



PRODUCTION OF ETHANOL FROM CELLULOSE (SAWDUST)

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<p>Abstract:</p> <p>The production of ethanol from food such as corn, cassava etc. is the most predominate way of producing ethanol. This has led to a shortage in food, inbalance in food chain, increased food price and indirect land use. This thesis thus explores using another feed for the production of ethanol- hence ethanol from cellulose. Sawdust was used to carry out the experiment from the production of ethanol and two methods were considered: SHF (Separate Hydrolysis and Fermentation) and SSF (Simultaneous Saccharication and Fermentation). The SHF proved more hazardous than SSF and also had waste products that are hazardous to the environment. However, it is less costly. On the other hand SSF produced more yield and the process took a longer duration compared to the SHF. The overall process was compared to previous work done on ethanol from an equal mass of corn and it was noted that more yield was experienced with corn (starch) and it was less costly. In conclusion, cellulose ethanol will only thrive well if strict bans are implemented on starch ethanol because evidently there is more yields from starch ethanol, safer and it is less expensive. However, a breakthrough in genetically engineering a microbe to directly convert cellulose to ethanol will be profitable.</p>	
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INTRODUCTION

The emission of greenhouse gases as a result of combustion of fossil fuels led to desire for an alternative or a fuel additive which has led to the increasing demand for ethanol. Ethanol production, however, traces back as far as the days of Noah who was believed to have built himself a vineyard in which he grew grapes that he fermented into some sort of alcoholic beverages. It is an important member of a class of organic compounds with the general name 'Alcohols'. During those early times, ethanol was used as a constituent of alcoholic beverages. With civilization and advancement in science and technology, the benefits derivable from ethanol have continued to multiply. These include solvent, germicide, as anti-freeze, fuel and versatile intermediate for other organic chemicals. For these enormous advantages of ethanol, researchers have been geared towards the production of ethanol from various raw materials (or feedstock). However, in an attempt to save the food chain and to reduce the inflation of food prices caused by ethanol from agricultural feed (sugar or starchy crops), researches are made on production of ethanol from cellulose. Cellulose as described by biologist and chemists is a complex carbohydrate. Cellulose is a linear polysaccharide polymer with glucose monosaccharide units (300 to over 10, 00units with the formula $C_6H_{10}O_5$).

Cellulose is the most common organic compound on earth. Cellulose sources include plant fibers (cotton (90% cellulose), hemp, flax, and jute, corn, and wood (about 42% cellulose). Since cellulose is insoluble in water and can be separated easily from other plant constituents. This acetyl linkage-beta makes it impossible for human beings to digest cellulose due to lack of necessary enzymes to break down cellulose into simpler units. This indigestible cellulose is referred to as roughages, they aid in the smooth working of the intestinal tract. Cellulose is useful in the production of paper and paper products, cotton, linen, and rayon for cloths, nitrocellulose for explosives, cellulose acetate for films, etc. it is also used as feedstock in the production of cellulosic ethanol. Asides from agricultural feed and cellulosic biomass other feed stock in the production of ethanol includes:

. Algae: rather than grow algae, harvest and ferment it, the algae grow in sunlight and produce ethanol directly which is removed without killing the algae. It is claimed the process can produce 6000 gallons per acre per year compared with 400 gallons for corn production.[17]

. Ethylene or Acetylene: these can be obtained from sources such as coal, calcium, carbide, oil gas and other resources. About 5% of the ethanol produced in the world in 2003 was actual a petroleum product.

While the basic steps in the production of ethanol remains the same, the process has been considerably refined in recent years leading to an efficient process. Ethanol can be produced industrially in the following ways:

1. Fermentation of sugar, starch or cellulose
2. Synthetically from ethylene or as by-product of certain industrial operation.

1.1 STATEMENT OF OBJECTIVE

The objectives of this research project are to:

1. Find out and describe ethanol production
2. Find out the use of ethanol production from waste products that are cellulosic e.g. sawdust, newsprint.
3. Comparing cellulosic (sawdust) ethanol to starch (corn) ethanol in technical and economic impact.
4. Production (experiment) of ethanol from cellulosic biomass (sawdust)
5. Comparing the economics of the production and yield of ethanol using separate hydrolysis and fermentation and simultaneous saccharification and fermentation.

1.2 LIMITATION OF CELLULOSIC ETHANOL

The limitations of the production of ethanol from cellulose include:

1. The resistance nature of biomass to breakdown.
2. The variety of sugar which are releases when the hemicellulose and cellulose polymers are broken.
3. The need to find or genetically engineer organisms to efficiently ferment these sugars.
4. Costs of collection and storage of low density biomass feedstock

1.3 SCOPE OF STUDY

For the cause of this research, the chosen cellulosic biomass for the production of ethanol from cellulose is sawdust. The extraction will be done by acid hydrolysis of sawdust to break it down to simple sugars and consequently fermentation of product in the presence of enzymes. Problems encountered in the course of the experiment will be noted, results will be compared to data of extraction of ethanol from the same mass of starch.

2 LITERATURE REVIEW

This part of the thesis contains the full analysis of Cellulose, Ethanol and Cellulosic ethanol

2.1 LITERATURE REVIEW

To satisfy the desire to make life better for man through science and technology and having in view the potentials ethanol has in this regard, there have been some concerted efforts directed at perfecting the fermentation techniques and feedstock used.

2.1.1 CELLULOSE

Cellulose was first discovered in 1838 by French chemist Anselme Payen, who isolated it from plant matter. He found that cellulose contains 44% to 45% carbon, 6 to 6.5% of hydrogen and the rest containing of oxygen. Based on these data, the empirical formula was deduced to be $C_6H_{10}O_5$. However, the actual macromolecular structure of cellulose was still unclear. Haworth proposed a chain-like macromolecular structure in the late 1920s. Staudinger delivered the final proof of the highly polymer nature of the cellulose molecule. This will be discussed under the structure of cellulose [16].

2.1.2 ETHANOL

Ethanol or ethyl alcohol has existed since the beginning of recorded history. The ancient Egyptians produced alcohol by naturally fermenting vegetative materials. Also in ancient times, the Chinese discovered the art of distillation, which increases the concentration of alcohol in fermented solutions. Ethanol was first prepared synthetically in 1826, through the independent effort of Henry Hennel in Britain and S.G in France. Michael Faraday prepared ethanol by the acid-catalyzed hydration of ethylene in 1828, in a process similar to that used for industrial synthesis of ethanol today [4].

Ethanol was used as lamp fuel in the United States as early as 1840, but a tax levied on industrial alcohol during the Civil War made this use uneconomical. This tax was repelled in 1906. In 1907, Henry Ford re-introduced ethanol to the Americans motoring public by producing his first vehicle to run on ethanol. The first Ford Motor Company Automobile was designed to use corn alcohol called ethanol. The most common substrate used for nearly 99%

of ethanol production in the United States today is starch from agricultural crops, primarily corn [4].

In 1940s the first fuel ethanol plant was built in the U.S. army built and operated an ethanol plant in Omaha, Nebraska, to produce fuel for the army and for regional fuel blending. Major quantities were not manufactured until the 1970s due to low cost of gasoline between 1940s and 1970s, however the ethanol industry began to reemerge when ethanol was used as a fuel extender during gasoline shortages caused by the OPEC oil embargoes [9].

In 1980s, after investing heavily in renewable fuels in the 1970s, Brazil kept the program alive during the 1980s. With its robust ethanol programs, Brazil developed an extensive ethanol industry. By the mid-1980s, ethanol-only cars accounted for almost 90% of all new-auto sales in Brazil, making the country the biggest alternative fuel market in the world. In 1988 ethanol began to be added to gasoline for the purpose of reducing carbon dioxide emissions. By 2000, Brazil deregulated the ethanol market and removed its subsidies. However on market conditions, all fuels are required to be blended with 20 to 25 percent of ethanol.

As the production has increased, the effect of biofuels on agricultural markets and the environment have become increasingly important topics, yet much uncertainty still remains. Biofuels have the potential to displace the use of petroleum as a transportation fuel and lower toxic emissions. The evolution of new biofuel production technologies could help alleviate some of the concerns regarding the use of food for fuel by facilitating the use of non-food feedstock's, and could alleviate some of the environmental concerns associated with grain ethanol production. In particular, cellulosic ethanol is believed to hold great promise in this regard, even though there are currently no commercial scale plants in the United State [12].

2.1.3 CELLULOSIC ETHANOL

Cellulosic ethanol i.e. ethanol from forestry or agricultural waste is considered a way to prevent displacement of crops to feed humans. Corn-based ethanol has been blamed by some for higher food prices and shortages because food producers are at times forced to compete with energy companies for grain. Some also argue that the growing demand for such crops is also responsible for indirect land-use change, the destruction of rain forest and wetlands to make room for more farmland. The joint study sees cellulosic ethanol as a viable alternative for reducing oil dependences while protecting food crops. Corn-Stover and switch grass are very potential cellulosic feed stock.

Gallagher et al (2003), also found that crop residues are likely the lowest cost biomass source. According to Atchison and Hettenhaus in 2003, over 240 million dry tons of corn Stover is produced each year in the United States. Brechbill and Tyner (2008) found through research that corn Stover collection risk soil loss from wind erosion and runoff from water erosion depending on the amount of corn Stover collected [12]. However, as cellulosic ethanol technologies advance the use of organic content of the municipal solid waste as a transportation fuel feedstock and simultaneously reduce externalities associated with waste disposal.

Wood is the most common cellulosic feedstock used to manufacture ethanol. Extraction of ethanol from wood alcohol dates as far back as 1819, a memorandum was published on wood alcohol by braconnet, after which numerous attempts have been made on the distillation of wood alcohol.

The various works on wood alcohol are briefly discussed below:

1. About eighty years after the memorandum by Braconnet, Simonsen in 1894 recommended the treatment of sawdust with dilute acids of about 0.3-0.7 at high pressure of about 7-8atm. It however did not become an industrial process because of excessive dilution of saccharine juices. Ekstrom attempted to solve the same problem by using strong sulphurous acid. [4].
2. In 1899, the hydrolysis of wood was studied by Classen who recommended sulphuric acid as the hydrolysis agent instead of sulphurous acid used by his predecessors because from his findings, volatile acids had better penetration of wood. Heating was carried out at 150⁰C, 7atm for 4 to 6hrs; residua are extracted by percolation and filtrate is neutralized and fermented. It was applied in America and abandoned due to corrosion, difficulty in stripping, consumption of coal and sulphuric acid. [6].
3. Beginning in the 1910, two chemical engineers, Messrs. Ewen and Tomlinson used the same procedure as Classen. They however improved the process by using a much shorter and wider converter (12 ft. by 18 ft.), and lining it with firebrick instead of with lead 1 sculpture dioxide gas to the extent of one percent, of the weight of wood treated is introduced into the converter, and steam passed in until a pressure of 100 lb. is obtained. The steam is then turned off and the cylindrical converter slowly revolved for forty-five minutes, the temperature is raised as quickly as possible to the critical point, between 135⁰C and 163⁰C, above which there is an excessive destruction of sugar and production of fermentable substances. The filtrate or juice obtained is partly

neutralized, filtered, cooled and sent on for fermentation using yeast as enzyme. This was implemented on a large scale, in America for the manufacturing of ethanol from sawdust. Industrial yield, under normal conditions, reached 7.3 liters of 100-degree alcohol per 100 kilograms of dry wood, and the factory's annual production is 20 000 hectoliters of alcohol [4].

4. Before 1914, in France, alcohol manufacture from sawdust was studied and implemented industrially in a distillery in the Ardeche region. Due to the need for alcohol for national defense during the World War. Wood alcohol was reconsidered during the 1914-1918 war, production of wood alcohol was achieved in Germany with either the classen or the Windesheim-ten-Doornkaat process. The latter involved heating sawdust with dilute hydrochloric acid in the presence of catalysts (metallic salt), in rotatory autoclaves, at 7 to 8 atmospheres for 20 to 30 minutes. Yield is 6 liters of alcohol per 100 kilograms of dry matter, but it is surely possible to improve this [4].

A research by Dubose led to the following conclusions:

- I. In saccharifying sawdust with 2 parts of sulphuric acid (90-95% H_2SO_4) per 100 parts of dry sawdust, maximum yield is obtained with a pressure of 7.5 atmospheres; yield decreases above or below this pressure.
- II. Conversion takes place as soon as 7.5 atmospheres is attained; in fact, maximum yield occurs within 15 minutes.
- III. Increasing duration does not increase sugar content, but rather reduces it; the sawdust is attacked and noxious secondary products are formed.
- IV. With pine sawdust, yields of 22 to 23% sugar are obtained, giving on average 100 to 115 liters of 95% alcohol per ton of wood treated.
- V. Besides sugar, acetic acid is produced at the rate of 1.4% of the wood treated; also formic acid. The latter acid is produced in quantities that increase with the duration of cooking at 7.5 atmospheres, and this fact is very important, considering the adverse effect of formic acid on alcoholic fermentation. [14].

5. Prodor process a new process based on the hydrolysis of sawdust by cold hydrochloric acid, which considerably reduces destruction of glucose during hydrolysis. It is a continuous process and about 37% HCL used is recovered. The yield is said to be 250 liters of 100% alcohol per tonne of dry sawdust. Residue contains non-fermentable pentose's, which can be converted to furfural, and lignin which, by dry distillation, gives as much methyl alcohol as would have been derived from all the wood from which it was extracted: as a matter of fact, we know that cellulose does not yield methyl alcohol on distillation. Process is still under economic and feasibility considerations. However, production of wood alcohol could only become economical if the wood, after decomposition, could be used for extraction of acetone and methyl alcohol by distillation, as intended in the Prodor process [14].

2.2 THEORETICAL PRINCIPLES

This part of the thesis contains the different type of ethanol production and a quick explanation on starch ethanol.

2.2.1 CELLULOSE: cellulose is the one of the most abundant polymer on earth, which makes it also the most common organic compound. It is also referred to as fiber. Cellulose is a complex carbohydrate or polysaccharide. It is a linear chain of glucose molecules. It is an organic compound with the formula $(C_6H_{10}O_5)_n$.

Structure of Cellulose.

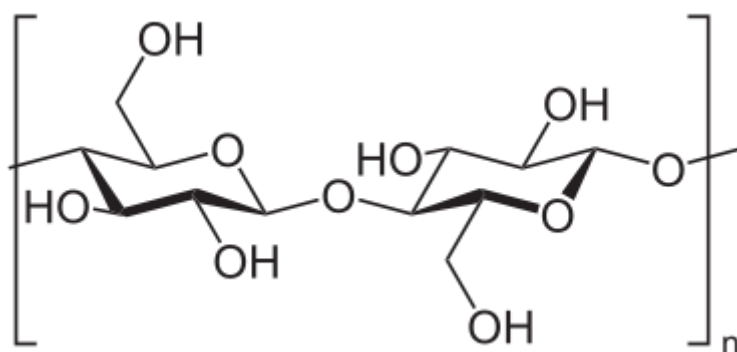


Figure 2.1: Structure of cellulose, Wikipedia

Each cellulose molecule is an unbranched polymer of 1000 to 1 million D-glucose units, linked together with β -1,4 glycoside bonds. The acetyl linkage-beta makes it different from starch. The multiple hydroxyl groups on the glucose from one chain, one chain form hydrogen bonds with oxygen molecule on the same or on a neighbor chain, holding the chains firmly together side by side. Several of these polysaccharide chains are arranged in parallel arrays to form cellulose micro fibrils. The individual polysaccharide chains are bond together in the micro fibrils by hydrogen bonds. Micro fibrils of cellulose are extremely tough and inflexible due to the presence of hydrogen bonds. Micro fibrils have crystalline properties and are bundled together to form macro-fibrils. Although, starch has the same basic structure as cellulose. It is also a polysaccharide; the glucose submits are bonded in such a way that allows the starch molecule to twist. In order words, the starch molecule is flexible, while the cellulose molecule is rigid. Cellulose from various sources are all the same at the molecular level, however, they differ in it crystalline structures and bindings by other biochemical.

2.2.2 PROPERTIES: Many properties of cellulose depend on its chain length or degree of polymerization, the number of glucose units that make up one polymer molecule. Cellulose from wood pulp has typical chain lengths between 300 and 1700 units: cotton and other plant fibers as well as bacterial cellulose have chain lengths ranging from 800 and 10,000 units [16]. The following are the basic properties of cellulose:

1. It is tasteless and odorless.
2. It is insoluble in water and most organic solvents.
3. It is hydrophilic.
4. It is biodegradable.
5. It can be broken down chemically into its glucose units by treating.
6. Very high tensile strength-this strength is important in cell walls, where they are meshed into a carbohydrate matrix, conferring rigidity to plant cells.
7. Cellulose is hard to digest because it has a beta 1.4 glycosidic linkage

2.2.3 PRODUCTION OF ETHANOL FROM CELLULOSE:

There are two methods of extraction of ethanol from cellulose:

Separate Hydrolysis and Fermentation (SHF) which can be sub-divided into

- I. Acid hydrolysis and fermentation.
- II. Enzymatic hydrolysis and fermentation

Simultaneous Saccharification and Fermentation (SSF)

The two methods are followed by distillation of fermented solution to extract pure ethanol. Thus the step by step process of producing ethanol is hydrolysis, fermentation and distillation.

2.2.3.1 SEPARATE HYDROLYSIS AND FERMENTATION

Hydrolysis is the conversion of cellulose to glucose also known as SACCHARIFICATION. In 1965, P.M Sinclair, in his book reported that the concentration of glucose in starch digests is 30-40 percent and highest glucose concentration in cellulose digest is 5 percent. Selby however obtained 17 percent glucose from cellulose. However, presently, using specific techniques 30 percent glucose can be obtained from cellulose. The major problems in obtaining high concentrations from cellulose are;

1. Susceptibility of substrate
2. Activity of enzymes on the system

Cellulose biomass is a mixture of three basic components: lignin, cellulose and hemicellulose. Wood contains 43 percent cellulose with softwood having 2-3 percent less than hardwood, 24-33 percent lignin and the remaining hemicellulose (xylan: 8-14 percent and glucomannan: 16-22 percent) (Browning B.L, 1967). Lignin serves as a sort of glue giving the biomass fibers its structural strength, while hemicellulose and cellulose polymers are the basic building blocks or the fibers. In order to break down the hemicellulose and cellulose to sugars, the basic structure of the biomass must be attacked. Once the structure of the biomass is disrupted, the hemicellulose and cellulose can be converted to sugars enzymatically. This can be done by the use of acid known as acid hydrolysis or by enzymes known as enzymatic hydrolysis.

2.2.3.1.1 ENZYMATIC HYDROLYSIS

The enzymatic hydrolysis reaction is carried out by means of enzymes that act as catalysis to break the glycosidic bonds. This is the degradation of cellulose chains into glucose molecules by *trichoderma viride* (cellulose) enzymes. There are two types of hydrogen bonds in cellulose molecules: those that form between the C₃OH group and the oxygen in the pyranose ring within the same molecules and those that form between the C₄OH group of one molecule and the oxygen of the glucosidic bond of another molecule. Ordinarily, beta-1,4 glycosidic bonds themselves are not too difficult to break. However, because of these hydrogen bonds, cellulose can form very tightly packed crystallites. These crystals are sometimes so tight that neither water nor enzyme can penetrate them; only *exoglucanase*, a subgroup of cellulase that attacks the terminal glucosidic bond, is effective in degrading it. The inability of water to penetrate cellulose also explains why crystalline is insoluble. On the other hand, amorphous cellulose allows the penetration of *endoglucanase*, another subgroup of cellulase that catalyzes the hydrolysis of internal bonds. The natural consequence of this difference in the crystalline structure is that the hydrolysis rate is much faster for amorphous cellulose than crystalline cellulose. The process of breaking the glucosidic bonds that hold the glucose basic units together to form a large cellulose molecule is called hydrolysis because a water molecule must be supplied to render each broken bond inactive. In addition to crystallinity, the chemical compounds surrounding the cellulose in plants, e.g. lignin, also limit the diffusion of the enzyme into the reaction sites and play an important role in determining the rate of hydrolysis. Sometimes, wood chips are pretreated with acid to strip hemicellulose and

lignin before they are treated with an enzyme or a mixture of enzymes. In general, 20 to 70 percent yield of glucose can be expected after 24 hours. Hence, it is sometimes referred to as Acid-Enzyme hydrolysis

Pre-treatment is necessary to break down the crystalline structure of the lignocellulose material isolating the cellulose away from the lignin in the cell walls for hydrolysis. Pre-treatment is carried out to increase the surface area and accessibility of the plant fiber to enzymes and thus, achieve high sugar yield for ethanol fermentation. The face stock is subjected to steam and acid whose temperature, concentration respectively and treatment time are mild so that the cellulose surface area is greatly increased as the fibrous feedstock is converted to a muddy texture with little conversion of the cellulose to glucose. This process is referred to as pre-treatment of the feedstock. The feedstock however becomes acidic and the pH. is adjusted by the addition of alkaline often NaOH to 4 to 6, which is the optimal pH range for celluloses. Higher pH is acceptable if alkalophilic cellulose is used. The pre-treated cellulose is then hydrolyzed with cellulose. Cellulose is a generic term denoting a multienzyme mixture comprising exocellobiohydrolases (CBH) endoglucanases (EG) and *Aspergillus luchunensis* that can be produced by a number of plants and micro-organisms. CBH and EG catalyze the hydrolysis of insoluble cellulose to cellooligosaccharides (majority cellobiose) which is then catalyzed to glucose by β G.

CBH and EG

β G

Cellulose-----→Cello oligosaccharides-----→ Glucose

CBH and EG bind to cellulose in the feedstock via carbohydrate-binding modules (CBMs), such as cellulose-binding domain (CBDs), while most β G enzymes, including *Trichoderma* and *Aspergillus* β -glycosidase enzymes, do not contain such binding modules and thus remain in solution.

Advantages:

1. Low utility costs since process can be run at low temperatures.
2. Process allows for very high rate of conversion.

However the problems with enzymatic hydrolysis are:

1. Cellulose accounts for up to 50 percent of the cost of hydrolysis due to the requirement of large amount of cellulose hence increased cost of process.
2. Presence of compounds that reduce rate of cellulose and/or micro-organisms in the subsequent fermentation of the sugar. E.g. glucose released during the process inhibits cellulose, particularly β G.

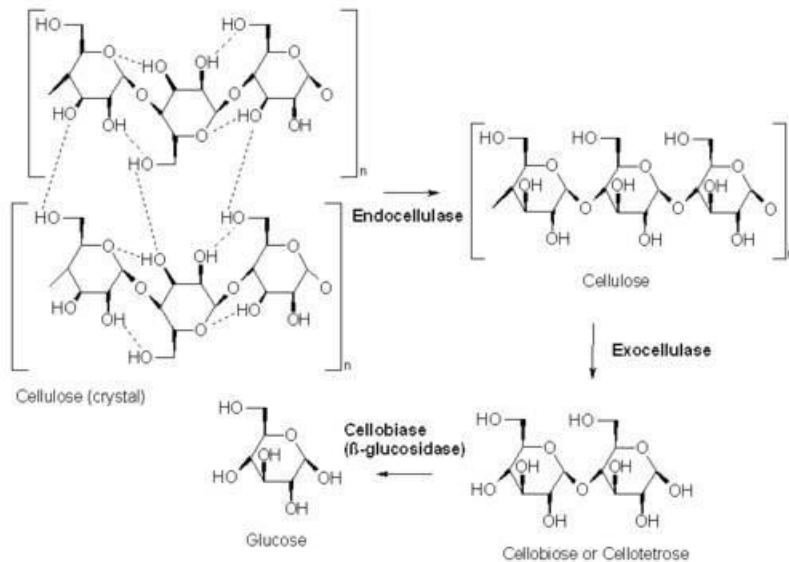


Figure 2.2. Reaction pathway from cellulose to glucose. Enzymeindia (2008).

2.2.3.1.2 ACID HYDROLYSIS

Acid hydrolysis is perhaps currently seen as the most technologically matured method of sugar release from biomass. Traditional methods developed in the 19th century and at the beginning of the 20th century, produced glucose from cellulose by usage of acid. No pretreatment is required if the end product (glucose) is to be fermented to alcohol. Depending on the concentration of the acid and the other parameters can be determined i.e. dilute acid maybe used at high temperature and pressure while concentrated acids maybe used at very low temperature and pressure. In the case when sulfuric acid can be concentrated (25-80%) or dilute (3-8%), measured as the weight of acid in the weight of acidified aqueous solution that is present with the feedstock [6].

The advantages of acid hydrolysis are:

1. It is a faster reaction requiring much less residence time in the reactor.

The disadvantages of acid hydrolysis are:

1. Lower conversion of cellulose to glucose because process is more equilibrium driven.
2. Sugar degradation to substances detrimental to fermentation occurs.
3. Mineral acid treatment makes possible utilization of the other lignocellulosic constituents is made more difficult.
4. Non-selectivity of mineral acids to lignocellulosic material.

The enzymatic hydrolysis is however preferred due to low utility costs and high conversion rates enzymatic hydrolysis can be improved by [15].

1. Development of pretreatment technology which is effective does not require expensive chemicals and/or high pressure expensive equipment.
2. Maintaining a high density of cells within the reactor so as to convert sugars to ethanol quickly
3. Combining enzymatic conversion of cellulose and hemi-cellulose polymer with fermentation as to keep sugar levels low, improving enzymatic conversion rates by minimizing product (sugar) inhibition of enzymatic conversion of the biomass to sugar.
4. Converting both the cellulosic (glucose) and hemicellulose (xylose) to ethanol.

2.2.3.1.3 THERMOCHEMICAL PROCESS

There are two ethanol production processes that currently employ thermochemical reactions in their processes. The first system is actually a hybrid thermochemical and biological system. Biomass materials are first thermo chemically gasified and the synthesis gas (a mixture of hydrogen and carbon oxide) bubbled through specially designed fermenter. A micro-organism that is capable of converting the synthesis gas is introduced into the fermenters under specific process condition to cause fermentation to ethanol.

The second thermochemical ethanol production process does not use any micro-organisms. In this process, biomass materials are first thermo-chemically gasified and the synthesis gas passes through a reactor containing catalysts, which cause the gas to be converted into ethanol. An intensive effort was made to develop these processes for fuel. Numerous efforts

have been made since then to develop commercially viable thermochemical-to-ethanol processes.

Ethanol yields up to 50% have been obtained using synthesis gas-to-ethanol processes. Some processes that first produce methanol and then use catalytic shifts to produce ethanol have obtained ethanol yields in the range of 80%. Unfortunately, like the other processes, finding a cost-effective all-thermochemical process has been difficult. (Badger, P.C. 2002)

2.2.3.2 SIMULTANEOUS SACCHARIFICATION AND FERMENTATION

According to Schell and Walter in 1991, simultaneous Saccharification and fermentation (SSF) is thought to be the best process for enzymatic conversion of cellulose to ethanol. The simultaneous Saccharification and fermentation process, firstly described by Takagi et al (1997), combines enzymatic hydrolysis of cellulose with simultaneous fermentation of its main derived sugar (glucose) to ethanol. In SSF, enzymatic cellulose hydrolysis and glucose fermentation to ethanol by yeast proceed simultaneously within one vessel. Compared with saccharification in the absence of yeast, simultaneous saccharification and fermentation (SSF) using *Trichoderma cellulose* and *saccharomyces cerevisiae* enhanced cellulose hydrolysis rates by 13-30 percent [8]. The optimum temperature for SSF was 35⁰C. The requirement for β G in SSF was lower than for saccharification. This is a very promising way of producing ethanol due to its ability to improve hydrolysis rates, yields and product concentration compared to SHF.

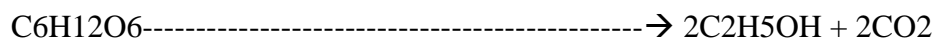
It is a combination of acid hydrolysis, enzymatic hydrolysis and fermentation. Here, the cellulose feedstock is pre-treated dilute acid pretreatment using H₂SO₄ or SO₂. The process is often run as a steam explosion treatment of acid impregnated material, where the hemicelluloses are removed and the digestibility of the cellulose is enhanced. H₂SO₄ is a strong catalyst that highly improves the hemicellulose removal but also easily yield inhibitors but also, a less extended hemicelluloses hydrolysis [10]. The choice of enzymes preparation used in SSF is subject of great importance due to very high cost of the enzymes. However presently a combination of cellulose and *Saccharomyces cerevisiae* (yeast) is used. Cellulose catalyzes the saccharification while the yeast is used to produce ethanol.

However, some species of bacteria have been found capable as direct conversion of a cellulose substrate into ethanol. An example is *clostridium thermocellum*, which uses complex cellulose to break down cellulose and synthesizes ethanol. However, other by-

products including acetate and lactate, in addition to ethanol, lowering the efficiency of the process. Some research efforts are directed to optimizing ethanol production by genetically engineering bacteria that focus on the ethanol producing pathway.

2.2.4 FERMENTATION

This is the chemical transformation of organic substance into simpler compounds by the action of enzymes. Originally the term fermentation was used to mean the enzymatic breakdown of carbohydrates in the absence of air. In industrial practice, fermentation refers to any process by which raw materials are transformed by the controlled action of carefully selected strains of organisms into definite products. Louis Pasteur used the term in a narrower sense to describe changes brought about by micro-organisms growing in the absence of air. However, for the cause of this thesis it is a biological method of producing ethanol. The fermentation reaction is caused by yeast or bacteria which feed on simple sugars. The glucose produced from the hydrolysis described above is fermented with yeast to produce ethanol. Carbon-dioxide is also produced as glucose is consumed. The simplified reaction equation is:



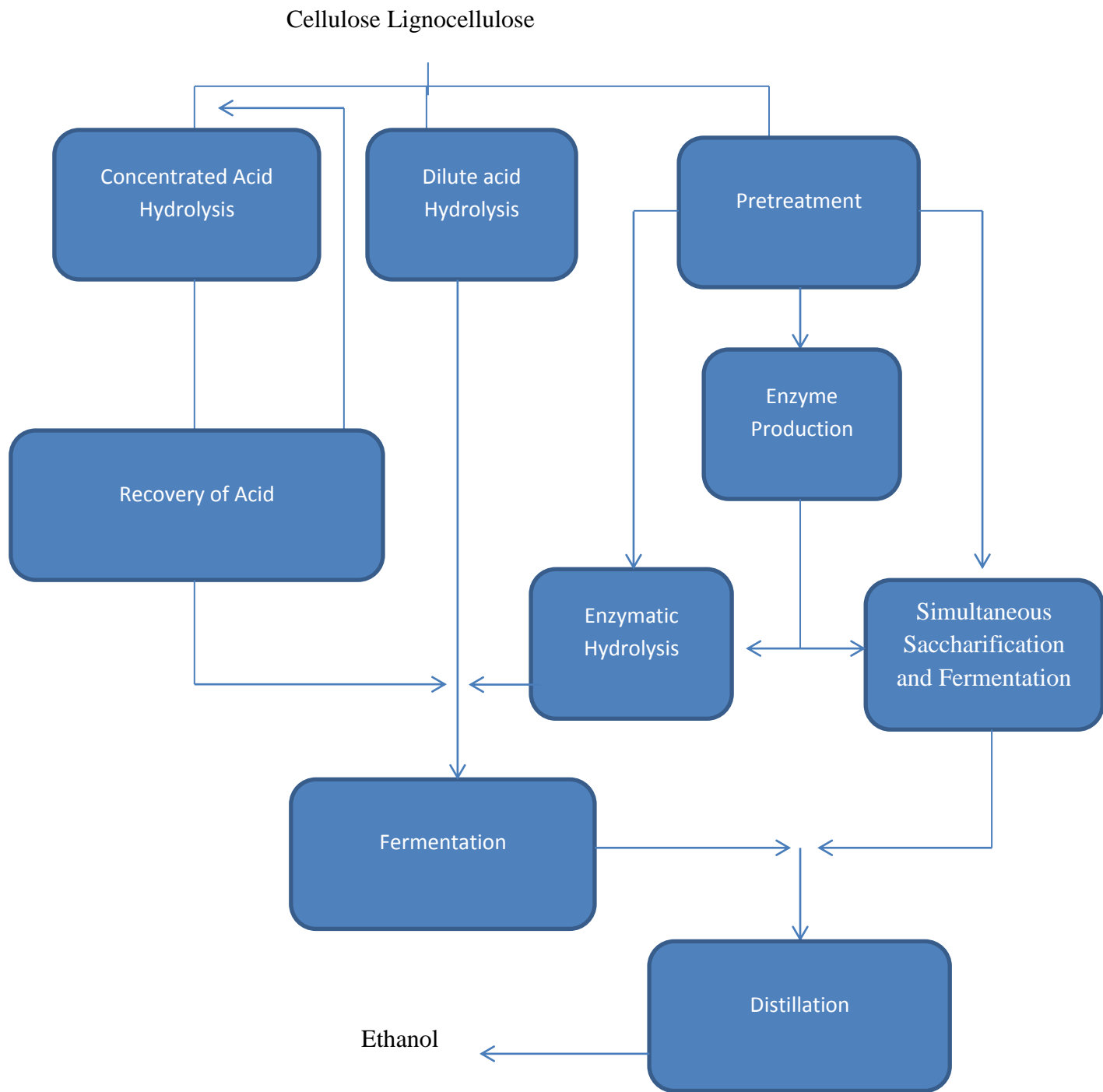


Figure 2.3: Cellulosic ethanol production process ZACCH (2002)

2.2.5 DISTILLATION

This is a separation of mixtures based on the volatilities (boiling points) of the individual components that make up the mixture. Distillation is often used only if one product is required. The product which is of low volatility is called distillate while the substances of high boiling point that remain in the flask is called residue or bottoms. In acid hydrolysis, part of the acid and water is recovered in distillation.

2.2.6 BRIEF DESCRIPTION OF STARCH ETHANOL

2.2.6.1 STARCH ETHANOL

Starch also known as carbohydrate is a polysaccharide consisting of many glucose units. It is second in abundance only to cellulose, a major structural component of plants. Starch, a polymer of glucose, is an alpha-glycan, predominantly containing alpha-1,4-glucosidic linkages with a relatively small amount of alpha-1,6-glucosidic linkage forming branch points. It is this linkage as stated earlier that differentiates it from cellulose and makes it more subject to break down compared to cellulose.

Starch are involved in important role in foods, either naturally occurring in an ingredient or added to achieve a desired functional characteristic. Papermaking amongst many is a non-food application of starch consuming millions of starch every year. Starch may be altered physically, chemically, or enzymatically to produce modified starches with improved functional properties. Sources of starch includes: cassava, wheat, rice, corn, yam, etc. starch granules vary in size (1 to 100 micron in diameter) and shape, which are characteristics of their specific plant origin.

Starch is the major energy reserve for plants; it is located mainly in the seeds, roots or tubers, stem pith, and fruit. Ethanol is made from sugar (sugarcane) however starch is the predominant feedstock for ethanol production. Corn is the most widely used starch for the production of ethanol referred to as corn ethanol while cassava is mostly used in Thailand, Nigeria and Ghana.

2.2.6.2 PRODUCTION OF STRACH ETHANOL

Ethanol is commercially produced in one of two ways, using either the wet mill or dry mill process. This is a feed preparatory process. Although wet mill facilities were common in the industry's early days, dry mill facilities now account for more than 80 percent of industry capacity.

WET MILLING: wet milling involves separating the grain kernel into its component parts (germ, fiber, protein, and starch) prior to fermentation. The process of wet milling takes the corn grains and steeps it in a dilute combination of sulfuric acid and water for 24 to 48 hours in order to separate the grain into many components. The slurry mix then goes through a series of grinders to separate out the corn germ. Corn oil is a by-product of this process and is extracted and sold. The remaining of the fiber, gluten and starch are segregated out using screen, hydrochloric and centrifugal separators. The gluten protein is dried and filtered to make a corn gluten-meals co-product and is highly sought after by poultry broiler operators as a feed ingredient. The steeping liquor produced is concentrated and dried with fiber and sold as corn gluten feed to the livestock industry. The heavy steep water is also sold as a feed ingredient and is used as an environmentally friendly alternative to salt in the winter months. The corn starch and remaining water can then be processed one of three ways:

- a) Fermented into ethanol, through a similar process as dry milling. This will be discussed below.
- b) Dried and sold as modified corn starch.
- c) Made into corn syrup.

DRY MILLING: here the entire grain kernel is ground into flour. The starch in the flour is converted to ethanol during the fermentation process.

The same procedure is used in the process for wet milling and dry milling. There are two methods which can be used: separate hydrolysis and fermentation or simultaneous hydrolysis and fermentation. The steps are the same and are only distinguished by whether the saccharification is carried out separately from the fermentation or simultaneously. Hence like cellulosic ethanol production we have:

- I. Separate Hydrolysis and Fermentation
- II. Simultaneous Hydrolysis and fermentation

The steps involved in the production of ethanol from cellulose are:

- I. Gelatinization: is the swelling of starch granule in the presence of heat and water. The starch loses its crystallinity and becomes an amorphous gel that can be attacked by enzymes.'
- II. Liquefaction: At this point, the starch or grounded grain slurry thickens considerably and would be difficult to process if an alpha- amylase were not added to partially hydrolyze the starch to dextrin. The pH is adjusted to about 5.8, and an alpha- amylase enzyme is added. The slurry is heated to 180-190 degree for 30-45 minutes to reduce viscosity. The alpha- amylase is a bacteria thermostable endo-amylase. It hydrolyzes 1.4 bonds at random points in the starch molecule to rapidly reduce the viscosity of gelatinized starch solutions. The dextrin solution is much more fluid; thus, we say the starch gel is liquefied. The alpha- amylase serves to reduce the viscosity of the solution and also to produce a lower molecular size substrate. This smaller substrate molecule is needed for the efficient action of glucoamylase.
- III. Saccharification: this smaller substrate molecule is needed for the efficient action of glucoamylase which hydrolyzes the dextrin to glucose. After the flash condensation cooling, the mixture is held for 1-2 hours at 180-190 degree to give the alpha- amylase enzymes time to break down the starch into short chain dextrin. After pH and temperature adjustment, a second enzyme, glucoamylase, is added as the mixture is pumped into fermentation tanks.
- IV. Fermentation: Yeast or candida tropical is added to convert the sugar to ethanol and carbon dioxide. The mash is then allowed to ferment for 50-60 hours, resulting in a mixture that contains about 15% ethanol as well as the solids from the grain and added yeast. However this will be applicable in SHF, in SSF, the glucoamylase and fermenter are added together so that saccharification can occur simultaneously.
- V. Distillation: this solution is then distilled to get ethanol.

2.2.7 ADVANTAGES OF CELLULOSIC ETHANOL OVER STARCH ETHANOL

The advantages of cellulosic ethanol over starch ethanol are as follow:

1. According to Regmi et al (2001), a 1% increase in food prices causes an average 0.75% decline in food consumption in developing countries. In addition to reducing caloric intake as food prices increase, low-income people also switch to less nutritious food by Von Braun (2007)
2. Cellulosic ethanol as a viable alternative for reducing oil dependence while protecting crops i.e. way to prevent the displacement of crops that feed humans.
3. Corn-based ethanol has been blamed by some for higher food prices and shortage because food productions are at times forced to compete with energy companies for the grain. Some also argue that the growing demand for such crops is also responsible for indirect land-use change, the destruction of rain forests and wetlands to make room for more farmland. On the other hand, proponents of cellulosic ethanol argue that because the fuel is produced from agriculture byproducts, it has no impact on the food supply or land use.
4. Cellulosic ethanol could help reduce air pollution- cellulosic ethanol not only emits less greenhouse gas than gasoline but emits fewer fine particles into the air.
5. From the analysis carried out, it is apparent that a shorter residence time is required for the production of ethanol from cellulose.
6. It provides a much greater energy yield.

Potential advantages of cellulosic biofuels are:

7. Reducing the amount of fertilizer and pesticide runoff into rivers and lakes.
8. Mascoma researchers are engineering microbes to combine the last two steps of the process: breaking down cellulose, and converting sugars into ethanol. They say that if they can get micro-organisms to make ethanol at sufficiently high rate, they can reduce the amount of expensive enzymes needed to break down cellulose, which can normally take up half of ethanol production costs. Thus making cellulosic ethanol cheap.

A report from the University of Minnesota said For each billion-equivalent gallons of fuel produced and combusted in the US, the combined climate-change and health costs are \$469 million for gasoline, \$472-952 million for corn ethanol depending on bio refinery heat source

(natural gas, corn Stover, or coal) and technology, but only \$123-208 million for cellulosic ethanol depending on feedstock.

2.2.8 DISADVANTAGES OF CELLULOSIC ETHANOL OVER STRACH ETHANOL

1. It involves more complex methods of production.
2. It is very expensive to produce.

2.2.9 USES OF ETHANOL

The following are some of the various uses of ethanol:

1. Raw materials: it is a raw material in the manufacturing of acetaldehyde, acetic acid, ethylene, glycol, dyes, detergents and cleaning solution.
2. Pharmaceutical preparation: ethanol is used in the preparation of cough syrups. It is also used in the preparation of antiseptics.
3. Fuel: It is used as an additive to extend gasoline for automotive fuel, has advantage of being renewable. Fuel blended with very high ethanol content has lower hydrocarbon emission than 100% gasoline.
4. Miscellaneous: It is used as anti-freeze in automobile radiator. It is also used in preparation of alcoholic beverages.

2.2.10 FACTORS FOR SELECTING SAWDUST AS CELLULOSIC FEEDSTOCK

Of the various possible feedstocks for production of cellulosic ethanol, corn Stover, wood and switch grass have significant potential. However, the choice of sawdust for the experiment is because:

1. Waste
2. Cheap and readily available
3. Although corn Stover may have tremendous potential as a feedstock, corn Stover collection can result in damage to the soil such as erosion.
4. Perlack et al (2005) reported that perennial crops, cultivated specifically for use in bioenergy production, were determined to potentially be available at quantities similar to corn Stover. However, an increase in the production of such crops would require a decrease in the areas of other agricultural crops or pastureland.
5. Previous study indicated that using waste products as an ethanol feedstock may be a more desirable option, since the production of both additional corn grain and switch grass for ethanol production increases GHG emissions by a substantial amount.

2.2.11 FACTORS LIMITING ETHANOL YIELD

The factors limiting ethanol yield include:

1. pH of the substrate: if the pH of the substrate must be within acceptable limits of fermenter (enzymes) otherwise it will destroy the enzymes or reduce ethanol that can be fermented.
2. Temperature of the substrate: An acceptable limit of substrate is between 30-45 degree for optimum yield.
3. Substrate concentration: the amount of substrate to be fermented is proportional to the ethanol that can be fermented from it, if all other condition is maintained.
4. Fermentation type: SHF or SSF.

2.2.12 ETHANOL PRODUCTION AND WORLD DEMAND

Dating back to antiquity when alcohol was only used as an alcohol beverage, the importance of ethanol to man has continued to rise. As a result of the myriad of use that ethanol could be put to, the demand for ethanol has increasingly gained prominence most especially in the process industries. The wide array of raw materials from which ethanol can be produce emphasizes the special role ethanol plays in the technological scheme of things. Efforts have been made to improve already established methods of producing ethanol for optimum yield and efficiency and more drives are on to open up new areas of study. With increasing global warming and desire of man to expand the supply of motor fuel and reduce pressure on world crude oil supplies needed to refine gasoline has led to the increasing world demand for ethanol.

TABLE 1.1 WORLD ETHANOL PRODUCTIONS (MILLION GALLONS)

Adapted from Ethanol World Production statistics (2011)

	2007	2008	2009	2010	2011	% Increase
Brazil	3,546	4,988	5,238	5,489	5,739	61.8
U.S.	5,896	6,198	6,858	7,518	8,178	38.7
China	970.5	1,075	1,101	1,128	1,154	45.8
India	447.3	531	551	571	591	32.2
France	203.8	285	301	317	333	63.4
Spain	112.4	163	184	206	227	101.9
Germany	184.9	319	381	444	506	173.7
Canada	184.9	230	276	322	368	99
Indonesia	74.9	76	76	84	100	33.5
Italy	46.4	50	53	55	60	29.2
ROW	1,627.5	2,302	2,548	2,794	3,040	86.8
Others	16,142.1	16,215	17,574	18,934	20,293	

From the table above, it can be deduced that with the rate of production of ethanol with the use of majority agricultural feed, the world is heading for one of the greatest tragedies ever. The world is facing the most severe food price inflation in history as grain and soybean prices climb to all-time highs. As a result, price of food products made from directly from these commodities such as bread, pasta, and tortillas, and those made indirectly, such as pork, poultry, beef, milk, and eggs, are everywhere on the rise. According to world watch institute and Centre for Ethanol progress, in Mexico, corn meal prices are up 60 percent. In Pakistan, flour prices have doubled; China is facing rampant food price inflation, some of the worst in decades, from 1990 to 2005, world grain consumption, driven largely by population growth and rising consumption of grain-based animal products, climbed by an average of 21 million tons per year. Then came the explosion in demand for grain used in U.S. ethanol distilleries, which jumped from 54 million tons in 2006 to 81 million tons in 2007. This 27 million ton jump more than double the annual growth in world demand for grain. If 80 percent of the 62 distilleries now under construction.

Historically the food and energy economies have been largely separate, but now with the construction of so many ethanol distilleries, they are merging. If the food value of grain is less than fuel value, the market will move the grain into the energy economy. Thus as the price of oil rises, the price of grain follows upward. The World Bank reports that for each one percent rise in food prices, caloric intake among the poor drops 0.5 percent. Millions of those living on the lower rungs of global economic ladder, people who barely hanging on will lose their grip and begin to fall down. [11]

Production of ethanol from sawdust to cellulose, thus, saves the food chain and the environment. Cellulose is contained in some waste product such as; human waste, citrus peels, sawdust or newsprint which reduces cost of raw material. Production from sawdust currently is facing challenges because its money intensive due to the fact that cellulose is a complex carbohydrate and will not be broken down to simple sugar.

3 METHOD

There are two methods in the production of ethanol from cellulose which are separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation. These methods have been extensively discussed in the previous chapter respectively and will be used in the experimental production of ethanol from 100g hardwood sawdust (cellulose) in this chapter.

3.1 LIST OF REAGENTS AND APPARATUS

The list of reagents and apparatus are presented below:

3.1.1 LIST OF APPARATUS

The following are the apparatus that will be used in the course of this experiment:

1. Weighing
2. Autoclave
3. Pipettes
4. Buchner funnel (this is used because the material is made up of is resistance to strongly acidic solutions).
5. Stirring rod
6. Shaker
7. pH meter
8. Thermometer
9. Conical flasks
10. Beakers
11. Density meter analyzer

3.1.2 LIST OF REAGENTS

Lists of reagents required are listed below:

1. Distilled water (pH 7.0)
2. Water (pH 9.7)
3. 18M H₂SO₄
4. 0.4m H₂SO₄
5. 8.5m NaOH
6. *Saccharomyces cerevisiac*
7. Hardwood sawdust
8. Tween 80
9. 0.01m Ca(OH)₂

3.2 EXPERIMENTAL PROCEDURE

3.2.1 Experiment 1: SEPARATE HYDROLYSIS AND FERMENTATION

Culturing saccharomyces cerevisiae: *Saccharomyces cerevisiae* is used to ferment the sugar in the ethanol and has to be cultured 48hours before commencing the experiment. 10g of potato dextrose Agar (PDA) is dissolve completely in 250ml water in a conical flask. The mixture is covered with cotton wool and foil paper and then sterilized in an autoclave at 121⁰ C for 5minutes. On removal it is allowed to cool and then poured into petri dishes and is set aside and allowed to solidify. The *Saccharomyces cerevisiae* are then introduced into the petri dishes with the aid of a sterilized inoculating loop. The petri dishes are then sealed and kept in an incubator for 48hours at a temperature of 25⁰ C.

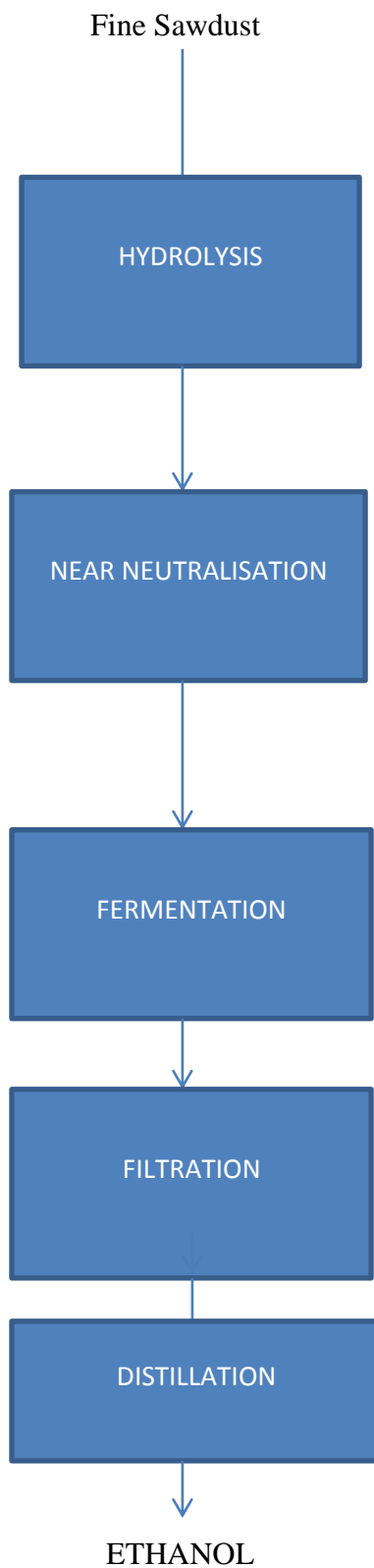
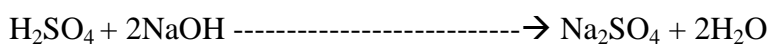


Fig 3.1 Simplified flow chart on the SHF production of ethanol from sawdust

Sawdust is sieved to create uniformity of particles. The sawdust is then dried for 12hours to remove moisture. A 250ml beaker is filled with 100g of dry sawdust and 100ml of 18M H_2SO_4 (sawdust to acid (w/v) ratio is 1:1) is added to it at standard conditions. The reaction is

spontaneous producing lignin (lignin is the substance that bonds sugar molecules to make cellulose out of them) which is seen as black residues, the conical flask also immediately become very hot and bubbles due to air pocket in sawdust. However, the pH is very low and *Saccharomyces cerevisiae* cannot function at this condition, it would function optimally at a pH of 4.5-6.0. Thus, there is need to increase the pH. In a 1000ml beaker add 200ml of water (water at pH of 9.7) and pour acidic solution into it and stir thoroughly. The pH was read to be 1.37. After which 100ml water was added again to the solution and stirred thoroughly, the pH was then read to be 1.91. 100ml water was added again to the solution and the pH read 2.35. However allowable dilution factor with water is 1:4. From the equation below it can be seen that 9M NaOH is required to form salt thus pH 7 and less will be required for an optimal reaction.



8.5M NaOH solution is prepared and added drop by drop until pH of 4.87 was attained. The solution is filtered bringing out the cellulose substrate as filtrate and lignin as residue using a Buchner funnel. Using a DMA 35 the sugar produced is measured to 32.4g. The cultures of *saccharomyces cerevisiae* in the agar slant tubes were dissolved with 10ml of distilled water containing a drop of tween 80. 10ml of the solution is then added to the cellulose substrate to ferment it. On a four-hourly basis the sample is tested for sugar content to determine rate of conversion of sugar to ethanol thereby determining the time required for fermentation and rate of fermentation. Ethanol fermentation was performed in a shaker incubator at 150rpm for 48-72 hours at 30⁰ C to allow it to ferment completely. Bubbles of CO₂ are seen to appear.

This 100ml of the filtrate is then distilled using a distillation bath. 5.9ml of ethanol is distilled at 78⁰ C and water can be distilled at 100⁰ C.

3.2.2 Experiment 2: SIMALTANOUS SACCHARIFICATION AND FERMENTATION

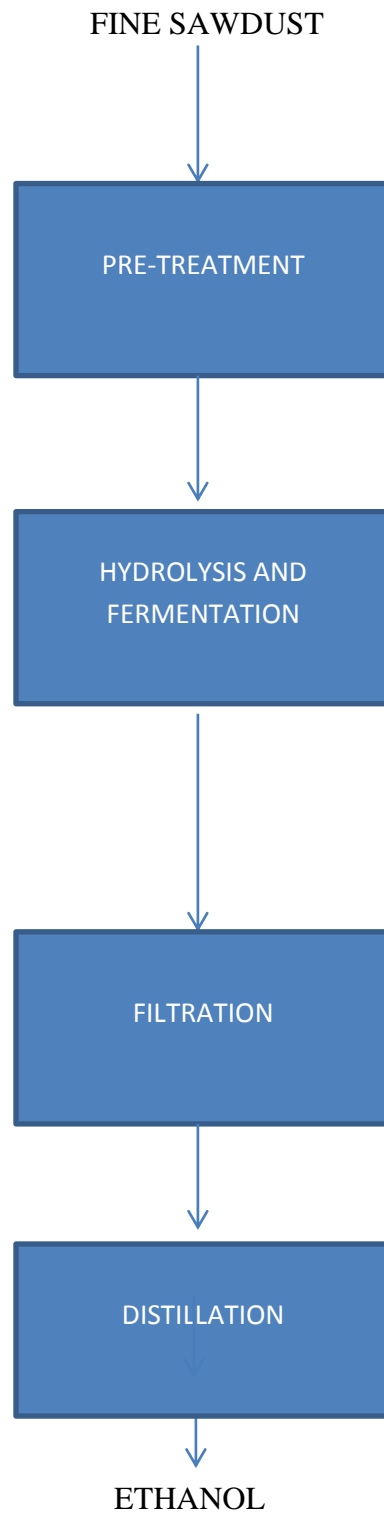


Fig 3.2: simplified flow chart for the SSF production of ethanol from sawdust.

100g of fine sawdust was added to 100ml conical flask and 100ml of 0.4M H₂SO₄ was added to it. The pH of the mixture was 3.1, thus, 0.01M Ca(OH)₂ was prepared and added in drops until pH of 4.61 was attained. The mixture was put in an autoclave and was subjected to a temperature of 120C for 10minutes. The mixture was then removed but because the temperature was too high for enzymes to be added, so it was cooled in a refrigerator until a temperature of about 30C was attained. The sugar content was tested for to be 24.7g. After which the 2.5g of cellulose was added and *Saccharomyces cerevisiae* was added. The mixture was kept in a shaker incubator at 150rpm for 48hours – 72 hours at 30⁰ C to allow it to ferment completely. On a four hourly basis, the mixture was tested for sugar content to determine rate of fermentation with time and the time required. 100ml of sample was distilled in a distillation bath and 6.3ml of ethanol was distilled at 78C,

Safety consideration:

1. Hydrolysis reaction is an exothermic reaction; care was taken in handling the reactor vessel.
2. Care was also taken in handling the H₂SO₄.
3. The acid was poured into water and not water into acid to avoid explosions of skin burns.
4. Overalls, gloves and goggles were worn in the course of the experiment.
5. In removing the hot liquids from the autoclave care was taken.

4 RESULTS AND DISCUSSION

4.1 ANALYSIS OF SEPERATION HYDROLYSIS FERMENTATION OF SAWDUST

The procedure for the production of ethanol from cellulose by SHF has been discussed in chapter 3; section 3.2.1, here 100g of hardwood sawdust is used to produce ethanol by acid hydrolysis using H₂SO₄ as the acid. 32.4g of sugar was obtained on completion of the hydrolysis; however, theoretically 52.2g of sugar can be produce from 100g of sawdust. The yield obtained by acid hydrolysis of hardwood sawdust can be calculated as follow:

$$1. \text{ Yield} = \frac{\text{actual value}}{\text{Theoretical value}} \times 100$$

Thus the sugar yield is :

$$2. \text{ Sugar yield} = \frac{32.4}{52.2} \times 100 = 62.07\%$$

Also, the experiment entailed a test of the sugar concentration on a four hourly basis to determine the rate of fermentation by checking the sugar disappearance and also to determine the exact time required for fermentation which is noticed when there is no significant change in concentration. The raw data is shown below.

Brix is the sugar content in an aqueous solution

Time (Hrs)	Sugar Concentration (Brix)
0	32.4
4	32.4
8	31.7
12	30.5
16	25.6
20	18.3
24	13.4
28	11.8
32	11.4
36	11
40	10.8
44	10.5
48	10.5
52	10.5

The raw data is plotted in the graph below:

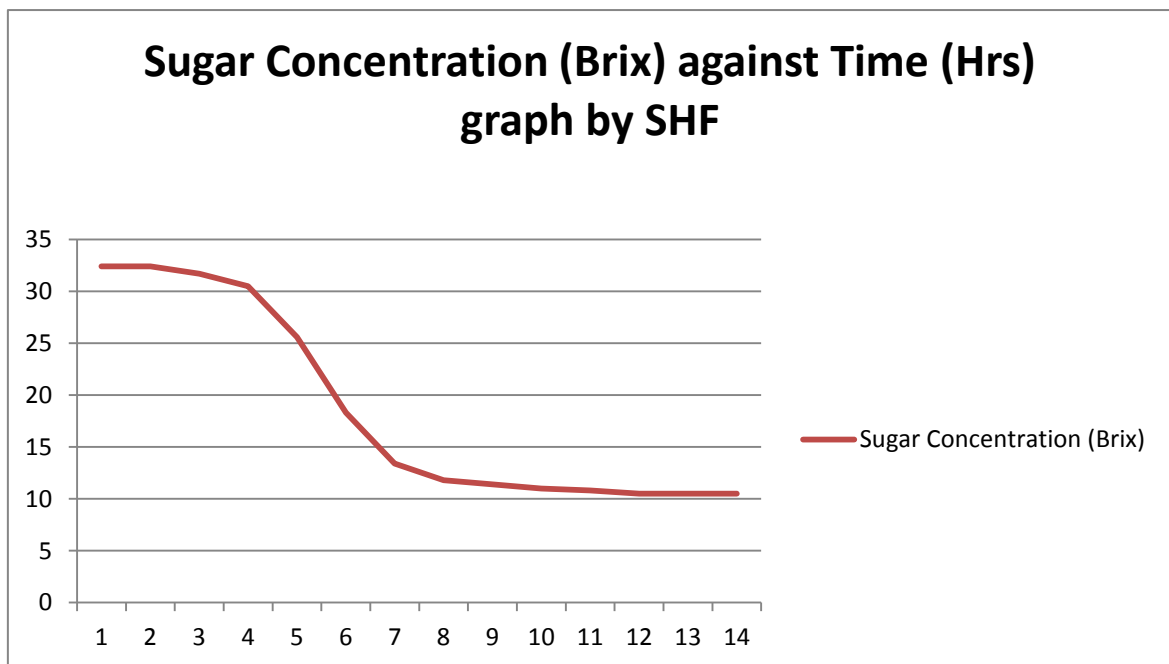


Figure: Graph showing sugar content against time by SHF

From the graph above it can be deduced that fermentation is completed after 44hours. It can also be seen that 10.5g of sugar was converted to ethanol. Sugar conversion can be calculated as follow:

$$\frac{\text{Sugar produced} - \text{sugar unconverted}}{\text{Sugar produced}} \times 100$$

Sugar produced

Thus, Sugar conversion for SHF = $\frac{32.4 - 10.5}{32.4} \times 100 = 67.59\%$

67.59% is converted by SHF

From experiment as described in section 3.2.1, 5.9ml of ethanol was produced from 100ml of a 400ml solution the total ethanol contained is $5.9 \times 4 = 23.6\text{ml}$

Ethanol yield = $23.6 \times 100 = 69.21\%$

34.1

The ethanol yield by SHF is 69.21%

Therefore, by Separate Hydrolysis and Fermentation using acid hydrolysis 23.6ml of ethanol is produced from 100g of sawdust

4.2 ANALYSIS OF SIMALTANEOUS SACCHARIFICATION AND FERMENTATION OF SAWDUST

The procedure for the production of ethanol by SSF is discussed in section 3.2.2, SSF method is used to produce ethanol from 100g of hardwood sawdust. Cellulose enzymes and yeast were used in the production. Theoretically 52.2g of sugar is present in 100g of hardwood sawdust.

Thus the yield can be calculated as follow:

$$\text{Yield} = \frac{\text{actual value}}{\text{Theoretical value}} \times 100$$

Theoretical value

The sugar yield by SSF:

$$\text{Sugar yield} = \frac{36.6}{52.2} \times 100 = 70.11\%$$

52.2

Also, on a four hour interval the sugar concentration of solution was tested to determine the rate of conversion of sugar to ethanol and also to determine the exact time required in SSF for the production of ethanol. The raw data obtained is shown below:

Time (Hrs.)	Sugar Concentration (Brix)
0	24.7
4	26.1
8	25.2
12	28
16	26.3
20	36.6
24	25.1
28	20.3
32	17.2
36	13.4
40	10.4
44	10.2
48	9.9
52	9.8
56	9.8
60	9.8

The raw data is plotted below:

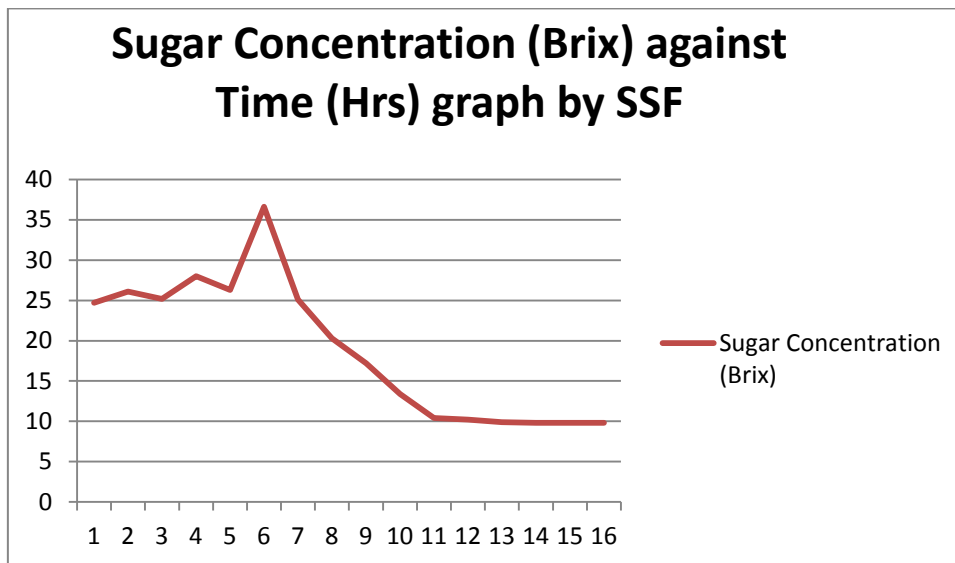


Figure: Graph showing sugar content against time by SSF

From the graph above, it can be deduced that because of the two processes occurring simultaneously there is an unsteady state which is responsible for the zigzag nature of the graph between 0-20 hours. The maximum sugar was produced at 20hours corresponding to 36.6 after which a steady state was obtained and only fermentation occurred. The time

required for the process was 52hours. After 52hours the sugar content 9.8g remained unconverted. Conversion of sugar can be calculated as follow:

$$\frac{\text{Sugar produced} - \text{sugar unconverted} \times 100}{\text{Sugar produced}}$$

Sugar conversion: $36.6 - 9.8 \times 100 = 73.22\%$

36.6

73.22% of sugar is converted by SSF.

6.3ml is distilled from 100ml of a 400ml solution. So the total ethanol content is 25.2ml.

Ethanol yield = $25.2 \times 100 = 73.9\%$

34.1

The ethanol yield by SSF is 73.9%

4.3 EFFECT OF THE METHODS ON SUGAR AND ETHANOL YIELD

Theoretically 52.2g of sugar can be produced from 100g of sawdust; however 32.4g was produced by SHF using acid hydrolysis and 36.6g by SSF. The acid which is highly concentrated destroy some sugar that may have been produced which is responsible for the low sugar production and inhibitors which hinder fermentation rate are also produced which is also responsible for the low sugar conversion of 67.59% and hence ethanol yield of 20.8ml compared with theoretical yield of 34.1ml. Theoretically 80 – 90% of sugar produced from sawdust can be converted to ethanol. Experimental error is also considered. From the graph it can be noted that in 44hours fermentation was completed.

In SSF however, the pre-treatment with dilute H₂SO₄ produced 24.7g of sugar which implies 47.3% sugar yield which shows dilute acid pre-treatment alone is not efficient for the production of sugar from sawdust. The addition of cellulose increased sugar production which depicted in the graph. From the graph it can be seen that because of the simultaneous

production of sugar and ethanol resulted in an unsteady state until the maximum sugar was produced which was 36.6g before steady state was achieved. An increased conversion of sugar was also achieved because dilute acid was used and the conversion of sugar immediately to ethanol. There was also higher yield in comparison with SHF od 25.2ml of ethanol produced. Fermentation was completed in 52hours

It can also be deduced that SSF is a more expensive method compared to the SHF owing largely to the enzyme – cellulose used. Although more water is required in SSF to dilute the acid.

4.4 WASTE PRODUCTS

The waste products from SHF is more hazardous to the environment as on a small scale, too much energy is required to distill H_2SO_4 for re-use, hence, its cost ineffective and thus has to be disposed of. 18M H_2SO_4 is highly concentrated and should not come in contact with the skin or be consumed. The acid in SSF is very small and may have little effect on the environment.

5 CONCLUSION AND RECOMMENDATION

This research work based on the extraction of sugar and subsequent fermentation of the sugar from cellulose. Study was carried out on ethanol from starch and ethanol and cellulose were compared. However for the experiment the cellulosic material or biomass used is sawdust. Two methods were used to produce the cellulosic ethanol: SHF and SSF.

It can be inferred that the SHF is a very dangerous method as highly concentrated acid is being use for the hydrolysis. However it is less costly compared to SSF method due to the use of cellulose enzymes. The SSF, however, produces more ethanol compared to SHF but the difference in the ethanol production doesn't account for the difference in cost of production making the SHF more cost effective. This may not be applicable on a large scale though.

Experiment data of production of ethanol from corn during the course of the literature was compared to the values for the production of ethanol from cellulose. It can be deduced from the data that from same mass of corn, more ethanol is produced using SHF (enzymatic though) and SSF. It is also seen that production of ethanol from cellulose is more costly compared to corn ethanol. Thus if ethanol is made from cellulose it will result in an increase in ethanol prices. Therefore, this will make the future of cellulosic ethanol very oblique.

Fuel derived from cellulosic biomass is essential in order to overcome our excessive dependence on petroleum for liquid fuels and also address the build-up of greenhouse gases that cause global climate change. The conversion offers the potential for radical technical advancement through application of powerful tools of modern biotechnology to realize truly low costs.

However, if strict bans are made on the production of food ethanol, cellulosic ethanol will thrive well and costs of enzymes may fall. Also, a breakthrough in genetically engineering an organism that will directly convert cellulose to ethanol will be more desirable in the production of cellulosic ethanol.

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