosic materials consisting of lignin, cellulose, and hemicellulose. Cellulose is a homogenous glucose polymer which accounts for 35-50% dry weight of agricultural lignocellulosic biomass. The chemical and crystalline structures of lignin and cellulose make them highly resistant to biodegradation, but they still constitute a large pool of potential raw material for bioethanol production.

Thus, the implementation of processes to hydrolyse the lignocellulosic materials is necessary in order to maximize yield while reducing costs. Already many industrial plants exist for the production of bioethanol in countries across the world, with Brazil currently being the world leader next to USA in the industry due to limited internal fuel resources and the fuel crisis of the 1970's.

Bioethanol is appropriate for the mixed fuel in the gasoline engine due to its high octane number, and its low cetane number and high heat of vaporization impede self-ignition in the

diesel engine. So, ignition improver, glow-plug, surface ignition, and pilot injection are applied to promote self-ignition by using diesel–bioethanol blended fuel (Kim *et al*., 2005). The most popular blend for light-duty vehicles is known as E85. In Brazil, bioethanol for fuel is derived from sugar cane and is used pure or blended with gasoline in a mixture called gasohol (24 percent bioethanol, 76 percent gasoline) (Oliveria *et al*., 2005). In several states of the United States, small amount of bioethanol (10 percent by volume) is added to gasoline, known as gasohol or E10. Blends having higher concentrations of bioethanol in gasoline are also used, e.g. in flexible-fuel vehicles that can operate on blends of up to 85 percent bioethanol E85 (Malca and Freire, 2006).

Some countries have exercised biofuel program involving both form bioethanol–gasoline blend program, e.g. the United States (E10 and FFV E85), Canada (E10 and for FFV E85), Sweden (E5 and for FFV E85), India (E5), Australia (E10), Thailand (E10), China (E10), Columbia (E10), Peru (E10), Paraguay (E7), and Brazil (E20, E25 and FFV any blend) (Kadiman, 2005).

Development of successful biofuel production processes requires striking a balance between ecological preservation, practicality and acceptance of the alternative fuel by the general public, i.e. the ultimate end users. In other words, a successful biofuel cannot compromise on performance, as compared to traditional fossil fuels, but must provide significant ecological advantages. Also, the energy balance must be positive in order for the production of biofuels to be feasible, i.e. the energy obtained from combustion of the biofuels must exceed the energy required for their manufacture.

2.2 Properties of ethanol

2.2.1 Physical properties

Ethanol is a volatile, colorless liquid that has a strong characteristic odor. It burns with a smokeless blue flame that is not always visible in normal light. It is also used in finger nail polish remover.

The physical properties of ethanol stem primarily from the presence of its hydroxyl group and the shortness of its carbon chain. Ethanol's hydroxyl group is able to participate in hydrogen bonding, rendering it more viscous and less volatile than less polar organic compounds of similar molecular weight.

Ethanol is a versatile solvent, miscible with water and with many organic solvents.

Table 2.1 Properties of ethanol

Molecular formula	C_2H_5OH
Molar mass	46.07 g mol^{-1}
Appearance	colorless clear liquid
Density	0.789 g/cm^3
Melting point	−114.3°C, 159 K,-174 °F
Boiling point	78.4°C
Solubility in water	Fully miscible
Acidity (pK_a)	15.9
Viscosity	1.200 mPa·s (cP) at 20.0°C
Dipole moment	5.64 fC·fm (1.69 D) (gas)

2.2.2 Chemical properties

Ethanol is classified as a primary alcohol, meaning that the carbon to which its hydroxyl group is attached has at least two hydrogen atoms attached to it.

2.2.2.1 Acid-base chemistry

Ethanol's hydroxyl group causes the molecule to be slightly basic. It is almost neutral like water. The pH of 100 percent ethanol is 7.33, compared to 7.00 for pure water. Ethanol can be quantitatively converted to its conjugate base, the ethoxide ion ($CH_3CH_2O^-$), by reaction with an alkali metal such as sodium:

$2CH_3CH_2OH + 2Na \rightarrow 2CH_3CH_2ONa + H_2$ …… (2.1)

2.2.2.2 Halogenation

Ethanol reacts with hydrogen halides to produce ethyl halides such as ethyl chloride and ethyl bromide:

$CH_3CH_2OH + HCl \rightarrow CH_3CH_2Cl + H_2O$ …… (2.2)

HCl reaction requires a catalyst such as zinc chloride. Hydrogen chloride in the presence of their respective zinc chloride is known as Lucas reagent.

$CH_3CH_2OH + HBr \rightarrow CH_3CH_2Br + H_2O$ …… (2.3)

HBr requires refluxing with a sulfuric acid catalyst.

2.2.2.3 Ester formation

Under acid-catalyzed conditions, ethanol reacts with carboxylic acids to produce ethyl esters and water:

$$RCOOH + HOCH_2CH_3 \rightarrow RCOOCH_2CH_3 + H_2O \quad \text{(2.4)}$$

For this reaction to produce useful yields it is necessary to remove water from the reaction mixture as it is formed.

Ethanol can also form esters with inorganic acids. Diethyl sulfate and triethyl phosphate, prepared by reacting ethanol with sulfuric and phosphoric acid respectively, are both useful ethylating agents in organic synthesis. Ethyl nitrite, prepared from the reaction of ethanol with sodium nitrite and sulfuric acid, was formerly a widely used diuretic.

2.2.2.4 Oxidation

Ethanol can be oxidized to acetaldehyde, and further oxidized to acetic acid. In the human body, these oxidation reactions are catalyzed by enzymes. In the laboratory, aqueous solutions of strong oxidizing agents, such as chromic acid or potassium permanganate, oxidize ethanol to acetic acid, and it is difficult to stop the reaction at acetaldehyde at high yield. Ethanol can be oxidized to acetaldehyde, without over oxidation to acetic acid, by reacting it with pyridinium chromic chloride. The direct oxidation of ethanol to acetic acid using chromic acid is given below.

$$C_2H_5OH + 2[O] \rightarrow CH_3COOH + H_2O \quad \text{(2.5)}$$

2.2.2.5 Chlorination

When exposed to chlorine, ethanol is both oxidized and its alpha carbon chlorinated to form the compound, chloral.

$$4Cl_2 + C_2H_5OH \rightarrow CCl_3CHO + 5HCl \quad \text{(2.6)}$$

2.3 Bioethanol and its application as a fuel

Bioethanol can be produced either from petrochemical feedstocks by the acid-catalyzed hydration of ethene, or from biomass feedstocks through fermentation. On a global scale, synthetic ethanol accounts for about 3-4 percent of total production while the rest is produced

from fermentation of biomass mainly sugar crops, e.g. cane and beet, and of grains (mainly corn) (Licht, 2006).

Ethanol as a neat fuel or even in the blended form with gasoline has a long history as automotive fuel. In 1860, German inventor Nicholas Otto used ethanol as a fuel in an early prototype of an internal combustion engine because it was widely available throughout Europe for use in spirit lamps. A few years later, Henry Ford built his first automobile with an engine that could run on ethanol. In 1908, Ford unveiled his Model T engine equipped with carburetors that could be adjusted to use alcohol, gasoline or a mixture of both fuels (Solomon *et al*., 2007). Ethyl alcohol as "the fuel of the future" was presented by him for the first time. In 1925, he told the New York Times: “The fuel of the future is going to come from fruit like that sumac out by the road, or from apples, weeds, sawdust almost anything. There is enough alcohol in one year's yield of an acre of potatoes to drive the machinery necessary to cultivate the fields for a hundred years. As an automotive fuel, hydrous ethanol can be used as a substitute for gasoline in dedicated engines. Anhydrous ethanol, on the other hand, is an effective octane booster when mixed in blends of 5 to 30 percent with no engine modification requirement (Licht, 2006).

2.4 World fuel ethanol production

USA is the world leading ethanol producer from corn, producing 14,300 million gallons by the year of 2014. Brazil is the second ethanol producer in the world and produces 6,190 million gallons in 2014. The table below shows world ethanol production of countries at different times.

Table 2.2 World fuel Ethanol production

World Fuel Ethanol Production by Country or Region (Million Gallons)								
Country	2007	2008	2009	2010	2011	2012	2013	2014
USA	6521	9309	10938	13298	13948	13300	13300	14300
Brazil	5019	6472	6578	6922	5573	5577	6267	6190
Europe	570	734	1040	1209	1168	1179	1371	1445
China	486	502	542	542	555	555	696	635
Canada	211	238	291	357	462	449	523	510
Rest of world	315	389	914	985	698	752	1272	1490
World	13,123	17,644	20,303	23,311	22,404	21,812	23,429	24,570

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

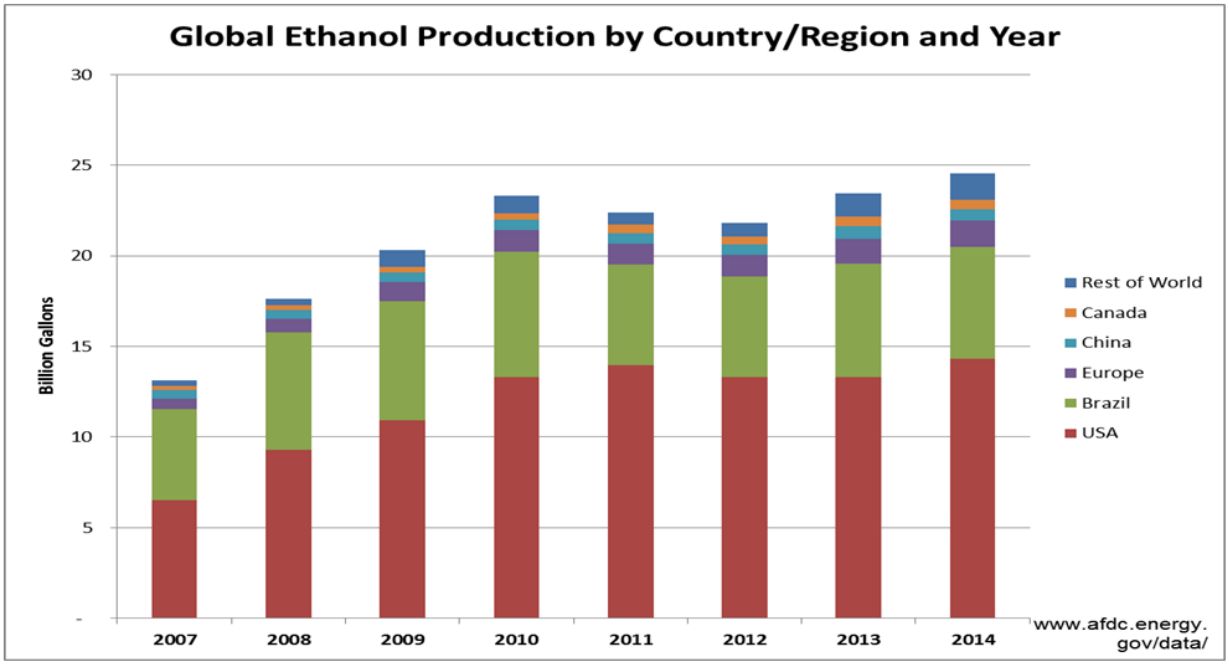


Figure 2.1 Annual ethanol production by the major producers, adapted www.afdc.energy.gov/data

2.5 Current ethanol production in Ethiopia

Bioethanol from sugarcane molasses was produced for the first time at Fincha Sugar Factory. Ethanol is manufactured from microbial conversion of biomass material 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.

Ethiopia has several sugar factories (Fincha, Metehara, Wonji Shoa, Tendaho and Welkait, and currently under construction Omo-kuraz-1, Kesem, two of the Tana Beles factories and Arjo Dedisa) which are run and administered by Sugar Development Agency. Among molasses derived products ethanol takes the largest part, but its utilization must attract the attention of the government policy makers in order to utilize as a bioethanol.

Table 2.3 Ethanol production in Ethiopia in liters

Source: Ethiopia Sugar Corporation

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

2.6 Feedstocks for bioethanol production

Fossil fuel is a non-renewable source of energy and the concern generated by its sustainability, economic and environmental impact led to the use of renewable sources for fuel (Schneider, 1989). Energy from biomass is renewable and can contribute to sustainable development. In addition, biomass resources are often available locally and processing is feasible without high capital investment. Furthermore, dependence on biomass energy can help reduce greenhouse gas emission (Lynd, 1996) thereby impacting on public health. The various raw materials used in the manufacture of ethanol via fermentation are classified into three main types: sugars, starches and cellulose materials (Lin and Tanaka, 2006). Sugars (from sugar cane, sugar beets, molasses and fruits) can be converted to ethanol directly by fermentation. The most widely used sugar for the production of ethanol via fermentation is molasses. Starches (from corn, cassava, potatoes and root crops) and cellulose (from wood, agricultural residues, waste sulphite liquor from pulp and paper mills) must first be hydrolyzed to fermentable sugars.

Enzymes from microorganisms can then ferment the sugars to ethanol. Most agricultural biomass containing starch can be used as potential substrate for ethanol fermentation by microbial processes. These substrates include corn (maize), wheat, oats, rice, potato and cassava.

The sugar and starch based feedstock used in the first and second generation biofuel respectively are still currently being used. However, these raw materials may not be sufficient to meet demands for biofuel production. Moreover, being food crops, the controversy over use in biofuel production lingers (Green *et al*., 2004). Lignocellulosic biomass (plant biomass), offers a great potential resource for the production of bio-fuels because it is largely abundant, inexpensive and its production is environmentally sound in terms of greenhouse gas emission. Cellulose materials represent the most abundant global source of biomass and have been largely unutilized.

Cellulose from wood, agricultural residues, waste sulphite liquor from pulp and paper mills as well dedicated energy crops must be converted to sugars, either by acid or alkali hydrolysis, or by action of cellulase enzymes and the sugar can be fermented to ethanol.

2.7 Lignocellulosic biomass

Lignocellulosic biomass is of various types, from grass and soft plants to hardwood and paper. Agricultural residues are a great source of lignocellulosic biomass which is renewable, chiefly

unexploited, and inexpensive. Such resources include: leaves, stems, and stalks from sources like corn cob, corn stover, sugarcane bagasse, rice hulls, woody crops, and forest residues. Also, other multiple sources of lignocellulosic waste from industrial and agricultural processes include citrus peel waste, sawdust, paper pulp, industrial waste, municipal solid waste, and paper mill sludge. In addition, dedicated energy crops for biofuels could include perennial grasses such as switch grass and other forage feedstocks such as Miscanthus giganteus, Bermuda grass, elephant grass, poplar etc. (Maki *et al*., 2009).

Lignocelluloses are complex substrates composed of a mixture of carbohydrate polymers i.e. cellulose and hemicelluloses tightly bound to lignin, a complex aromatic polymer, mainly by hydrogen bonds but also by some covalent bonds. Lignin interferes with cellulose hydrolysis because it acts as a physical barrier that prevents the contact of cellulase to cellulose in enzymatic hydrolysis. The first step in biofuel production from lignocelluloses therefore is delignification to liberate cellulose and hemi celluloses from their complex with lignin.

2.7.1 Cellulose

Cellulose was first described by Anselme Payen in 1938 as a resistant fibrous solid that remains behind after treatment of plant tissues with acid and ammonia (Brown, 2007). Cross and Bevan in early 1900's designated plant materials which do not dissolve in concentrated sodium hydroxide solution as alpha cellulose, meaning true cellulose; and the soluble materials were designated beta and gamma cellulose. The beta and gamma cellulose were later shown to be simple sugars and other carbohydrates, but not cellulose.

Cellulose is a complex carbohydrate consisting of 1000 – 3000 or more glucose units in a linear chain structure that can pack into fibers of great tensile strength. Cellulose is the most abundant renewable bio-resource produced in the biosphere. It is a major component of the cell wall of plants; about 25 – 30 percent of mosses and seaweeds and 40 – 50 percent of trees. Cotton fibers consist of about 90 – 98 percent of cellulose.

The cellulose chain has a large number of free hydroxyl groups which allow it to react with acid to form esters as well as with alcohol to form ethers (Shanbhag *et al*., 2007). Industrially, it is used mainly to produce paper and converted to a wide variety of derived products such as cellophane, rayon, guncotton, methyl cellulose and carboxyl methyl cellulose.

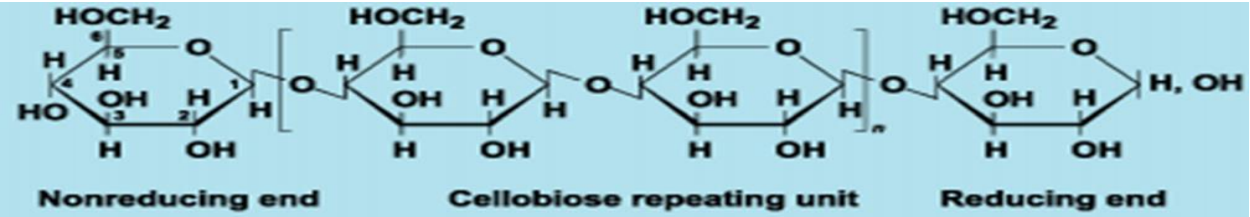


Figure 2.2 Chemical structure of cellulose showing the repeating unit of anhydrocellobiose (Demirbas, A., 2005)

2.7.2 Hemicelluloses

Hemicellulose is a heterogeneous polymer, which varies in composition from plant to plant and within different parts of the same plant. It is made up of mainly pentoses (D-xylose, D-arabinose), hexoses (D-mannose, D-glucose, and D-galactose) and sugar acids (Maki *et al*., 2009). In hardwoods hemicellulose contains mainly xylans, while in softwood, mainly glucomannans are present. Hydrolysis of hemicelluloses requires various types of enzymes. For example, xylan degradation alone requires endo-1-4,-β-xylanase, β-xylosidase, α-glucuronidase, α-L-arabinofuranosidase, and acetylxylan while in glucomannan degradation β-mannanase and β-mannosidase are required to cleave the polymer backbone (Maki *et al*., 2009).

2.7.3 Lignin

Lignin is the third component of lignocellulosic biomass, its main function is to surround and protect the cellulose and hemicellulose from degradation of microorganisms in plants. It constitutes around 20-30 percent of plant and wood biomass. Lignin is a complicated structure of various compounds. The alcohols p-coumaryl-, coniferyl- and sinapyl-alcohol are the precursors of the most common building blocks in lignin. Softwood lignins are predominantly polymers of coniferyl alcohol. Hardwood lignins are made from coniferyl and sinapyl alcohol and lignin in grass contain all the three.

Table 2.4 the contents of cellulose, hemicellulose and lignin in common bioethanol feedstocks (Adapted from Chen at al., 2007)

Lignocellulosic material	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Hardwoods stems	40-55	24-40	18-25
Softwoods stems	45-50	25-30	30-40
Nut shells	25-30	25-30	30-40
Corn cobs	45	35	15
Grasses	25-40	35-50	10-30
Paper	85-99	0	0-15
Wheat straw	30	50	15
Sorted refuse	60	20	20
Leaves	15-20	80-85	0
Cotton seed hairs	80-95	5-20	0
Newspaper	40-55	25-40	18-30
Waste paper	60-70	10-20	5-10
Primary wastewater solids	8-15	NA	24-29
Swine waste	6.0	28	NA
Solid cattle manure	1.6-4.7	1.4-3.3	2.7-5.7
Coastal Bermuda grass	25	35.7	6.4
Switch grass	45	31.4	12

2.8 Waste paper materials to ethanol production

Among cellulosic biomass, waste paper presents a unique opportunity for bioethanol production for several reasons. First, it is a cost competitive feedstock due to the available infrastructure and networks to collect and process waste paper (Dale & Musgrove, 2009). Second, although cellulosic materials are less expensive than corn, they are more costly to convert to ethanol because of the extensive processing required associated with feedstock preparation and pretreatment to open lignocellulosic structures (Di Pardo, 2004). Third, converting recovered

paper biomass to ethanol also has environmental benefits. It not only increases the net energy balance of ethanol compared to converting corn to ethanol (Di Pardo, 2004), but also reduces the volume of landfilled MSW and it avoids generating hazardous chemicals during anaerobic digestion (Jeffries & Schartman, 1999).

Besides recycling waste paper materials as a main or supplemented feedstock for paper production, waste paper is also considered as a potential candidate for bio-fuel production (Duff & Murray, 1996).

Waste paper to ethanol has been studied in terms of pretreatment techniques, enzymatic hydrolysis and fermentation conditions based on different paper grades. Generally, wastepaper fiber from chemical pulps contains 60-70% cellulose, 10-20% hemicellulose and 5-10% lignin (Sun & Cheng, 2002).

2.8.1 Office paper

Office paper has high cellulose and low lignin content since it is mostly bleached grade paper. It also contains ash in the range of 5-30% since inorganic fillers like calcium carbonate and clay are added to improve printing properties (Wayman *et al*., 1992). Office paper with low ash content generally requires no pretreatment (Wayman *et al*., 1992) or very mild acid neutralization (Shi *et al*., 2009) to achieve high sugar conversion. It was believed that ultrasonic cavitation disassembled the relatively smooth surface of cellulose fibers into component micro fibrils, which enabled better enzyme digestibility.

Waste paper consists of a considerable share of municipal and industrial waste even though recycling efforts have been strengthened in recent years by legal provisions like the packaging directive (Forum for Agricultural Research in Africa, 2008). However, the recycling rate of waste paper is low and the recycled waste paper has a low grade paper product because of shorted fiber length. Since the shortening of paper fibers decreases the quality of paper, the maximum ratio of paper-to-paper recycling is said to be 65 percent (Ikeda *et al*., 2006). This means that a certain fraction of paper would always be sent to disposal. Still, waste paper is considered as one of the prospective and renewable biomass materials to produce bioethanol.

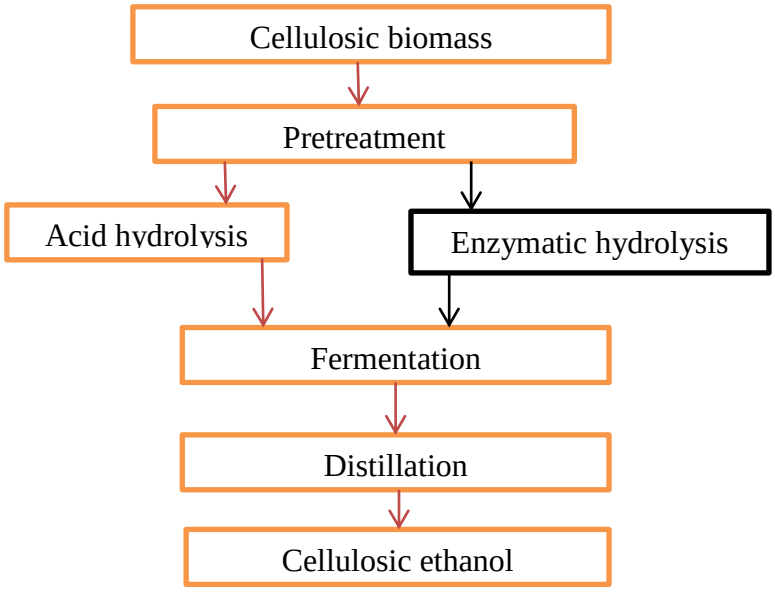


Figure 2.3 Cellulosic ethanol production processes (Zacchi and Galbe, 2002)

2.9 Pretreatment of biomass to ethanol

An ideal pretreatment for lignocellulosic biomass should provide the following desirable properties:

- Produce accessible and reactive fibers
- Yield carbohydrates in non-degraded form
- Avoid formation of carbohydrate and lignin-derived byproducts that inhibit subsequent hydrolysis and fermentation
- Produce no solid residues
- Work in reactors of reasonable size with moderate cost
- Be cost-effective with a high degree of simplicity
- Be effective at low moisture contents

The categories of available pretreatment options are: physical, physico-chemical, chemical, and biological processes. Since different lignocellulosic materials have different physico-chemical characteristics, it is necessary to adopt suitable pretreatments technologies based on the lignocellulosic biomass properties of each raw material.

2.9.1 Physical pretreatment

The purpose of physical pretreatment is to mechanically reduce biomass particle size, cellulose crystallinity and degree of polymerization as well as increasing specific surface area.

Physical approaches on biomass to ethanol involves mechanical size reduction, internal delamination and surface fibrillation such as chipping, grinding, milling, extrusion, refining, pyrolysis etc. (Jones *et al*., 2013; Sun & Cheng, 2002). In addition, irradiation by gamma ray, electron beam and microwave have also been evaluated (Taherzadeh & Karimi, 2008). Generally, chemicals are not required when doing physical pretreatment. However, most methods are high energy-demanding with little lignin removal. Hence, physical pretreatment like refining is combined with chemical pretreatments (usually as a post-treatment) to lower the overall energy and chemical demand, while still achieving desirable conversions without too much sugar degradation (Chen *et al*., 2012; Wu *et al*., 2010).

2.9.2 Physico-chemical pretreatment

Physico-chemical pretreatment usually refers to the explosion method for pretreatment of lignocellulosic materials. Steam explosion is one of the common and simple methods that involve only water with a reaction temperature ranging from 160 to 260ºC (Sun & Cheng, 2002). This process mainly converts hemicellulose into soluble oligomers with little impact on lignin degradation (Fernandez-Bolanos *et al*., 2001). It also generates byproducts such as acetic acid (hydrolysis of acetyl groups linked to sugars), furfural (product of pentose dehydration – xylose and arabinose) and hydroxyl methyl furfural (HMF) (dehydration of hexoses – glucose, galactose and mannose) (Gamez *et al*., 2006). Furfural could be further degraded into formic acid and HMF into formic and levulinic acid (Taherzadeh & Karimi, 2007). Since these degradation products are inhibitory to enzymatic hydrolysis, and fermentation, a washing step is necessary to remove the inhibitory materials prior to enzymatic hydrolysis (Sun & Cheng, 2002). Auto hydrolysis or liquid hot water (LHW) is another pretreatment that does not require chemicals. Compared to stream explosion, auto hydrolysis is conducted at a similar temperature range of 130 to 260ºC (Lee *et al*., 2010). However, it requires higher water demand and does not require rapid decompression. As a result, higher hemicellulose recovery and lower inhibiting byproducts are obtained (Alvira *et al*., 2010).

To improve pretreatment efficiency, steam explosion with the addition of chemicals such as H_2SO_4 (or SO_2), CO_2 and ammonia fiber explosion (AFEX) can be used (Sun & Cheng, 2002). SO_2 explosion is a method using either SO_2 or H_2SO_4 impregnation prior to steam explosion at a temperature of 160 to 230ºC with the aim of mainly degrading hemicellulose (Eklund *et al*., 1995). It was reported that the addition of SO_2 yielded higher glucose compared to H_2SO_4 even though higher hemicellulose loss occurred (Eklund *et al*., 1995). CO_2 explosion is based on the utilization of CO_2 as a supercritical fluid to effectively remove lignin and disrupt cellulose structure with low inhibitor formation (Alvira *et al*., 2010). AFEX is a kind of alkali physico-chemical pretreatment process where the biomass is exposed to liquid ammonia at 90-100°C for a certain period of time (e.g. 30 min) and followed by an immediate reduction of pressure, leaving only the pretreated solid material. The AFEX process does not significantly solubilize hemicellulose and can partially remove or modify lignin fraction. Although inhibitory byproducts are not produced in this process, washing is still recommended due to the existence of lignin fragments and cell wall extractives on the cellulose surface. In addition, the AFEX method was reported to have little effect on substrate with high lignin content (above 20%) and the cost of the process is also relatively high (Sun & Cheng, 2002).

2.9.3 Chemical pretreatment

2.9.3.1 Acid pretreatment

Inorganic acids such as H_2SO_4, HCl and HNO_3 and organic acids such as fumaric and maleic acid are used to treat lignocellulosic materials. Early studies show that concentrated acid (30-70 percent) at low temperature (e.g. 40°C) is effective in terms of breaking down lignocellulosic structure and achieving high sugar conversion (Sivers & Zacchi, 1995). However, interest has been reduced in this process due to corrosive conditions, high cost on non-metallic or alloy equipment and the challenge of acid recovery.

Dilute acid pretreatment using H_2SO_4 appears to be more favorable for practical application and have been studied for pretreating a wide range of lignocellulosic biomass materials. This is typically conducted using low acid concentration and can be performed at lower temperatures (less than 160°C) for longer periods of time (30-60 min); or at higher temperature (e.g. 160-240°C) for shorter periods of time (e.g. 10 min) (Taherzadeh & Karimi, 2007). The mechanism behind this process is the solubilization of hemicellulose, and also converts solubilized hemicellulose to fermentable sugars. It is not effective in lignin removal, but can disrupt lignin

to a certain extent to increase enzyme accessibility to cellulose (Yang & Wyman, 2004). High hydrolysis yields have been reported when pretreating lignocellulosic biomass with diluted H_2SO_4. However, several draw-backs are present in the dilute acid pretreatment method. First, the pH of the process stream following pretreatment must be neutralized to the optimum range of enzymes for hydrolysis and further adjustments are necessary for fermentation. This process also suffers from low yields because of sugar decomposition. Sugar degradation in a single stage dilute acid pretreatment process tend to form furfural, HMF, carboxylic acids, furans and phenolic compounds, which are potentially inhibitory to fermentation. In order to reduce sugar degradation, studies have been done using two or more stages of dilute acid hydrolysis with mild conditions in the first stage and more severe conditions in the subsequent stage(s) (Taherzadeh & Karimi, 2007).

2.9.3.2 Alkali pretreatment

Alkali pretreatment refers to the utilization of base chemicals in the pretreatment process. A wide range of alkaline chemicals have been studied for pretreatment use such as: NaOH, Ca $(OH)_2$ (lime), Na_2CO_3, $NaHCO_3$, ammonia and furthermore, SO_2 in alkaline conditions have also been applied (Jin *et al*., 2010; Taherzadeh & Karimi, 2008). The mechanism behind the alkali pretreatment process is to swell lignocellulosic materials, causing disruption in lignin structures and linkages between lignin and carbohydrates. An increase of internal surface area and decrease in cellulose crystallinity and degree of polymerization was observed (Sun & Cheng, 2002). Alkali pretreatment has been shown to cause less sugar degradation than acid pretreatment and it was reported to be more effective on non-woody (agricultural residues) than on woody biomass (Alvira *et al*., 2010).

There is also a consensus that dilute alkaline pretreatment is more effective on hardwood than softwood (Sun & Cheng, 2002; Wu et al., 2010).

To achieve higher lignin removal, alkali pretreatment can be combined with ammonia, O_2 or H_2O_2 at temperature of 100-220°C. High sugar yields in enzymatic hydrolysis with effective lignin removal (40-85%) has been reported in these alkali based wet oxidation techniques (Iyer et al., 1996; Koo et al., 2011; Martin et al., 2007; Saha & Cotta, 2007).

2.9.3.3 Ozonolysis

Ozonolysis pretreatment by ozone is very effective in degrading lignin in a short period of time. It can be performed in high (e.g. 35 percent) or low (e.g. 1 percent) substrate consistency. Since ozone reacts with lignin in the gaseous phase, it was reported that ozone pretreatment conducted with high substrate consistency was more effective than with low consistency (Neely, 1984). Neither high temperatures nor pressures are involved in the ozonolysis pretreatment process and inhibitory residues are not generated. Thus the process is exceedingly well suited for scaling-up or scaling-down with design simplicity. However, the main drawback of ozonolysis is the large amount of ozone required that makes this process economically undesirable (Alvira et al., 2010).

2.9.3.4 Ionic liquids (ILs) pretreatment

ILs (e.g. 1-ethyl-3-methylimidazolium acetate) are salts, typically composed of large organic cations and small inorganic anions, which exist as liquids at wide ranges of temperatures (usually lower than 100°C) (Alvira *et al*., 2010). Due to their high thermal stability and nearly complete non-volatility, non-flammability, low vapor pressures and non-toxicity, ILs are effective.

ILs can be used as a "green solvent" to break the internal lignin and hemicellulose bonds (Lee *et al*., 2009). In addition, ILs pretreatment is one of the few processes that enable lignin and hemicellulose solubilization and recovery. A lower degree of crystallinity on dissolved cellulose was observed using this process. However, some concerns that ILs residue on the substrate may negatively affect enzyme and yeast performance. Development of energy efficient recycling methods for ILs is a prerequisite for both economic and technical concern since ILs recovery is necessary for cost reduction. Due to the immaturity of this technique and high cost of overall operation, this process is still commercially unavailable (Alvira et al., 2010).

2.9.3.5 Organosolv

Organosolvation is a pretreatment process utilizing organic or aqueous solvent mixtures to solubilize lignin and possibly a part of hemicellulose that results in easier access to cellulose for enzymatic hydrolysis (Pan *et al*., 2006). A number of solvents can be used including methanol, ethanol, acetone, ethylene glycol and tetrahydrofurfuryl alcohol. Acid catalysts (e.g. HCl, H_2SO_4, oxalic or salicylic) have been studied in order to lower the reaction temperature and

obtain high yields of xylose. High temperatures (above 18°C) facilitate delignification to an ideal extent that acid catalysts become unnecessary (Alvira et al., 2010). One advantage of Organosolv pretreatment is that this process produces two relatively pure byproducts, high - quality reactive lignin and an aqueous hemicellulose stream. However, the Organosolv pretreatment process is relatively more expensive than steam explosion and the commercial feasibility highly depends upon complete recovery of the organic solvents (Duff & Murray, 1996).

2.9.4 Biological pretreatment

Among all the methods, fungal pretreatment provides the mildest environmental condition with low capital and energy cost, no chemical requirements and unit operation simplicity. Microorganisms such as brown, white and soft-rot fungi have been used. Brown rots mainly attack cellulose, while white and soft rots attack both cellulose and lignin (Sun & Cheng, 2002). Lignin degradation by white-rot fungi is the most effective for biological pretreatment of lignocellulosic materials. It degrades lignin and hemicellulose with major cellulose preservation (Shi *et al*., 2008). The major drawbacks for moving this biological pretreatment forward is the low hydrolysis rate in the processes (Sun & Cheng, 2002). Thus, biological study of more basidiomycete's fungi or even genetic modification can enable more efficient fungi delignification.

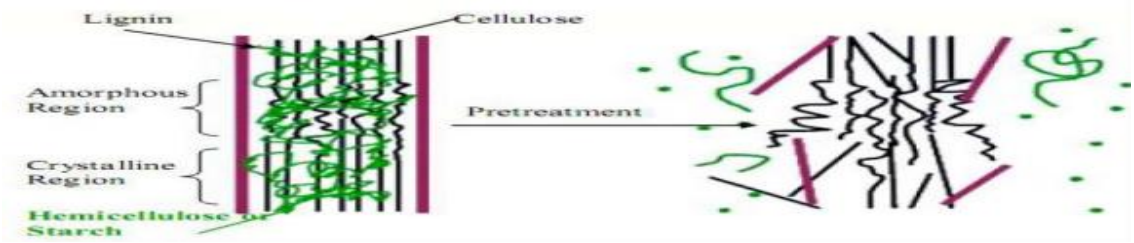


Figure 2.4 Schematic representation on biomass pretreatment (Mosier et al, 2004

Table 2.5 Summary of various processes used for the pretreatment of lignocellulosic materials (Adapted from Parveen Kumar *et al.*, 2009)

Pretreatment process	Advantages	Limitations and disadvantages
Mechanical comminution	Reduces cellulose crystallinity	Power consumption usually higher than inherent biomass energy
Steam explosion	Causes hemicellulose degradation and lignin transformation; cost-effective	Destruction of a portion of the xylan fraction; incomplete disruption of the lignin carbohydrate matrix; generation of compounds inhibitory to microorganisms
AFEX	Increases accessible surface area, removes lignin and hemicellulose to an extent; does not produce inhibitors for downstream processes	Not efficient for biomass with high lignin content
Ozonolysis	Reduces lignin content; does not produce toxic residues	Large amount of ozone required; expensive
Acid hydrolysis	Hydrolyzes hemicellulose to xylose and other sugars; alters lignin structure	High cost; equipment corrosion; formation of toxic substances
Alkaline hydrolysis	Removes hemicelluloses and lignin; increases accessible surface area	Long residence times required; irrecoverable salts formed and incorporated into biomass
Organosolv	Hydrolyzes lignin and hemicelluloses	Solvents need to be drained from reactor, evaporated, condensed, and recycled; high cost
Pyrolysis	Produces gas and liquid products	High temperature; ash production
Biological	Degrades lignin and hemicelluloses; low energy requirements	Rate of hydrolysis is very low

2.10 Hydrolysis of lignocellulosic materials

After the pretreatment process, there are two types of processes to hydrolyze the feedstocks for fermentation into ethanol, most commonly used are acid (dilute and concentrated) and enzymatic hydrolysis (Demirbas, 2005).

The cellulose molecules are composed of long chains of glucose molecules. In the hydrolysis process, these chains are broken down to free the sugar, before it is fermented for alcohol production. There are two major hydrolysis processes: a chemical reaction using acids, or an enzymatic reaction.

2.10.1 Acid hydrolysis

Acid hydrolysis is a possible process for treating lignocellulosic materials such as waste papers, wood chips (Silva 1996), rice straw (Almeida 1991), sugar beet pulp and wheat straw. According to Parisi (1989), the mineral acids act simply and rapidly as reaction catalyzers of polysaccharide fractions. Sugarcane bagasse can be hydrolyzed using dilute acid to obtain a mixture of sugars with xylose as the major component.

However, in the hydrolyzate some byproducts generated in the hydrolysis, such as acetic acid, furfural, phenolic compounds, or lignin degradation products, can be present. These are potential inhibitors of a microbiological utilization of this hydrolyzate.

Processes such as two-stage acid hydrolysis can be employed to produce xylose and glucose (Beck, 1986). Treatment with dilute sulphuric acid at moderate temperatures (the first stage of acid hydrolysis) has proven to be an efficient means of producing xylose from hemicellulose.

In general, acid treatment is effective in solubilizing the hemicellulosic component of biomass. Proper combinations of pH, temperature, and reaction time can result in high yields of sugars, primarily xylose from hemicellulose (Elander and Hsu 1995).

2.10.2 Enzymatic hydrolysis of cellulose

Enzymatic hydrolysis of cellulose is carried out by cellulase enzymes, which are highly specific. The products of the hydrolysis are usually reducing sugars including glucose.

Utility cost of enzymatic hydrolysis is low compared to acid or alkaline hydrolysis because enzyme hydrolysis is usually conducted at mild conditions (pH 4.8 and temperature 45–50°C) and does not have a corrosion problem. Both bacteria and fungi can produce cellulase for the hydrolysis of lignocellulosic materials. These microorganisms can be aerobic or anaerobic, mesophilic or thermophilic. Bacteria belonging to Clostridium, Cellulomonas, Bacillus, Thermomonospora, Ruminococcus, Bacteriodes, Erwinia, Acetovibrio, Microbispora, and Streptomycescan produce cellulases. Although many cellulolytic bacteria, particularly the

cellulolytic anaerobes such as Clostridium thermocellumand and Bacteroides cellulosolvens produce cellulases with high specific activity, they do not produce high enzyme titres. Because the anaerobes have a very low growth rate and require anaerobic growth conditions, most research for commercial cellulase production has focused on fungi.

Cellulases are usually a mixture of several enzymes. At least three major groups of cellulases are involved in the hydrolysis process: (1) endoglucanase which attacks regions of low crystallinity in the cellulose fiber, creating free chain-ends; (2) exoglucanase or cellobiohydrolase which degrades the molecule further by removing cellobiose units from the free chain-ends; (3) β-glucosidase which hydrolyzes cellobiose to produce glucose. During the enzymatic hydrolysis, cellulose is degraded by the cellulases to reducing sugars that can be fermented.

2.11 Fermentation process

The fermention of the biomass is conducted under standard fermention 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* (Cellulosic ethanol, 2010).

A variety of microorganisms, generally either bacteria or yeast, ferment carbohydrates to ethanol under oxygen-free conditions.

$$3C_5H_{10}O_5 \longrightarrow 5C_2H_5OH+5CO_2 \quad \text{..... (2.7)}$$

$$C_6H_{12}O_6 \longrightarrow 2C_2H_5OH+2CO_2 \quad \text{..... (2.8)}$$

Methods for C_6 sugar fermentation were already known at least 6000 years ago, when Sumerians, Babylonians and Egyptians began to perfect and describe the process of making beer from grain (starch). After it became possible to free the C6 sugars in lignocellulosic crops (end 19^{th} century), conversion of the C_5 sugars became interesting. They represent a high percentage of the available sugars, the ability to recover and ferment them into ethanol is important for the efficiency and economics of the process. Only in the 1980s research on xylose fermentation began to bear fruit, when a number of wild type yeast were identified that could convert xylose to ethanol.

Bacteria have drawn special attention from researchers because of their speed of fermentation. In general, bacteria can ferment in minutes as compared to hours for yeast.

All microorganisms have limitations: either in the inability to process pentoses and hexoses, the low yields of ethanol, or the co-production of cell mass at the cost of ethanol. Furthermore, the oxygen free condition of fermentation slowly exterminates the microorganism population. When hydrolysis and fermentation reactions are connected directly, intermediate inhibitive products are avoided, and the yield is potentially higher. Also, genetic engineering and new screening technologies will bring bacteria and yeast that are capable of fermenting both glucose and xylose, although fermentation of xylose and arabinose remains problematic. Near-term fermentation using genetically engineered yeast or bacteria may even utilize all five of the major biomass sugars–glucose, xylose, mannose, galactose and arabinose. Mid- to long-term technology will improve the fermentation efficiency of the organism (yielding more ethanol in less time), as well as its resistance, requiring less detoxification of the hydrolysate.

The fermenting bacteria and yeast are grown in series of aerated seed reactors. These consume a side-streamed carbohydrate fraction (9 percent of the cleaned hydrolysate), and some protein nutrients. The consolidation of conversions in fewer reactors has impact on the total process integration.

The hydrolysis and fermentation can be performed in two separate units, which is referred to as separate hydrolysis and fermentation (SHF), or a combined unit, which is referred to as simultaneous Saccharification and fermentation (SSF). Two principal approaches for SSF can be taken:

(1) The process can take place with one microorganism. This approach is often referred to as consolidated bioprocessing (CBP);

(2) The process can take place with an enzyme for hydrolysis of lignocellulosic material in combination with a microorganism to produce ethanol from different sugar monomers.

2.11.1 Effect of fermentation parameters

Microorganisms for ethanol fermentation can best be described in terms of their performance parameters and other requirements such as compatibility with existing products, processes and

equipment. The performance parameters of fermentation are: temperature, pH, alcohol tolerance, and inhibitor tolerance (Demirbas, 2005).

2.11.1.1 Temperature

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

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

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

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

2.11.1.2 pH

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

2.11.1.3 Sugar

The concentration of sugar can affect the microbial ethanol fermentation in various ways. The amount of ethanol produced is proportional to the amount of sugar added; thus, high sugar concentrations are desired. However, too high sugar concentrations can inhibit metabolism due to increased osmotic pressure. Very low levels of sugar, on the other hand, may limit the rate of ethanol production. Hence, each fermentation process will have an optimal glucose or equivalent sugar concentration (Sofer and Zaborsky, 1981). A sugar concentration of 10-18% is usually satisfactory, although other concentrations are used (Dunn, 1959).

2.11.1.4 Inhibitor Tolerance

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

1) Compounds originating in the biomass by hydrolysis. These include organic acids such as acetic, glucuronic and galacturonic acids from the hemicellulose, and 28 phenolic compounds from the lignin. The most inhibitor of these for both yeast and bacteria is acetic acid and solubilized lignin.

2) Compounds formed by degradation of the products resulting from pretreatment and hydrolysis of the biomass. Furfural from xylose and HMF from glucose lead this group. It is completed by an assortment of aldehydes, acids and alcohols from lignin, sugar and protein degradation.

3) Compounds from other sources. Metal ions resulting from equipment corrosion, sulfites, SO_2 and lactic acid introduced with other streams containing nutrients, cleaning solutions and backset.

2.12 Distillation

After fermentation, the fermented product is transferred to a distillation process where the ethanol is separated from the remaining stillage (residue non-fermentable solids and water). Distillation is the process in which a liquid or vapor mixture of two or more substances is separated into its component fractions of desired purity by the application or removal of heat.

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 azeotrope of 95

percent ethanol which has the lowest boiling point. If an efficient fractionating column is used, 95 percent 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 percent alcohol cannot be further concentrated by distillation because the vapor has exactly the same composition as the liquid; towards distillation, then, 95 percent alcohol behaves exactly like a pure compound (Jackman, 1987).

2.13 Dehydration

Ethanol from distillation process is sent to the molecular sieves column for further dehydration to produce 99.9 percent v/v ethanol. After distillation, about 5 percent 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 inhomogeneous fuel is not acceptable. Thus, dehydration can be another issue (Onuki, 2005). 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 95-96 percent due to the formation of a low boiling water-ethanol azeotrope. 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 azeotrope distillation.

3 MATERIAL AND METHODS

3.1 Materials used for the experiment

3.1.1 Equipment

The following equipments were used during the experiment.

Plastic bags to collect and transport samples to the laboratory, scissor for cutting the waste paper in to pieces, oven (GALLENKAMP) to dry the sample, crushers to crush the dried sample, vacuum filter (model BN 3 STAATLICH, Berlin), balances (ADAM, PW 124) to weigh samples. digital pH meter (pH meter 3310, JENWAY) to measure the pH of the hydrolyzate before fermentation, thermostats to control temperature of the sample under experiment (fermentation and distillation) isothermally at the set point, rack:- to hold samples, vessels to hold samples and additives for hydrolysis, fermentation and distillation experiments, graduated cylinders of different volumes for volume measurement, autoclave (Sanoclave) for sterilization and hydrolysis, pycnometer for density measurement, shaker (EXCELLA E24R) to shake sample and its additives after hydrolysis and before fermentation and fermentation and distillation set ups to ferment and distill respectively.

3.1.2 Chemicals

The following chemicals were used during the experiment.

Sulfuric Acid (98% H_2SO_4, England, bought from Neway plc.) used as catalyst for hydrolysis of waste paper, sodium hydroxide (NaOH, min. assay 98%, England, bought from Neway plc.) used to adjust the pH of soluble cellulose and hemicelluloses before fermentation, benedict's reagent to estimate glucose concentration, yeast extracts (Agar) used as nutrient in media preparation, urea used as nutrient in media preparation, dextrose sugar used as nutrient in media preparation, Mg SO_4.7 H_2O used as nutrient in media preparation, peptone used nutrient in media preparation and yeast *Saccharomyces cerevisiae* (Arif instant).

3.1 Experimental procedures

The study was intended at investigation of acid hydrolysis parameters in the production of ethanol from waste paper. Waste paper (used office paper) was obtained from Addis Ababa

institute of technology. It was collected in plastic bags and transported to the laboratory for ethanol production.

The followings were the basic steps for the production of ethanol alcohol

- ✓ Sample collection.
- ✓ A pre- treatment phase (size reduction) to make the waste paper agreeable to hydrolysis.
- ✓ Hydrolysis to break down the molecules of cellulose and hemicelluloses into simple sugars.
- ✓ Yeast fermentation of the resulting sugar solution.
- ✓ Distillation to produce alcohol.

3.1.1 Sample preparation

The sample that was needed had to be prepared and conditioned for pretreatment, hydrolyze, fermentation and distillation. Sample preparation process include: manual size reduction (scissor cutting), and grinding after the samples was collected. 2 kg of Waste paper (used office paper) was used for experiment. The waste paper was size reduced to about 3 mm size.

3.1.2 Steam pretreatment

The purpose of pretreatment is to remove lignin, reduce cellulose crystallinity, and increases the porosity of the materials (Prasad, 2003). Pretreatment must meet the following requirements: improve the formation of sugar, avoid the degradation or loss of carbohydrate, avoid the formation of by-product inhibitors and must be cost effective.

3.1.2.1 Procedure in steam pretreatment

First distilled water was prepared and then 50 g of the sample was soaked in a distilled water of 500 mL in conical flasks for 24 hours.

The conical flasks were capped with the help of aluminum foil. Then the lignocellulosic biomass was rapidly heated at 121°C by high-pressure steam without addition of any chemicals in autoclave. The biomass/steam mixture was held for 15 minutes to promote hemicellulose hydrolysis, and terminated by an explosive decompression. After finishing the given pretreatment time and temperature the sample in autoclave was allowed to cool and the soluble

portion was separated from the non-soluble portion. The non-soluble portion was hydrolyzed in the next steps and the soluble solution was placed in another conical flask.

3.2 Dilute acid hydrolysis

The cellulose molecules which are composed of long chains are broken down to simple sugar, before it is fermented for alcohol production. Even though there are many types of hydrolysis types, dilute acid hydrolysis is an easy and productive process and the amount of alcohol produced in case of acid hydrolysis is more than that of alkaline hydrolysis.

Each sample had to pass through five primary experiments that were in series to get the final result ethanol, that is: size reduction, pretreatment, hydrolysis, fermentation and distillation. The two-parameter were applied to hydrolysis step of the experimentation. The hydrolysis experiments for ethanol production and optimization were conducted in a completely randomized design using Design expert® 7 software. 50 g of ground waste paper was used for each experiment and the factors for hydrolysis were time (45, 60 and 75 minutes), hydrolysis temperature (120,130 and 140^{o}C), and acid concentration (3% v/v) was used throughout the experiment.

Table 3.1 General factorial design for hydrolysis stage

Std	Run	Block	Temperature,^{o}c	Time, min
16	1	Block 1	130	75
1	2	Block 1	120	45
4	3	Block 1	130	45
18	4	Block 1	140	75
12	5	Block 1	140	60
6	6	Block 1	140	45
2	7	Block 1	120	45
10	8	Block 1	130	60
5	9	Block 1	140	45
13	10	Block 1	120	75
11	11	Block 1	140	60
3	12	Block 1	130	45
7	13	Block 1	120	60
15	14	Block 1	130	75
9	15	Block 1	130	60
17	16	Block 1	140	75
14	17	Block 1	120	75
8	18	Block 1	120	60

Procedures for acid hydrolysis

- 3% (v/v) diluted sulfuric acid was added to the non-soluble component obtained from pretreatment steps in the order of experimental design.
- The waste paper was then hydrolyzed in the reactor at a temperature of 120, 130 and 140^{o}C and at a time of 45, 60 and 75 min.
- After hydrolysis, the solid part was separated from the liquid in the hydrolyzate by vacuum filtration (to remove the non-fermentable lignin portion).
- After the solid part was separated, it was again washed by distilled water. The washing was performed in order to extract all soluble sugars from the solid waste paper material.
- The filtered hydrolyzate was neutralized with 10 M NaOH until the pH became in a range of 4.9-5.2

- Then the soluble component mixed with the previously filter solution from the pretreatment step for the next procedure.


Figure 3.1 a. Hydrolysis in autoclave reactor b. sample ready for hydrolysis c. sample after hydrolysis

3.3 Filtration

The lignin and degraded cellulose which is called monomeric sugar was separated in pressurized filter or bag filter. Then, the sugar solution (hydrolyzate) was neutralized and introduced into fermentation. The lignin which obtained from this filtration process was dried and weighed before use for another purpose.

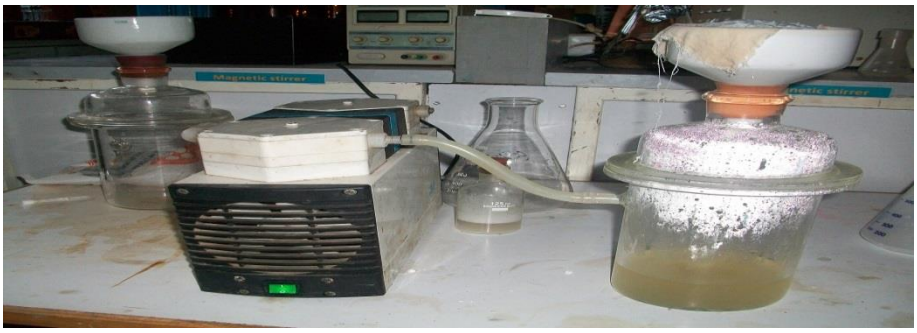


Figure 3.2 Vacuum filtration machine

3.4 pH adjustment

Before addition of any micro-organism to the above prepared samples, pH of these samples has to be adjusted. Otherwise the micro-organism will die in hyper acidic or basic state. A pH of around 4.9 -5.2 is maintained.

Procedures in pH adjustment

- First the pH meter was calibrated by using buffer solution.
- The hydrolyzate solution is acidic, so it needs highly basic solution to bring the pH in the range of 4.9-5.2.
- Sodium hydroxide solution was added drop wise to the other flask with constant stirring until the pH reaches to a range of 4.9-5.2.
- If suppose the pH goes beyond 4.9-5.2, concentrated sulfuric or hydrochloric acid was added drop wise to maintain the pH in the range.

3.5 Fermentation

The aim of the experiment was to measure the ethanol production by the yeast type (Saccharomyces cerevisiae) using waste paper hydrolyzate as energy and carbon source. The clear solutions then go to fermentation. The fermentation was carried out under anaerobic condition at a temperature of 30^{o}C and 200 rpm stirring condition for 3 days. Before conducting fermentation, preparation of media for the yeast is a must. In order to prepare the media the favorable condition for yeast growth must be established to supply the required amount of nutrients. For this, the following nutrients were mixed in their desired proportion.

Media preparation

For preparing 100 ml media, the following were added.

Sugar (Dextrose) 2 g, yeast extract 1 g, urea 1 g, distilled water 100 ml, peptone 2 g and Mg SO_4.7 H_2O 1 g.

Procedures in media preparation

To the 100 ml media prepared as above, 0.5 g of yeast, *Saccharomyces cerevisiae* (arif instant) was added in a 250 ml conical flask. The conical flask was properly covered with aluminum foil. The conical flask was then placed in a shaking incubator for 24 hours at a temperature of 30^{o}C and 200 rpm.

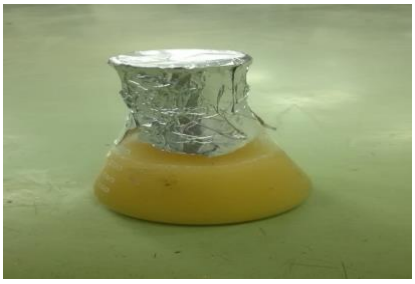


Figure 3.3 Cultured media

Procedures for fermentation

The sample was conditioned to temperature of 30^{o}C before fermentation step was started. The adapted media with the proportion of 1:10 to the soluble sample was added. The shaker incubator (fermenter) was set at parameters of fermentation time, yeast concentration (yeast proportion) and fermentation temperature of 72 hour, 10% (with the proportion of 1:10 that is the prepared media and sample) and 30^{o} C respectively. After 72 hours of fermentation; the samples were taken out and distilled.

3.6 Ethanol separation

Distillation was the final step in the production of ethanol. It is the purifications steps. Distillation is the method used to separate two liquid based on their different boiling points. However, to achieve high purification, several distillations are required. In this experiment separation was achieved by simple distillation at a temperature of 85^{o}C for 2 hrs.

Figure 3.4 Distillation setup

3.7 Experimental analysis

3.7.1 Moisture content determination

Stainless steel moisture dishes were weighed and desired amount of each sample was added. The dishes were then placed in an oven with the lids off at 60°C and dried to a constant weight. The lids were replaced and the dishes were transferred into a desiccator for 1 h. The dishes were re-weighed and the percentage moisture was determined.

$$\% \text{ Moisture content} = \frac{(W1-W2)}{(W1-W0)} * 100 \quad \text{.......} \quad (3.1)$$

Where, W_0, W_1 and W_2 are weight of dish, weight of dish plus sample and weight of dish plus dried sample respectively.

3.7.2 Sugar content determination after hydrolysis

The concentration of total reducing sugar content of Hydrolyzate which was obtained from hydrolysis was determined using a spectrophotometer by measuring absorbance vs. sugar concentration. Benedict solution and standard glucose solution was used to plot the calibration curve to show the % of absorbance of red light by the standard glucose solutions.

Benedict's solution is designed to detect the presence of reducing sugars. In hot alkaline solutions, reducing sugars reduce the blue copper (II) ions to brick red copper (I) oxide precipitate. As the reaction proceeds, the color of the reaction mixture changes progressively from blue to green, yellow, orange and red. When the conditions are carefully controlled, the coloration developed and the amount of precipitate formed depends upon the amount of reducing sugars present. Hence, in most conditions, a sufficiently good estimation of the concentration of glucose-equivalent reducing sugars present in a sample can be obtained.

Plotting calibration curve

- Standard glucose dilution series solution was prepared at different concentration of 15, 12, 9, 6, 3, 1 and 0%.
- 1ml of each of the standard glucose solution is added into labeled test tubes, each containing 5 ml of benedict's solution and mixed by shaking.
- The labeled test tubes were heated in 90^{o}C water bath for 5 minutes.

- The test tubes removed from the water bath and filtered the samples using filter paper to remove any red precipitate formed when reducing sugar in the samples reacted with benedict reagent.
- The absorbance of the samples was read using spectrophotometer.
- Finally, plot a calibration curve to show the % of absorbance of red light by the standard glucose solution.

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

$$\text{Yield of TRS (\%)} = \frac{glucose\ concentration\ of\ sample}{gram\ of\ sample\ used} * \text{volume of hydrolyzate} * 100 \ldots\ldots\ldots (3.3)$$

Specific gravity (density) measurement

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

$$\text{Sp. gravity} = \frac{(W1 - Wo)}{(W2 - Wo)} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3.4)$$

Where: W0- weight (g) of empty bottle

W1- weight (g) of bottle + sample (ethanol)

W2 - weight (g) of bottle + water

Yield of ethanol

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

$$\text{Volume of 99.9\% Ethanol yield } (V_{AE}) = \frac{(\text{VHE} * \rho\text{HE} * \text{XE})}{\rho\text{AE}} \quad \ldots\ldots\ldots\ldots\ldots\ldots (3.5)$$

Where

V_{HE} = volume of hydrous ethanol formed.

V_{AE} = volume of anhydrous ethanol formed

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

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

X_E = mass fraction of ethanol (% alcohol by weight)

3.8 Design of the experiment

Data analysis for this study was carried out by design expert software (Full factorial design) to evaluate the effects of the process variables; hydrolysis temperature (120^oC, 130^oC and 140^oC), reaction time (45min, 60min and 75 min) at an acid concentration of 3%v/v. This design of the experiment helps us to Optimize of Process Parameters Using Response Surface Methodology (RSM). Significance of the result was set from analysis of variance (ANOVA).

4 RESULTS AND DISCUSSION

4.1 Determination of Moisture Contents

The collected waste paper(used office paper) of different weight (10, 15 and 20 g) were prepared to determine the moisture content at 60^{o}c and the moisture contents of the sample were tabulated in table 4.1 using equation (3.1)

Table 4.1 Moisture content determination

Drying time, hrs	0	2	4	6
Weight of sample1 (g)	10	9.80	9. 61	9.60
Weight of sample2 (g)	15	14.75	14.43	14.43
Weight of sample3 (g)	20	19.81	19.23	19.22

The percentage of moisture content of the three samples was 4%, 3.8%, and 3.9% respectively. Therefore, the average moisture content of the three samples was 3.9 %.

4.2 Total reducing sugar determination

Total reducing sugar of the hydrolyzate sample was determined using Spectrometer, which measures the intensity of red light. Standard curve was plotted from known concentration of standard glucose and benedict solution reagent in digital spectrophotometer at 540nm wavelength and the corresponding concentration of sample was determined using equation (3.2).

From table 4.2, it is clear that the absorbance and concentration of sugar has inverse relationship. The sample which have less sugar concentration will remain with high amount of CU (II) oxide, blue color which is unreacted with the benedict's reagent due to this absorbance of the sample increases, but in the sample which have high sugar concentration cu (II) oxide is completely react with the Benedict's reagent and form cu (I) oxide red color precipitate and absorbance of the sample decreases.

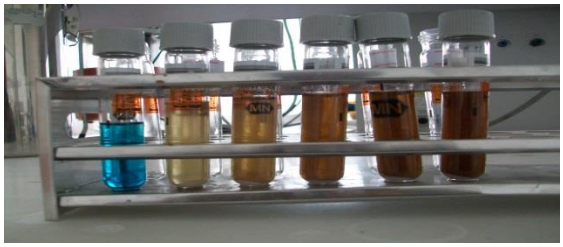


Figure 4.1 Standard glucose solutions after being reacted with benedict's solution (reagent)

Table 4.2 Concentration of Standard glucose and its absorbance

Glucose conc.(g/ml)	0	0.01	0.03	0.06	0.09	0.12	0.15
Absorbance	0.70	0.62	0.54	0.44	0.32	0. 21	0.115

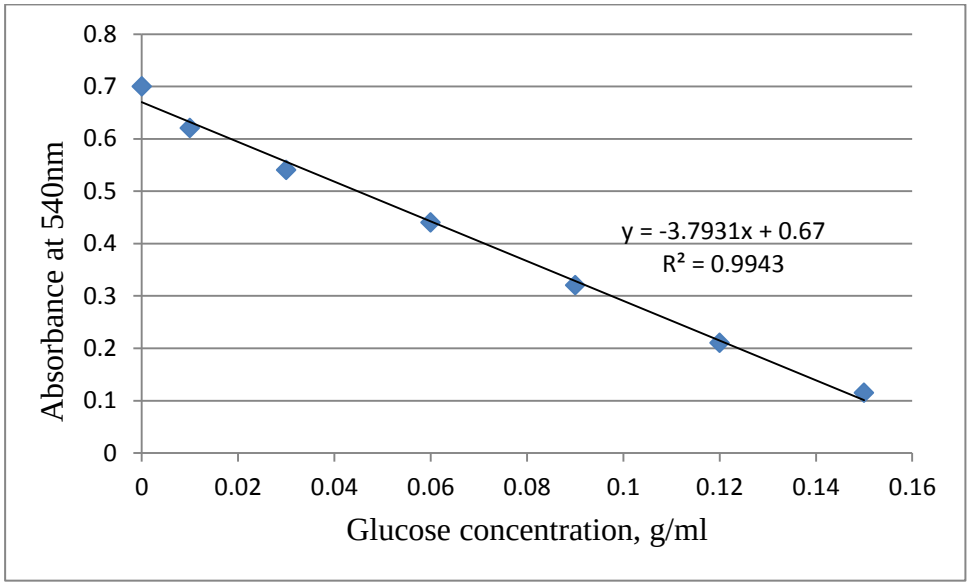


Figure 4.2 Calibration curve of standard glucose concentration

Table 4.3 Yield of total reducing sugar (TRS) of samples

Run no.	Temperature(^{o}c)	Time (min)	Absorbance	Yield of TRS after hydrolysis(%w/w)
1	130	75	0.3950	58.00
2	120	45	0.5219	27.00
3	130	45	0.5277	30.00
4	140	75	0.4374	49.05
5	140	60	0.3379	70.04
6	140	45	0.5348	28.50
7	120	45	0.5219	27.00
8	130	60	0.5087	34.00
9	140	45	0.5348	28.50
10	120	75	0.4708	42.00
11	140	60	0.3381	70.00
12	130	45	0.5277	30.00
13	120	60	0.5514	25.00
14	130	75	0.3950	58.00
15	130	60	0.5087	34.00
16	140	75	0.4375	49.03
17	120	75	0.4708	42.00
18	120	60	0.5514	25.00

The highest yield of total reducing sugar (70.04% w/w) was obtained at 140^{o}C temperature, 60 min hydrolysis time.

Ethanol yield

The yield of ethanol was determined using equation (3.5).The specific gravity of ethanol was determined using Pycnometer and this specific gravity was related with concentration of ethanol using standard table from Perry chemical engineering handbook(chap 2, page 112).

Table 4.4 Density and yield of ethanol

Run No.	Volume of hydrous ethanol(ml)	Density of hydrous ethanol(g/ml)	Ethanol Con. (w/w %)	Yield of anhydrous ethanol(ml/50g wp)
1	23.63	0.91823	0.48	13.20
2	19.98	0.94305	0.36	8.60
3	20.78	0.93919	0.38	9.40
4	23.70	0.92897	0.43	12.00
5	24.20	0.90936	0.52	14.50
6	20.40	0.94114	0.37	9.00
7	19.98	0.94305	0.36	8.60
8	22.67	0.93720	0.39	10.50
9	20.40	0.94404	0.37	9.00
10	23.83	0.93518	0.40	11.30
11	24.45	0.91160	0.51	14.41
12	20.78	0.93919	0.38	9.40
13	18.62	0.94404	0.35	7.80
14	23.20	0.91604	0.49	13.20
15	22.67	0.93720	0.39	10.50
16	24.70	0.93314	0.41	12.00
17	23.83	0.93518	0.40	11.30
18	18.62	0.94494	0.35	7.80

From the result shown above (table 4.4) the highest yield ethanol which is 14.50ml/50g_{wp} (0.23g/g) was obtained at 140^{o}C hydrolysis temperature and 60 min reaction time.

Table 4.5 Analysis of variance (ANOVA) for the cubic model

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	77.10	7	11.01	42.68	< 0.0001
A-Temperature	44.29	1	44.29	171.63	< 0.0001
B-Time	14.44	1	14.44	55.96	< 0.0001
AB	0.045	1	0.045	0.17	0.6851
A^2	1.03	1	1.03	3.99	0.0736
B^2	0.45	1	0.45	1.74	0.2166
A^2B	0.60	1	0.60	2.33	0.1578
AB^2	24.85	1	24.85	96.29	< 0.0001
A^3	0.000	0			
B^3	0.000	0			
Residual	2.58	10	0.26		
Lack of Fit	2.58	1	1.09	4.21	0.6705
Pure Error	4.050E-003	9	4.500E-004		

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 Square divided by Residual Mean Square. Here the Model F-value of 42.68 implies the model is significant. There is only a 0.01% 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 A (teperature) and B (time)are significant model terms. And there is no interaction between the two factors. Values greater than 0.1000 indicate the model terms are not significant.

The lack of fit F-value of 4.21 implies the lack of fit is not significant relative to the pure error. There is a 67.05% chance that a lack of fit F-value this large could occur due to noise. Non significant lack of fit is good.

Table 4.6 Model adequacy measures

Std. Dev.	0.51	R-Squared	0.9676
Mean	10.70	Adj R-Squared	0.9449
C.V. %	4.75	Pred R-Squared	0.9121
PRESS	7.00	Adeq Precision	19.651

The "Pred R-Squared" of 0.9121 is in reasonable agreement with the "Adj R-Squared" of 0.9449. "Adeq Precision" measures the signal to disturbance ratio due to random error. A ratio greater than 4 is desirable. Here ratio of 19.651 indicates an adequate signal.

Therefore, this model can be used to navigate the design space.

Table 4.7 Regression coefficients and the corresponding 95% CI High and Low

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High
Intercept	11.26	1	0.27	10.66	11.85
A-Temperature	3.33	1	0.25	2.76	3.89
B-Time	1.90	1	0.25	1.33	2.47
AB	0.075	1	0.18	-0.33	0.48
A^2	-0.51	1	0.25	-1.07	0.058
B^2	-0.34	1	0.25	-0.90	0.23
A^2B	-0.47	1	0.31	-1.17	0.22
AB^2	-3.05	1	0.31	-3.75	-2.36

Final Equation in Terms of Coded Factors:

Ethanol yield = $+11.26+3.33 * A+1.90 * B+0.075 * A * B-0.51* A^2-0.33* B^2-0.48* A^2 * B-3.05*A*B^2$ ……(4.1)

The actual versus predicted values using model in the above equation (in terms of actual factors) are tabulated in table 4.8 on the appendix.

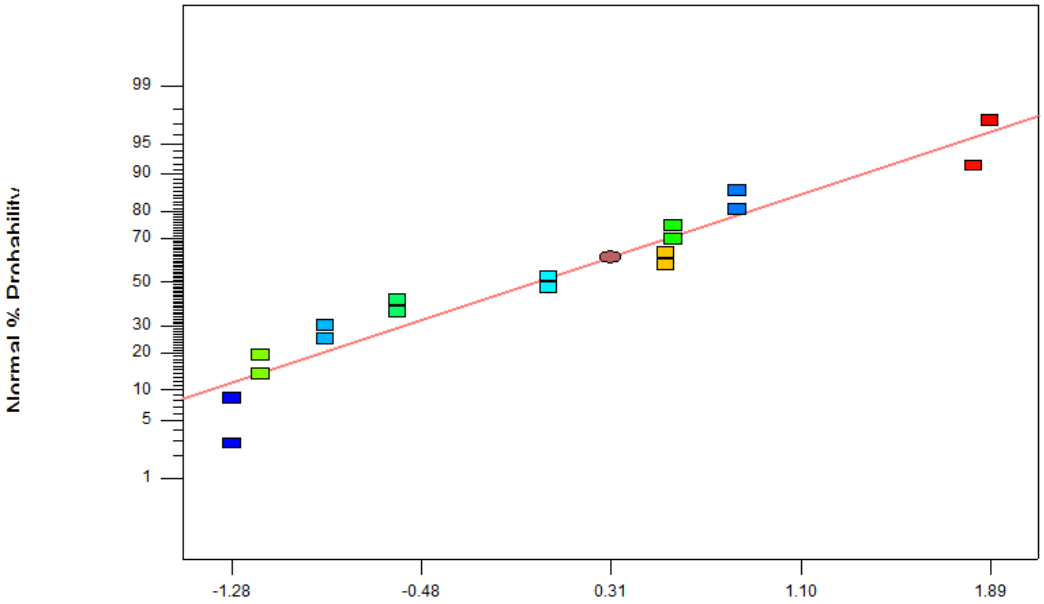


Figure 4.3 Normal plots of residuals

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

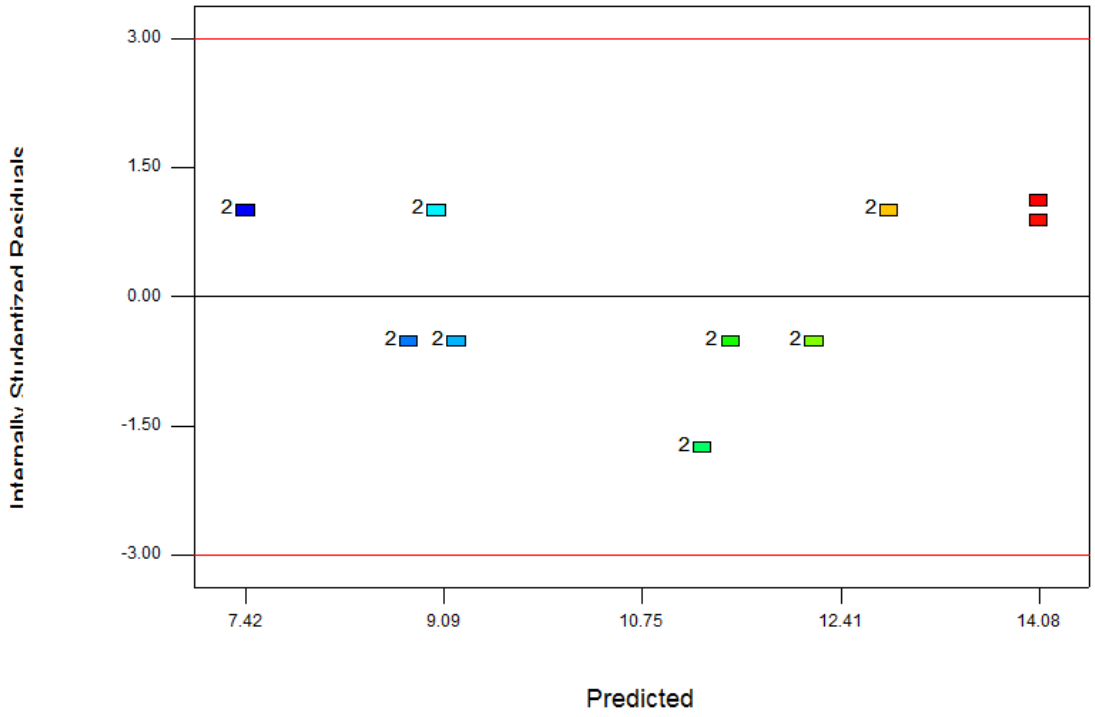


Figure 4.4 Residual versus predicted values

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

4.3 Effects of experimental variables on hydrolysis

The best way of showing the effects of this parameter for the yield of ethanol are to generate response surface plots of the equation. The three dimensional i.e. interactions, contours and response surfaces effect were plotted in the figure below.

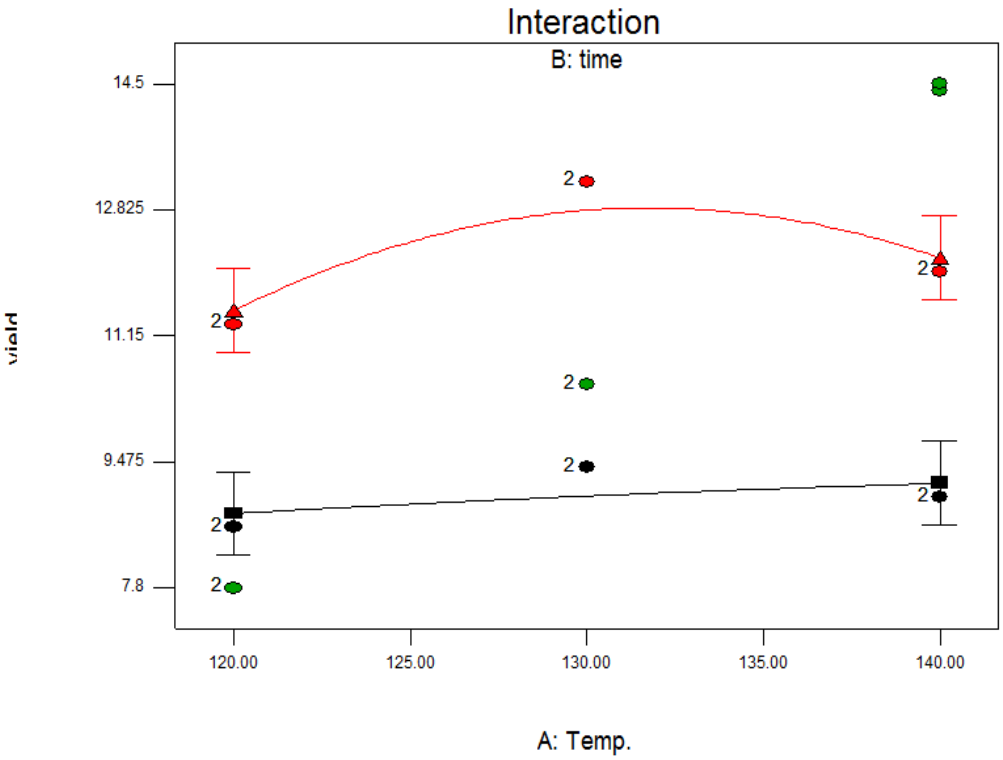


Figure 4.5 Interaction between temperature and time

The fig. 4.5 shows that there is no interaction between temprature and time since its p value is greater than 0.05.

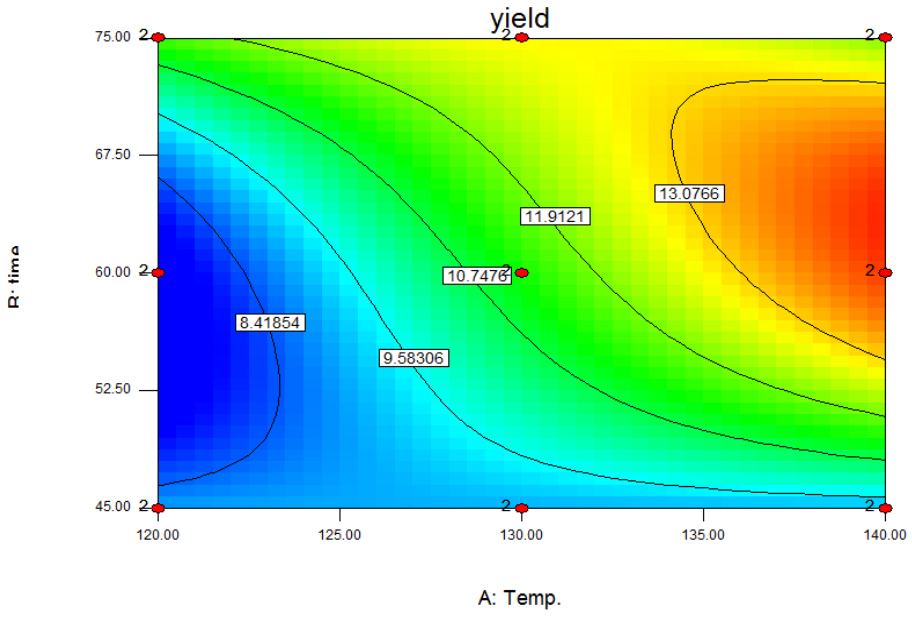


Figure 4.6 Contour plots of the effects of temperature and time on ethanol yield

Contour plot graph showing predicted response of ethanol yield as a function of hydrolysis time

and temperature was shown in figure 4.6. In the figure above the red region identifies the higher ethanol yield. The contour levels revealed that there was high ethanol yield in the vicinity of 60 minutes time and 140^{o}c temperature.

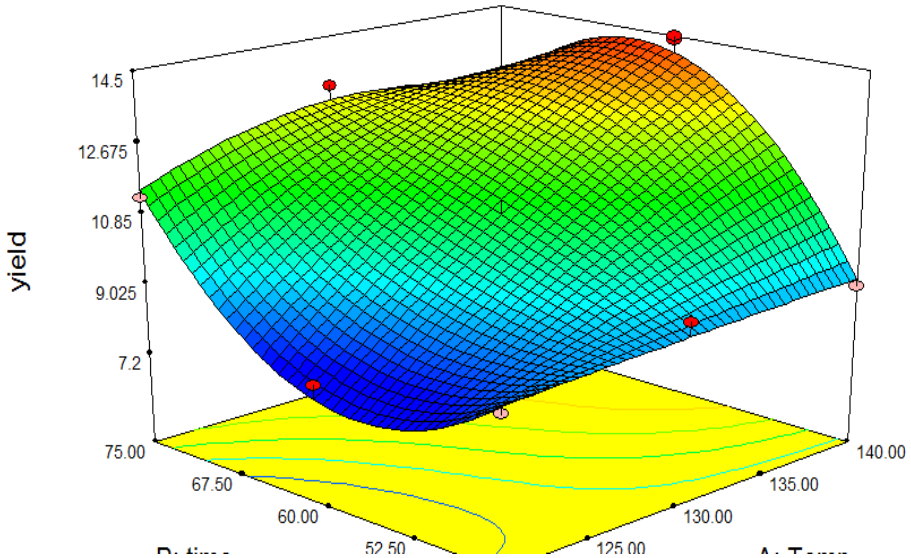


Figure 4.7 Response surface plots of the effects of temperature and time on ethanol yield

Figure 4.7 is the response surface obtained from hydrolysis time and temperature. Hence from the result, there were well defined optimums operating conditions. This graph has also the same message as that of countour plot. At high hydrolysis temperature and at a middle hydrolysis time high amount of ethanol was obtained

4.4 Optimizations

The optimization of hydrolysis criteria for ethanol production from waste paper using dilute acid hydrolysis are summarized as follows:

Table 4.8 Optimization criteria for optimum yield of ethanol

Parameter	Goal	Lower limit	Upper limit
Temperature	is in range	120	140
Time	is in range	45	75
Ethanol yield	maximize	7.8	14.5
TRS	maximize	25	70.04

Table 4.9 Optimum possible solutions

Number	Temperature	Time	Ethanol yield	TRS	Desirability
1	140.00	63.24	14.2426	66.466	0.938(selected)
2	140.00	63.22	14.2426	66.4663	0.938
3	140.00	63.23	14.2426	66.4662	0.938
4	140.00	63.21	14.2425	66.4665	0.938
5	140.00	63.21	14.2425	66.4663	0.938
6	140.00	63.26	14.2427	66.4657	0.938
7	140.00	63.27	14.2427	66.4656	0.938
8	140.00	63.30	14.2427	66.4647	0.938
9	140.00	63.32	14.2427	66.4641	0.938

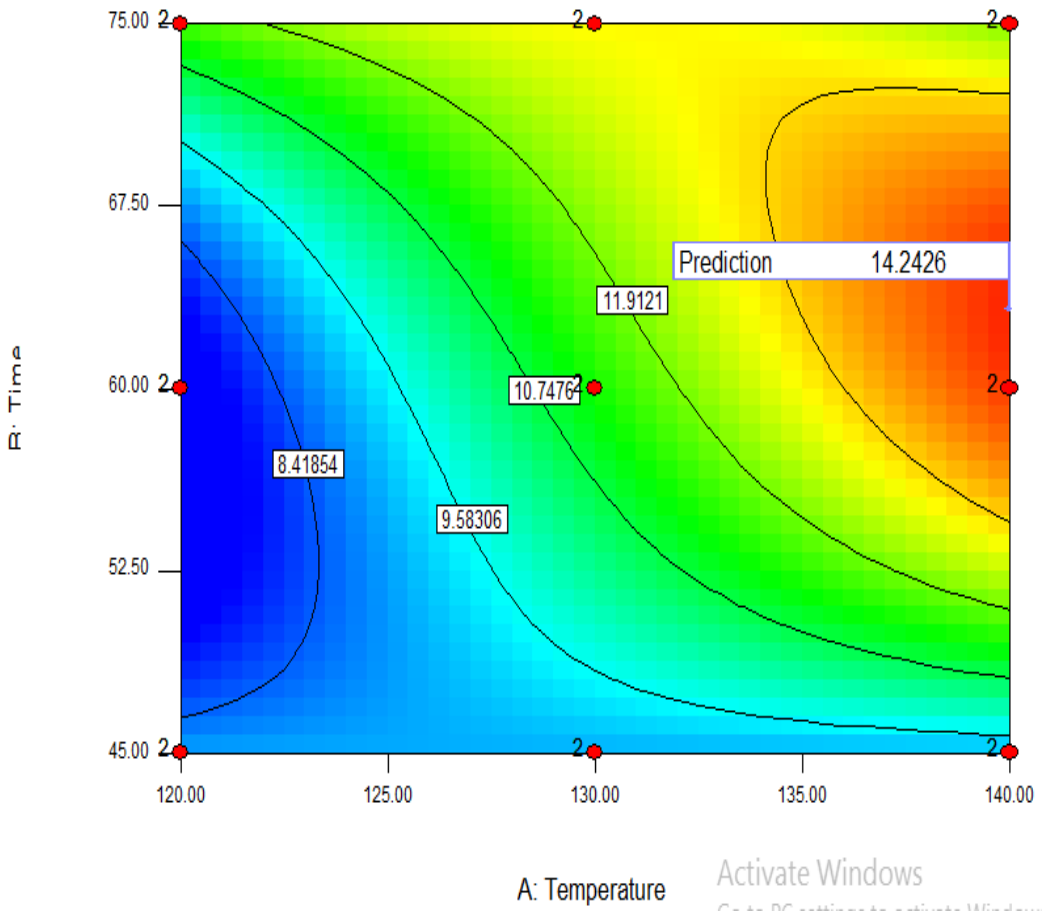


Figure 4.8 Countur plot of the possible optimum solution

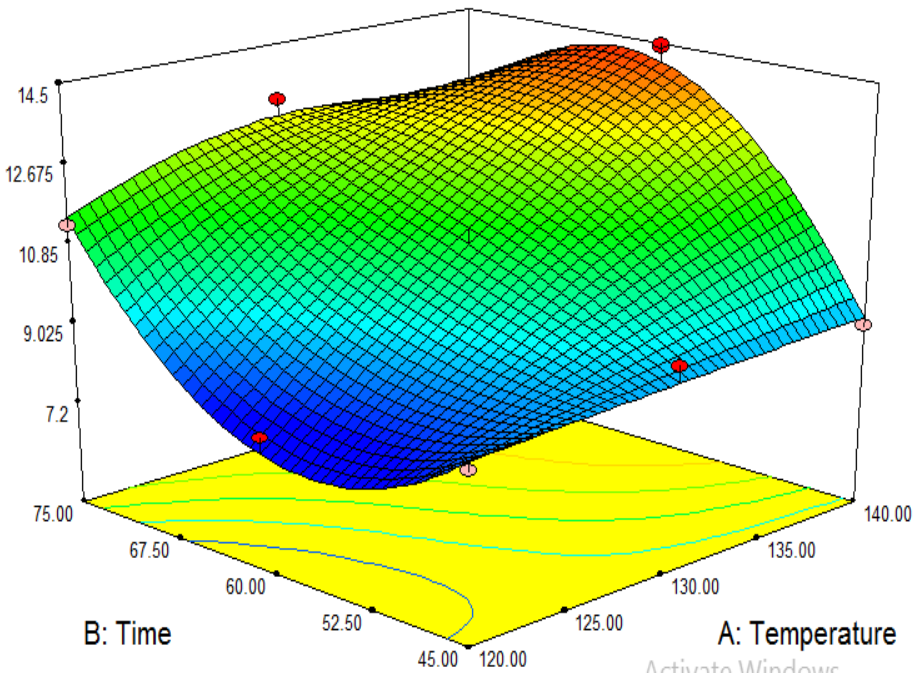


Figure 4.9 Surface plot of possible optimum solution

4.5 Model validation

According to the three-level factorial design result using Design-Expert® 7 software, an experiment with hydrolysis temperature and time were conducted in order to study the outcome or effect of the factorial design. The experiment was carried out at the optimized conditions. As it can be seen from figure 4.8 and 4.9 above, ethanol yield of average predicted value was 14.2426ml/50g sample. As a result, the model was considered to be accurate and reliable for predicting the yield of ethanol from waste paper using dilute acid hydrolysis. The optimal values of variables were calculated as 63.24 min and 140°C.

4.6 Bioethanol characterizations by FTIR

Alcohols have characteristic IR absorptions associated with the O-H, C-O and the C-H stretching vibrations. When run as a liquid film the region 3500-3200 cm-1 with a very intense and broad band indicated the O-H stretch of alcohols, while the region 1260-1050 cm-1 confirms the C–O stretch. The bands at around 2880 and 2930 cm-1 were assigned as the symmetric stretching modes of the $–CH_2$ and$–CH_3$ groups, respectively (Coates and Meyers,

2000; Yu et al, 2007). This assures that the product obtained from waste paper (used office paper) is exactly ethanol due to the confirmation of these regions as shown below.

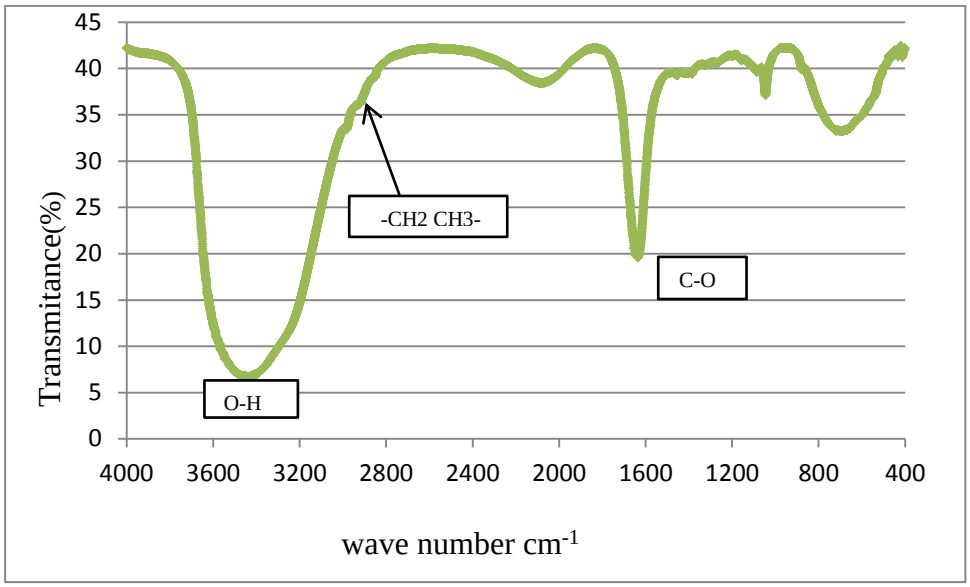


Figure 4.10 Fourier transform Infrared spectra of bioethanol from waste paper (used office paper)

5 MATERIAL AND ENERGY BALANCE

5.1 Material balances

Basis: 330 working days annually

Production rate: 4.0x10^6 lit/year of ethanol (99.9%) with plant operation of 330 days per year and the Plant attainment (i.e. the percentage of the available hours in a year) was calculated as

$$\% \text{ Plant attainment} = \frac{\text{operation hours}}{\text{hours of the year}} x100 \ldots\ldots (5.1)$$

$$= \frac{7920}{8760} x100\% = 91\%$$

The percent of plant attainment was acceptable because the recommended % of plant attainment for chemical industry is between 90-95%.

The production rate in term of mass flow rate was

$$\varrho = m/v \ldots\ldots (5.2)$$

m = ϱ x v=0.789kg/lit *4.0x10^6lit/yr.

m =3,156,000kg ethanol per year or 3,156 ton/yr.

Material balance for Molecular sieve

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

Anhydrous Ethanol (M_{AE}) =3,156 ton

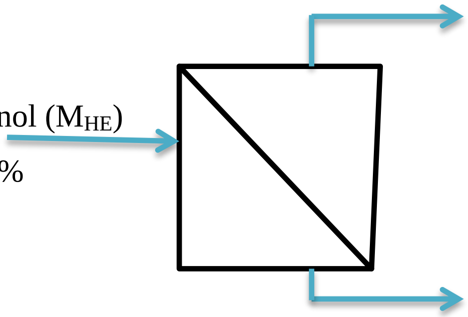
X_{AE}=99.9%

Xw=0.1%

Hydrous ethanol (M_{HE})

X_{HE}=96%

X_w=4%



Water (W) Xw=1, Xe=0%

Where X_{HE}= Percentage of hydrous ethanol

Xe= Percentage of Ethanol

X_{AE}= Percentage of anhydrous ethanol

Xw= Percentage of water

Over all mass balance

$M_{HE}=W + M_{AE}$ ..... …… (5.3)

Ethanol mass balance

$M_{HE}* X_{HE}= M_{AE}* X_{AE}$..... ….. (5.4)

From equation 5.3

$$M_{HE}=\frac{MAE* XAE}{XHE} = \frac{3{,}156{,}000\ x\ 0.999}{0.96} = 3{,}284.21 ton$$

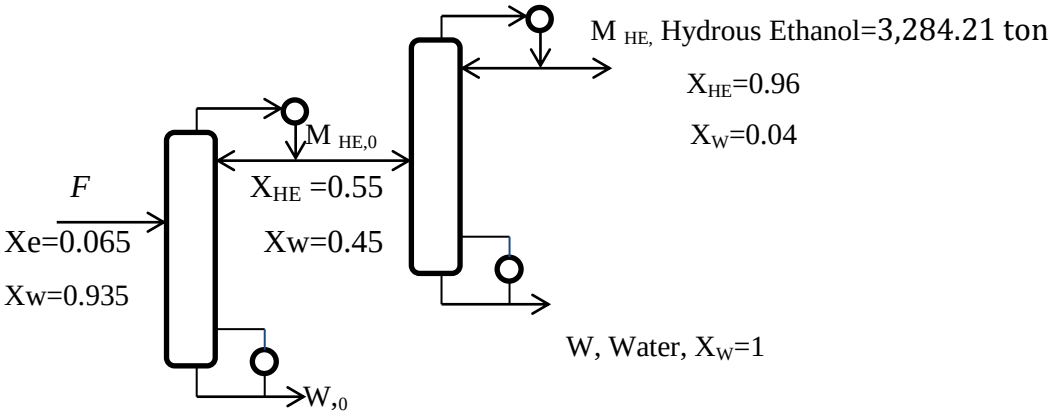
The water that is trapped by the molecular sieve is calculated from equation 5.2

$W= M_{HE} - M_{AE}$=3,284.21-3,156.00

W =128.2 1ton of water

Material balance on distillation

Distillation is used for recovery of ethanol from water-ethanol mixture. The mixture of ethanol water which obtained from fermentation process was separated using distillation column at 85^{o}C and recovered 96% ethanol from the mixture of 6.5% ethanol.



Where: X_{DE} is fraction of diluted hydrous ethanol

X_W is fraction of dilute hydrous ethanol and hydrous ethanol

X_{HE} is fraction of hydrous ethanol

Ethanol mass balance on the overall boundary

$F*X_F = W*X_E +D*X_{HE}$

$$F=\frac{W*XE +D*XHE}{Xf} = \frac{0+3{,}284.21*0.935}{0.065} = 47{,}242.10\ ton$$

Thus, $W= F- D=47{,}242.10 - 3{,}284.21$

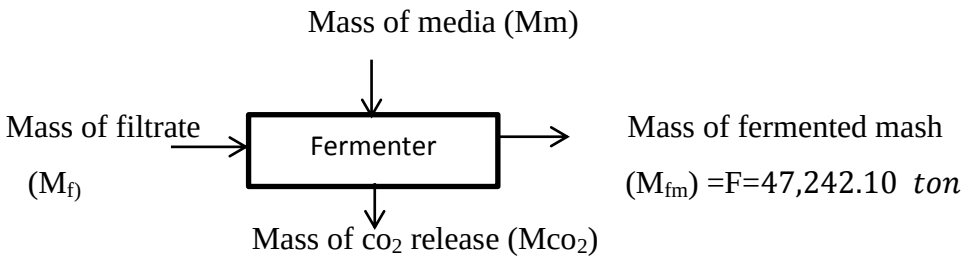
W =43,957.90 ton of water

Material Balance on fermentation

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

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

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



Where: M_{fm} is mass of fermented mash

Mm is mass of media

Mco_2 is mass of carbon dioxide

M_f is mass of filtrate

Assume 92% glucose can be converted into ethanol.

$$\frac{0.92MG}{180} = \frac{\text{HEx}0.96}{92}$$

$0.92M_G$= (3,284.21x0.96x180)/92

M_G=6,705 ton of glucose is required yearly

Where: HE is hydrous ethanol

M_G is mass of glucose

The amount of carbon dioxide produced is

M_{CO2}= (6,705 x88)/180

M_{CO2} = 3,278 ton of CO_2 released per year

Over all mass balance on the fermentation process is

$M_f + M_m = M_{fm} + M_{CO2}$

$M_m = 0.1M_f$

$M_f + 0.1M_f$ =47,242.10 ton +3,278 ton

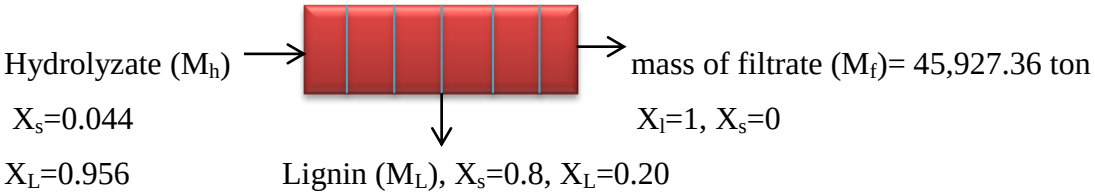
$1.1M_f$ = 50,520.10 ton

M_f = 45,927.36 ton of filtered Hydrolyzate is required

Mm = 4,592.74 ton of inoculum is required

Material balance on pressurized bag filter

This is used for separating insoluble solid (lignin) from liquid (sugar solution). Moisture content of sludge (lignin) was 20%.



Over all mass balance

$$M_h = M_L + M_f \quad \text{..... (5.5)}$$

Mass balance on the solid

$$M_h * 0.044 = M_L * 0.8$$

$$M_h = 18.18\ M_L \quad \text{..... (5.6)}$$

Substitute equation (5.6) into equation (5.5) to calculate mass of lignin or total solid remain after hydrolysis

$$17.18\ M_L = M_F$$

$$M_L = (45{,}927.36 \text{ ton})/17.18$$

M_L = 2,673.30 ton of solid or lignin was generated per year.

The amount of Hydrolyzate is calculated from equation (5.4)

M_h=48,600.67 ton of Hydrolyzate is required per year.

Material balance on hydrolyzer

The pretreated sample is soaked and treated with 3% sulfuric acid to convert cellulose to glucose and serve as catalyst meaning it has no any reaction with cellulose.

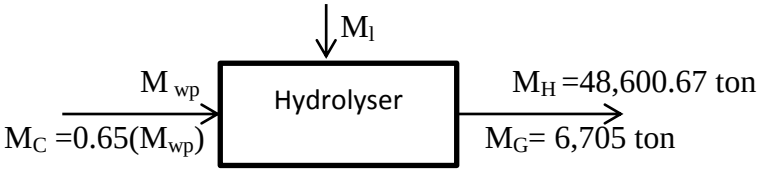
Hydrolysis reaction is

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

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

But not 100% conversion and assume that 95% of cellulose converts to glucose. According Dehnavi, (2009) 60-70 % of waste paper is cellulose.

And an average of 65% is taken.



Where: M_C is mass of cellulose

M_G is mass of glucose

M_{wp} is mass of waste paper

M_l is mass of liquid

By using stoichiometry equation the required dry waste paper (M_{wp}) is calculated as

$$\frac{0.65 * 0.95\ Mwp}{162} = \frac{MG}{180}$$

$$0.65*0.95*\ M\,wp = (162*\ 6{,}705)/180$$

Mwp =9,772.47 ton of waste paper is required yearly

Overall material balance

$$M_{wp} + M_l = M_h$$

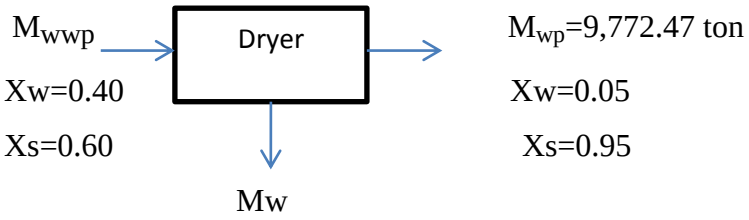
M_l=48,600.67 -9,772.47=38,828.20 ton

Mass of sulfuric acid is 3% of mass of liquid.

Therefore, M_{SA}= 3% *38,828.20 ton =1,164.85 ton of sulfuric acid is required yearly.

Mass balance on dryer

It is used to dry the pretreated waste paper i.e. to make agreeable for easily crushing.



Over all mass balance

$$M_{wwp} = Mw + M_{wp}$$

Component mass balance on the WP

$$0.6\ M_{wwp} = 0.95\ M_{wp}$$

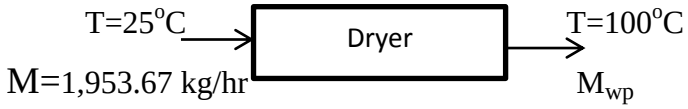
$$M_{Wwp} = (0.95*9{,}772.47)/0.6$$

M_{Wwp} =15,473.07 ton of wet waste paper is required

From the above over all mass balance mass of water is 5,700.6 ton.

5.2 Energy balance

The basis of the calculation is one hour.



The required energy is calculated as

$$Q = m\ Cp\ \Delta T$$

Where

Q= required energy

M=mass of wet waste paper

Cp =specific heat of, Cp of waste paper = 1.4 kJ/kg k

Cp = CPw*Xw + Cps *Xs

= 4.18*0.4 +1.4*0.6

=2.512kJ/kg k

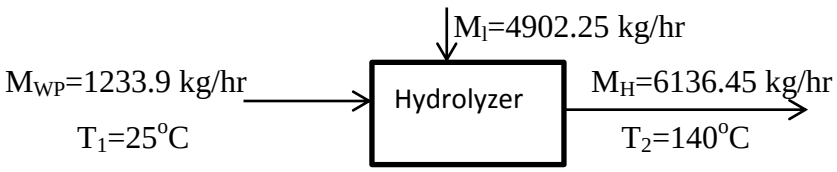
$Q = m\ Cp\ \Delta T$

Q = 1,953.67 kg/hr*2.512 kJ/kg k (100-25) K

Q=368,071.51 kJ/hr

Energy balance on hydrolysis

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



The heat required for hydrolysis is

$Q = M_H Cp_m \Delta T$

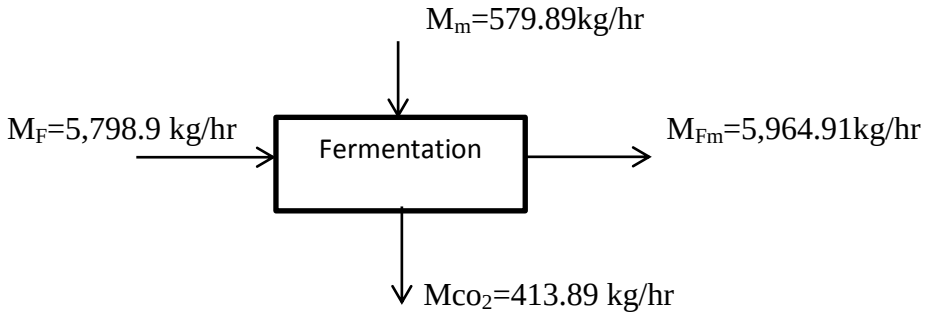
$Cp_m = \frac{1233.9 * 1.4 + 4902.25 * 4.18}{1233.9 + 4902.25} = 3.62$kJ/kg k

$Q = M_H Cp_m \Delta T$

Q =6136.45 *3.62*(140-25)

Q = 2,555,294.39KJ/hr.

Energy balance on fermentation



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

Cp of mix at 30^oC = 4.026kJ/kg k

Cp of CO_2 at 30^oC = 0.846kJ/kg k

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

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

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

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

Xco_2=413.89 / (5,798.9 +579.89) =0.065

X_{Fm}=1-0.065=0.935

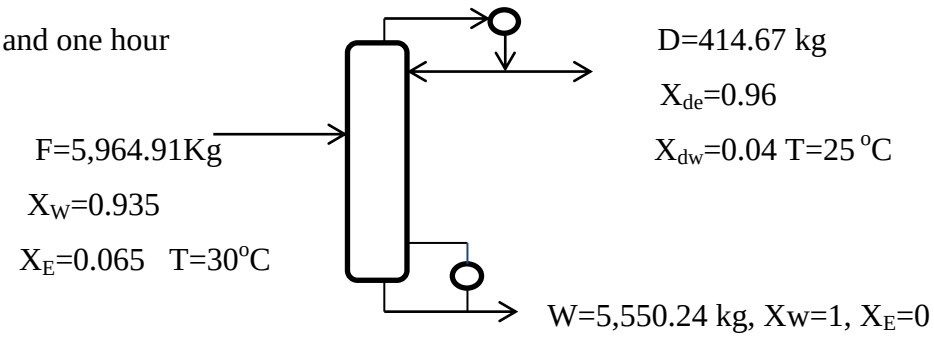
Cp_{Fm}= 4.04kJ/kg k

Q=6,378.80*4.026*(30-25)-413.89*0.846*(30-25) - 5,964.91*4.04 *(30-25)

Q =6,163.30 KJ

Energy balance on distillation

Basis =25^oC and one hour



Specific heat capacity of distillate (Cp_d)

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

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

Cp_w =4.18kJ/kg k

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

L=2.5D=2.5*414.67 kg/hr = 1,036.67 kg/hr

V=L+D=1,036.67 kg/hr+414.67 kg/hr = 1,451.345 kg/hr

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

At steady state condition

Input=Output

$H_F = H_D + H_L + Q_C$

$Q_C=H_F-H_D-H_L$

Assume complete condensation, therefore

Enthalpy of Vapor = Latent + Sensible heat

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

H_V =1,451.34*789+1,451.34*2.778(78.13-25) =1,222,220.9 kJ/hr

Overall balance to determine Q_B

Input = Out put

$Q_B + H_F = Q_C + H_D + H_W$

$Q_B=Q_C+H_D+H_W-H_F$

$H_F = m_FCp\Delta T$

Cp=0.065*2.72+0.935*4.18 = 4.08 kJ/kg k

H_F=5,964.91kg/hr*4.08* (30-25) = 121,836.27kJ/hr

$H_W = m_wCp\Delta T$=5,550.24 *4.18 (100-25) =1,740,000 kJ/hr

$Q_B = Q_C + H_D + H_W - H_F$

Q_B=1,222,220.9 kJ/hr + 0 + 1,740,000 kJ/hr-121,836.27kJ/hr =3,084,057.41 kJ/hr

Amount of steam required

Latent heat of steam at 274KN/m^3, λv=2174kJ/kg, therefore mass of steam required,

Q_B=Ms λs

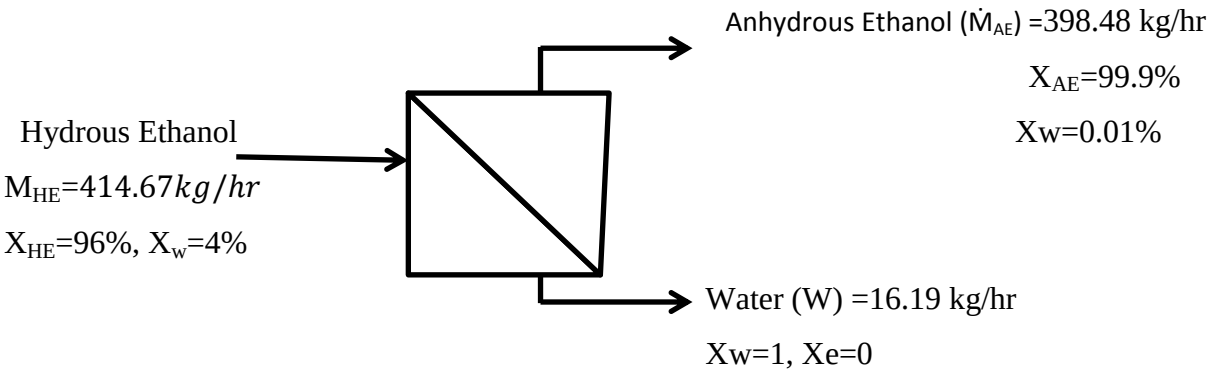
Ms=QB/ λs = (3,084,057.41 kJ/hr) / (2174 kJ/kg) = 1,418.61 kg/hr

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

Q_C=Mw Cp ΔT, M= $\frac{Qc}{Cp\Delta T} = \frac{1,222,220.9}{4.18*30}$ =9,746.6 kg/hr

Energy balance on molecular sieve

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



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

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

The temperature entering the molecular sieve is 25^{o}C and exit is at a temperature of 95^{o}C

Q=m Cp ΔT= 414.67kh/hr * 2.78kJ/kgk*(95-25) k = 80,694.78kJ/hr

6 SIZING OF MAJOR EQUIPMENT

Assumption:

- Sizing of equipment is depending on the material balance of the plant.
- All tanks are 85% full or there is 15% safety factor.

Sizing of waste paper storage tank

Material of construction carbon steel

Material handled waste paper

Density of waste paper: 0.8g/cm3=800kg/m3

Required mass 1,233.89 kg

$\rho = m/v$

$v = m/\rho$

$v = 1233.89/800$

$v = 1.54 m^3$

Required storage volume, $V = 1.54/0.85 = 1.81 m^3$

Sizing of storage tank for Sulfuric acid

Material handled Acid

Material of construction stainless steel

Density of slurry $1840 kg/m^3$

Required mass 147.07 kg /hr

$V = m/\rho$

$= 147.08/1840$

$= 0.08 m^3/0.85$

$= 0.095 m^3$

Sizing of water storage tank

Material handled Water

Density of slurry $1000 kg/m^3$

Required mass 4,755.48 kg /hr.

$V = m/\rho$

$= 4,755.48/1000$

$= 4.75 m^3/0.85 = 5.6 m^3$

Sizing of storage tank for hydrolyzate

Material of construction	carbon steel
Material handled	slurry waste paper
Density of slurry	0.2*800 + 0.8*1000= 960kg/m3
Required mass	6,136.44 kg /hr

$V = m/\rho$

$= 6{,}136.44/960$

$= 6.4\ m^3$

Therefore, the required storage volume is $V= 6.4/0.85= 7.5\ m^3$

Sizing of distillation column

A distillation column is to design to separate 5,964.91kg/hr of ethanol water mixture with 6.5% ethanol and 93.5% water entered to the first distillation column and produced 55% ethanol and 45% mixture which is the feed to the second distillation column so as to give 99.9% ethanol. A reflux ratio of 2.5 of product is assumed to be used. The column works at 0.6 atm with a vapor velocity of 0.7 m/s.

Molecular weight of ethanol = 46g/mol

Molecular weight of water = 18g/mol

Boiling point of ethanol = 78.13^oC=351.13k

Boiling point of water=100^oC= 373k

Feed mean molecular weight = 0.55*46+0.45*18

= 33.4g/mol

Specific heat of feed= 0.55*2.72+0.45*4.18

=3.38kJ/kg.k

Water mole fraction on feed

$$X_{W,F} = \frac{\frac{45}{18}}{\frac{45}{18}+\frac{55}{46}} = 0.67$$

Water mole fraction in distillate

$$X_{W,D} = \frac{\frac{4}{18}}{\frac{4}{18}+\frac{96}{46}} = 0.096$$

Water mole fraction in bottom, $X_{W,B}=1$

Ethanol mole fraction in feed

$$X_{E,F} = \frac{\frac{55}{46}}{\frac{55}{46}+\frac{45}{18}} = 0.3235$$

Ethanol mole fraction in distillate

$$X_{E,D} = \frac{\frac{96}{46}}{\frac{96}{46}+\frac{4}{18}} = 0.9$$

Ethanol mole fraction in bottom, $X_{E,B} = 0$

Reflux ratio = 2.5

Molar latent heat of mixture in feed

$\lambda = 0.55*790 + 0.45*2376.7$

$= 1{,}504 kJ/kg$

$= 1{,}504 kJ/kg * \frac{1kg}{0.021kmol} = 71{,}619 kJ/kmol$

$= 71{,}619\ kJ/kmol * \frac{0.239cal}{kJ}$

$= 17{,}117\ \frac{cal}{kmol}$

Boiling point (average) of feed

$= \sum X_i T_i$

$= 0.3235*303 + (1- 0.3235)*303$

$= 303k$

Heat capacity of feed

$CP_F = 3.38 kJ/kg.k$

$= 3.38 kJ/kg.k * 0.239 kg\ cal/kJ$

$= 0.78 kcal/kg.k$

$$q = \frac{Cpdt+\lambda}{\lambda} = \frac{0.78(303-298)+17{,}117}{17{,}117} = 1.00$$

Vapor equation data of ethanol water solution given below

Table 6.1 VLE data for ethanol water solution

X(liq.)	0	0.019	0.0721	0.0966	0.1238	0.1661	0.2337	0.2608	0.3273	0.3965
Y(vap.)	0	0.17	0.3891	0.4375	0.4704	0.5089	0.548	0.558	0.583	0.6122

X(liq.)	0.5079	0.5198	0.5732	0.6763	0.74472	0.8954	0.93	0.97	1.00
Y(vap.)	0.6564	0.6599	0.6841	0.7385	0.7815	0.8954	0.92	0.96	1.00

Slope of q line=$\frac{q}{q+1}$=$\frac{1}{1+1}$=0.5

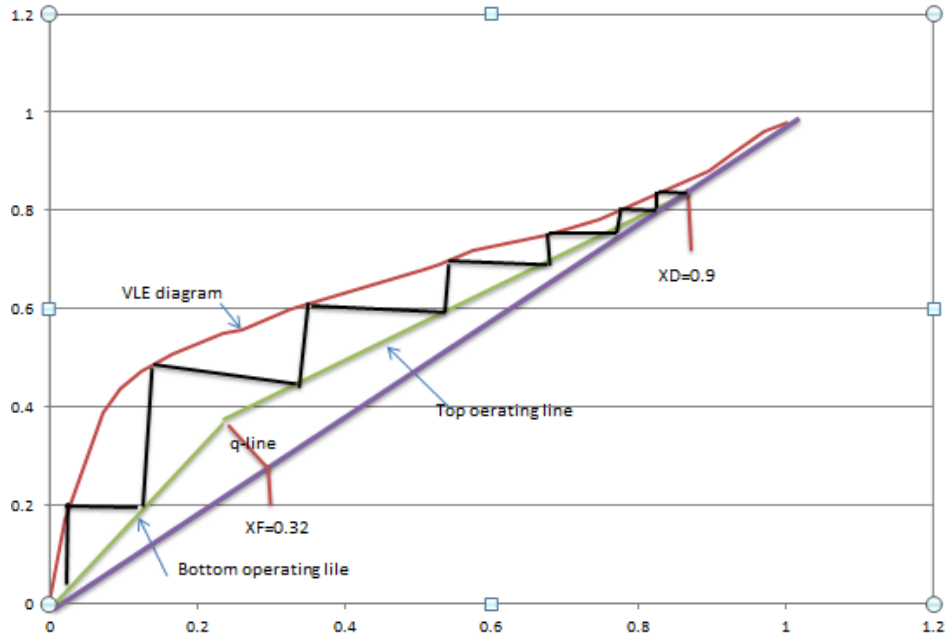


Figure 6.1 McCabe-Thiele diagrams to determine number of stage

With the above information, number of theoretical plate is 7 and feed is introduced at the 6 stage.

Temperature at the top is 85℃ (358k), assumed

Assuming ideal gas behavior, R= 0.082atm.m^3/kmol.k, P= 0.6atm

V =$\frac{nRT}{p}$ where n is taken the vapor load at V = L+D =1,451.345 kg/hr

n=$\frac{1,451.345*0.96}{46}+\frac{1,451.345*0.04}{18}$=33.18kmol/hr

V= $\frac{33.18*0.082*358}{0.6}$= 1,623m^3/hr

Cross sectional area

$$A_c = \frac{v}{linear\ velocity} = \frac{1{,}623 m3/hr}{\frac{0.7m}{s} * \frac{3600s}{hr}} = 0.64 m^2$$

Then the diameter, $D = \sqrt{\frac{4AC}{\Pi}} = \sqrt{\frac{4*0.64}{\Pi}} = 0.9m$

Sizing of storage tank for Fermenter

Liquid to be handled	filtered hydrolyzate
Required mass per hour	6,378.80 kg
Density of liquid	960kg/m3

$$v = m/\rho$$
$$= 6{,}378.8/960$$
$$= 6.64\ m^3/0.85 = 7.8\ m^3$$

Sizing of storage tank for Ethanol

Liquid to be handled	Ethanol
Required mass per hour	398.5 kg
Density of liquid	789kg/m3

$$v = m/\rho$$
$$= 398.5kg/789kg/m3$$
$$= 0.5\ m^3/0.85$$
$$= 0.59\ m^3$$

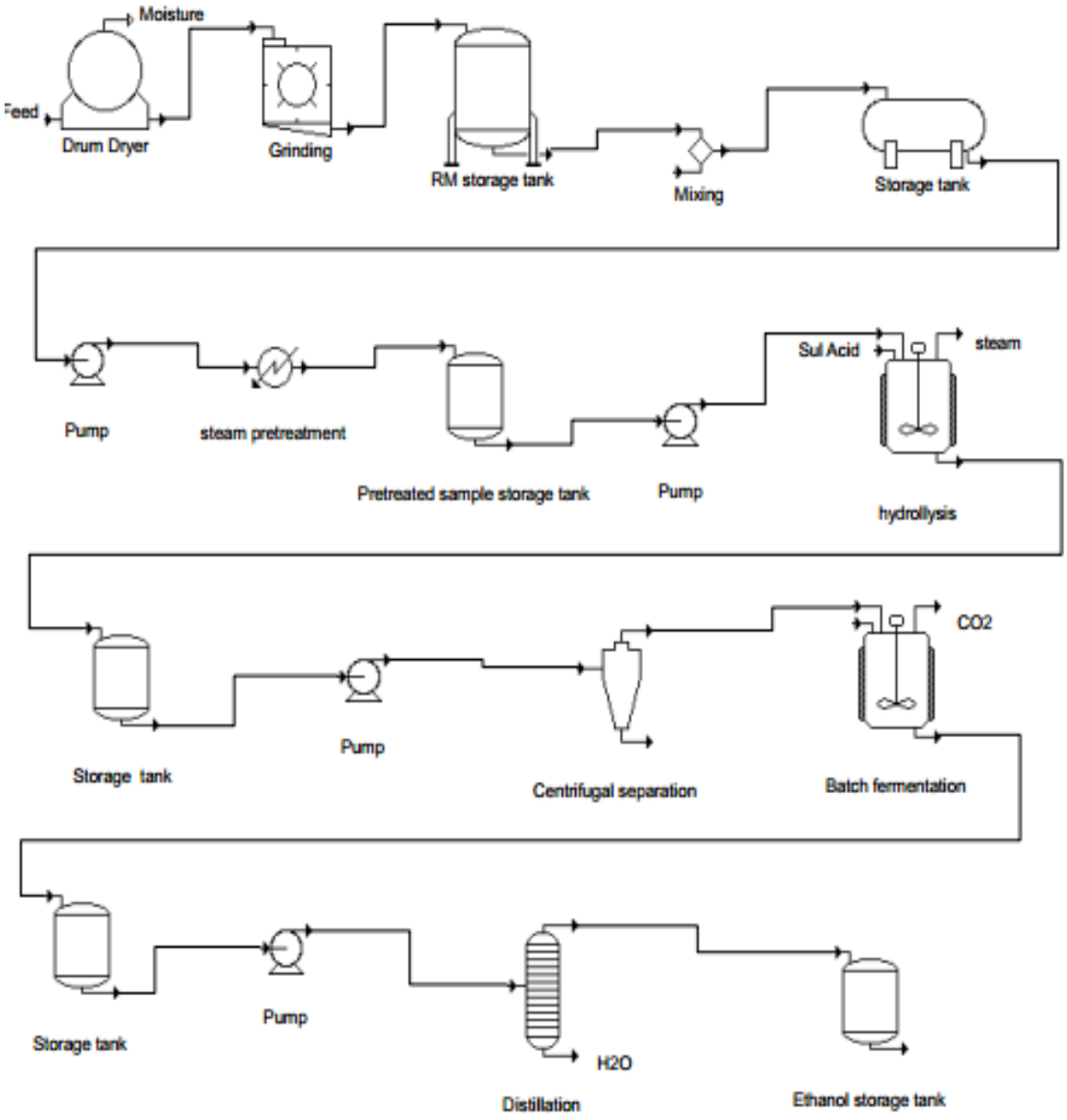


Figure 6.2 Process flow diagram of bioethanol production from waste paper

7 PRELIMINARY ECONOMIC ANALYSIS

Total capital investment

Total Capital Investment = Fixed Capital Investment + Working Capital Investment

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

Fixed capital cost = f (purchased equipment cost)

Table 7.1 Total purchased equipment cost

(Sources: http: //www. mhhe.com./engics/chemicals/peters/data)

Equipment name	Quantity	Capacity	Unit price ($)	Total price ($)
Drum drier	2	1.954 kg/hr	37,100	72,200
Waste paper storage tank	2	284m^3/day	17,196	34,392
Water Storage Tank	3	5.6m^3/day	31,070	93,200
Hydrolyzate St. Tank	3	6.5m^3/batch	33,020	99,060
Mixer	1	5m^3	14,920	14,920
Fermenter	3	7.8m^3/batch	48,500	145,500
Distillation	1	21m^3/hr	7,000	7,000
Molecular sieve	1	45.27kg/hr	12,584	12,584
Ethanol St. Tank	1	0.6m^3/week	31,059	31,059
Centrifugal pump	4	0.004m^3/s	8,450	33,800
Total purchased equipment cost				556,299$

Estimation of fixed capital investment

Table 7.2 fixed capital investment

Direct cost (DC)	Factor	Price ($)
Purchased equipment	1	556,299
Purchased equipment installation	0.3	166,890
Instrumentation and control	0.2	111,260
Piping (installed)	0.1	55,630
Electrical(installed)	0.1	55,630
Building	0.1	55,630
yard improvement	0.1	55,630
Service facilities	0.1	55,630
Land	0.06	33,378
A. Total direct cost (TDC)		1,145,977
Indirect cost (IC)		
Engineering and Supervision	0.33	183,579
Construction expenses	0.41	228,083
Contractors fee's	0.20	111,260
Contingency	0.40	222,520
B. Total indirect cost (TIC)		745,442
C. Fixed capital investment (FCI)= A +B		1,891,419

TCI=FCI+WC

The working capital cost = (10-20) % of total capital investment

TCI =FCI+0.15TCI

TCI = FCI/0.85

TCI = 1,891,419/0.85

= 2,225,199 **$**

Direct production costs (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 determined using the material balance.

Table 7.3 Direct production cost (variable cost)

Item			Cost($)
Raw material cost		0.2$/kg*9,772,470 kg	1,954,494
1	Operating labor	90*150$*12	162,000
2	Direct supervisors and clerical labor	0.1L	16,200
3	Utilities (electric & water cost)	0.025$/Kwh*6,644,880	166,122
4	Maintenance and repair	0.06FCI	113,485
5	Operating supplies	0.01FCI	18,914
Direct production cost			2,433,015

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.

Table 7.4 Fixed Operating Costs

S no.	Item		Cost($)
1	Depreciation	0.1 FCI	189,142
2	Local tax	0.02 FCI	37,828
3	Insurance	0.005 FCI	94,571
4	Rent	0.08 (land + building)	10,513
Total fixed charges			332,054

Table 7.5 Plant overhead cost

No.	Item		Cost($)
1	Plant overhead cost	0.1 DPC	243,301

Manufacturing cost = direct production cost (variable) + fixed operating cost + plant overhead cost

= 2,433,015+ 332,054+ 243,301

= 3,008,370 $

Table 7.6 General expenses

S no.	Item		Cost($)
1	Administrative cost	0.5L	90,000
2	Distribution and sell cost	0.2L	36,000
3	Research and development		Not Applicable
4	Financing interest (self-finance)		Not Applicable
Total general expense			126,000

Total production cost = manufacturing cost + general expense

TPC = 3,008,370 + 126,000

TPC = 3,134,370$

Variable cost = 2,433,015

Fixed costs = fixed charges (except depreciation) + plant overhead cost

= 142,912 + 243,301

= 386,213 $

Depreciation cost = 189,142 $

General expense = 126,000 $

Profit Analysis

Net income, Payback time and return on investment

Gross earn cost

Assuming the current price of 99.9% ethanol =1$/L

Annual revenue = 1$/L*4,000,000L

= 4,000,000$

Total production cost = 3,134,370$

Gross annual profit = 4,000,000 - 3,134,370

= 865,630$

Income tax on gross profit (34%) = 0.34*865,630

= 294,314$

Net income = 865,630- 294,314

= 571,316$

Percent profit

% profit = $\frac{Net\ income}{TPC}$*100

$$= \frac{571,316}{3,134,370} * 100$$

$$= 18.23\%$$

Percent rate of return

Net income = 394,010$

Total capital investment = 3,575,417$

$$\text{Rate of return, \% ROR} = \frac{\text{Net income}}{\text{TCI}} * 100$$

$$= \frac{571,316}{2,225,199} * 100$$

= 26% which is greater than 12%.

Payback period

$$\text{PBP} = \frac{\text{FCI}}{\text{profit} + \text{depreciation}}$$

$$= \frac{1,891,419}{865,630+189,142}$$

= 1.8 years

So, the payback period of the project is estimated to be 1.8 year.

8 CONCLUSION AND RECOMMENDATION

8.1 Conclusion

Due to the diminishing of fossil fuel resources, production of ethanol from lignocellulosic material has acquired significance as a fuel for the future. This study examines the possibility of waste paper (used office paper) for ethanol production. The conversion of waste paper to ethanol was carried out with steam pretreatment, dilute acid hydrolysis, fermentation and distillation process steps.

It is showed that wastepaper is promising lignocellulosic feedstock for bioethanol production. From the result obtained, it can be concluded that the yield of ethanol decreases at very low hydrolysis temperature at very high and very low hydrolysis time. The optimum values of the two factors chosen for maximum ethanol yield of 14.50ml/50g sample (0.23g/g) were 140°C (hydrolysis temperature), 60 minutes (hydrolysis time) and 3% v/v acid concentration.

According to the rough preliminary economic analysis of bioethanol production, the project is profitable since the rate of return on investment was 26% and a payback period of 1.8 year.

Finally it can be concluded that utilization of waste paper as thereby production of biomass based fuels both enhances the economic potential. The ethanol synthesis from waste papers may replenish the fuel availability and it may lead to the sustained development.

8.2 Recommendation

Producing ethanol from renewable resources is becoming an important issue for the whole world. Therefore, the work needs to be continued for scaling up of ethanol production from waste paper. Future studies should include optimization of pretreatment process, optimization of fermentation process and optimization of distillation process variables to obtain maximum yield of bioethanol from waste paper. Finally, it is recommended that further researches should be done on bioethanol production from different types of waste papers such as old newspaper, magazines and cardboards.

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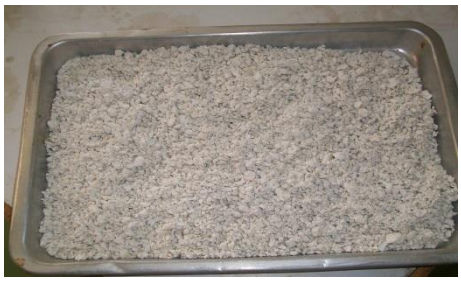
APPENDICES

Appendix A: Laboratory photos



Pretreated waste paper

Sample ready for Hydrolysis

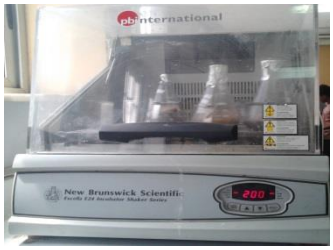
Autoclave (reactor)

Sample after hydrolysis

Vacuum filtration machine

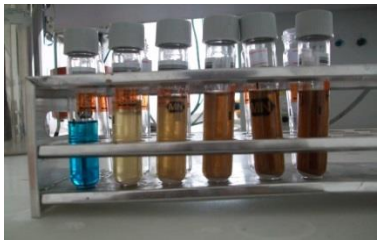
Filtered sample

Cake (byproduct)



Shaker (fermenter)

Distillation Apparatus



Std. glucose solution after being reacted with Benedict's reagen

Appendix B: Properties of Ethanol

Molecular formula	C_2H_5OH
Molar mass	46.07 g mol^{-1}
Appearance	colorless clear liquid
Density	0.789 g/cm^3
Melting point	−114.3 °C, 159 K, -174 °F
Boiling point	78.4 °C, 352 K, 173 °F
Solubility in water	Fully miscible
Acidity (pK_a)	15.9
Viscosity	1.200 mPa·s (cP) at 20.0 °C
Dipole moment	5.64 fC·fm (1.69 D) (gas)

Appendix C: VLE data for Ethanol water solution, total pressure: 101.3kPa

X(liq.)	0	0.019	0.0721	0.0966	0.1238	0.1661	0.2337	0.2608	0.3273	0.3965
Y(vap.)	0	0.17	0.3891	0.4375	0.4704	0.5089	0.548	0.558	0.583	0.6122

X(liq.)	0.5079	0.5198	0.5732	0.6763	0.74472	0.8954	0.93	0.97	1.00
Y(vap.)	0.6564	0.6599	0.6841	0.7385	0.7815	0.8954	0.92	0.96	1.00

Appendix D: Densities of Mixtures of C2H5OH and H2O at 20°C

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

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

Appendix E: Actual versus model Predicted value of ethanol yield

Std. Order	Actual Value	Predicted Value	Residual	Leverage	Internally studentized Residual	Externally studentized Residual	Influence on Fitted Value	Cook's Distance
1	7.80	7.42	0.38	0.444	0.999	0.999	0.894	0.100
2	10.50	11.26	-0.76	0.278	-1.753	-1.998	-1.239	0.148
3	9.00	9.19	-0.19	0.486	-0.519	-0.500	-0.486	0.032
4	12.00	12.19	-0.19	0.486	-0.519	-0.500	-0.486	0.032
5	7.80	7.42	0.38	0.444	0.999	0.999	0.894	0.100
6	9.00	9.19	-0.19	0.486	-0.519	-0.500	-0.486	0.032
7	8.60	8.79	-0.19	0.486	-0.519	-0.500	-0.486	0.032
8	14.41	14.08	0.33	0.444	0.880	0.870	0.778	0.078
9	12.00	12.19	-0.19	0.486	-0.519	-0.500	-0.486	0.032
10	9.40	9.02	0.38	0.444	0.999	0.999	0.894	0.100
11	9.40	9.02	0.38	0.444	0.999	0.999	0.894	0.100
12	8.60	8.79	-0.19	0.486	-0.519	-0.500	-0.486	0.032
13	11.30	11.49	-0.19	0.486	-0.519	-0.500	-0.486	0.032
14	13.20	12.82	0.38	0.444	0.999	0.999	0.894	0.100
15	11.30	11.49	-0.19	0.486	-0.519	-0.500	-0.486	0.032
16	14.50	14.08	0.42	0.444	1.118	1.134	1.014	0.125
17	13.20	12.82	0.38	0.444	0.999	0.999	0.894	0.100
18	10.50	11.26	-0.76	0.278	-1.753	-1.998	-1.239	0.148