

## Turning Waste to Wealth: A mini review on Bioethanol Production from Renewable Biomass

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### Abstract

*The growing concern over the effect of global warming and environmental pollution coupled with dwindling supply of fossil fuels and security issues on petroleum exploration has become a global issue particularly in Nigeria. Biorenewable fuels such as bioethanol, biogas, biohydrogen and biodiesel could provide the much desired alternative and source of sustainable rural revitalization in most developing countries, Nigeria inclusive. In fact, biofuel is considered to be ideal an alternative energy because it could be a less expensive option for low income communities. Myriads of organic wastes including agro-waste could be utilized as a substrate in the production of sustainable clean energy at a fraction of the standard cost. This paper has presented a concise but informative review on the utilization of agro-waste biomass as a substrate for bioethanol production, thus turning the waste into wealth. The paper reviewed different approaches observed by researchers in waste biomass pretreatment to enhanced the release of much needed sugars required for the fermentation process to produce the bioethanol. It is hope that the paper will serve as a platform of reference for researchers and students alike.*

**Key words:** Bioenergy, Biomass, Waste, Renewable Energy, Hydrolysis

### 1.0 Introduction

Energy is one of the major building blocks of modern society. Demand for energy and its resources are continuously increasing in tandem with the rapid growth of population and urbanization. Energy supplies can be divided into two classes: nonrenewable and renewable energy. Non-renewable energy are obtained from taxed stores of energy that remain bound unless it is released by human interaction (Dombek & Ingram, 1987). Among the popularly used energy source globally are the fossil fuels (petroleum, natural gas and coal based fuels), which are known to be non-renewable energy sources. Of all the energy sources, oil accounts for 32.6% of global primary consumption, followed by coal (22.2%), natural gas (21.2%) and biomass with other renewable energy accounting for 12.9% (Boyle, 2004).

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The continuous use of the fossil fuels based energy have raised severe concern on global warming and other environmental consequences, resulting in environmental pollution through the emission of carbon monoxide (CO), nitrogen oxides (NO<sub>x</sub>), sulphur oxides (SO<sub>x</sub>) and particulate matter. At current global consumption rates it is shown that world coal reserves should last for about 200 years; natural gas for 60 years and oil for approximately 40 years (Boyle, 2004). Similarly, insufficient supply of petroleum and security and political issues on petroleum exploration has become a concern globally. This necessitates recent surge in research on alternative renewable sources of energy to cope with the increasing demand (Hindatu *et al.*, 2017). Renewable energy could provide the much desired sustainable rural revitalization in most developing countries. It is an ideal alternative because it could be a less expensive option for low income communities.

Renewable energy is energy generated from natural resources such as biological fermentation, sunlight, wind, rain, tides wave and geothermal heat. This energy is not substantially depleted by continual usage, and does not cause significant pollutions or other environmental problems. Additionally, it caused reduction to greenhouse gas emission. In fact, bioethanol based on sugarcane is reported to caused greenhouse gas reductions of more than 80% compared to fossil fuel use (Macedo *et al.*, 2008). Renewable energy derived from biological resources (algae, plants oils, organic biomass including agro material wastes etc.) is termed as 'Bioenergy'. The biological resources can be burned directly to produce heat or power, or can be fermented to produce biofuels such as biogas and bioethanol (Moser, 2009). As at 2016, biofuels have curved a global market of \$100.76 billion and the market is projected to reach \$132.67 billion by 2023 (Statista, 2016), of this whopping amount, bioethanol is anticipated to reach \$68.95 billion by 2022 (Rohan, 2018).

This review has portrayed recent advances on the bioconversion of agro waste to produce bioethanol as sustainable alternative energy. It is hope that the review will serve as reference platform for starting researchers and enthusiasts on bioenergy generation.

## 2.0 BIOMASS A SOURCE OF RENEWABLE ENERGY

Biomass can be defined as any hydrocarbon material which consists of carbon, hydrogen, oxygen, nitrogen and small amount of sulfur. The production of biomass is estimated at 146 billion metric tons a year, mostly of wild growth (Demirbas, 2009). Biomass is the fourth largest source of energy in the world; it is about 12.9% of the world's primary energy consumption and about 38% of the primary energy consumption in the developing countries (Demirbas, 1998). They include wood and herbaceous species, wood wastes, bagasse, agricultural crops and their waste byproducts, municipal solid waste, animal wastes, waste from food processing and aquatic plants and algae. The average majority of biomass energy is produced from wood and wood waste (64 %), followed by municipal solid waste (24 %), agricultural waste (5 %) and landfill waste (5 %) (Demirbas, 1998).

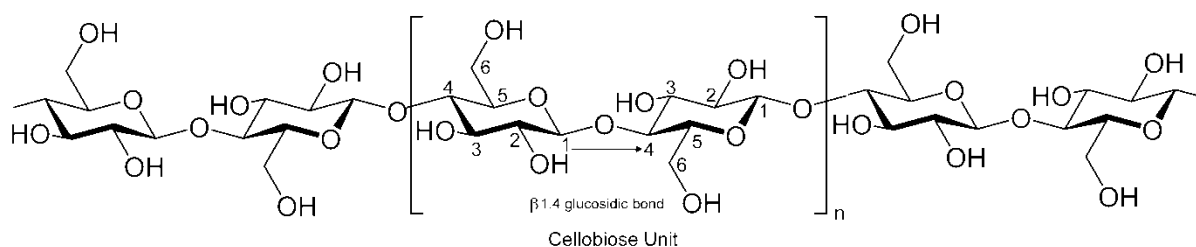
Biomass is an organic matter, in which the energy of sunlight via the photosynthesis process is stored in chemical bonds. The chemical energy stored would be release when, the bonds between adjacent carbon, hydrogen and oxygen molecules are broken by digestion, combustion, or decomposition (Mckendry, 2002). Biomass is unique as it can provide the only renewable source of fixed carbon, which is a crucial component in production of fuel (Bridgwater, 1999). Among the biomass, those of plant origin composed of a mixture of hemicelluloses, cellulose,

lignin and minor amounts of other organics. The relative concentrations differs enormously between plant species (Demirbas & Balat, 2007).

Lignocelluloses biomass has a complex internal structure. It is comprised of a number of major components that have, in their turn, also complex structures. To obtain a clear picture of the material, an analysis of the structure of each main component is made in this section, concluding with the description of the structure of lignocelluloses itself. Also addressed are the physical properties of each of the components of lignocelluloses, and how each of these components contributes to the behavior of the complex structure as a whole. The study is oriented towards breaking down the complex of lignocelluloses and utilizing its components to produce sugars.

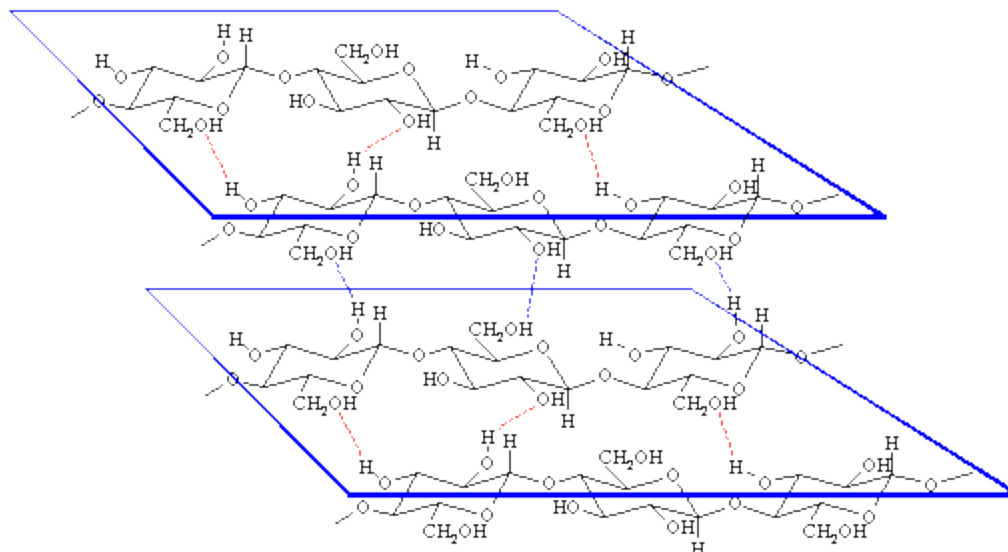
## 2.1 Cellulose

Cellulose is the  $\beta$ -1, 4-polyacetal of cellobiose (4-O- $\beta$ -D-glucopyranosyl-D-glucose). Cellulose is more commonly considered as a polymer of glucose because cellobiose consists of two molecules of glucose. The chemical formula of cellulose is  $(C_6H_{10}O_5)_n$  as presented in Figure 1.



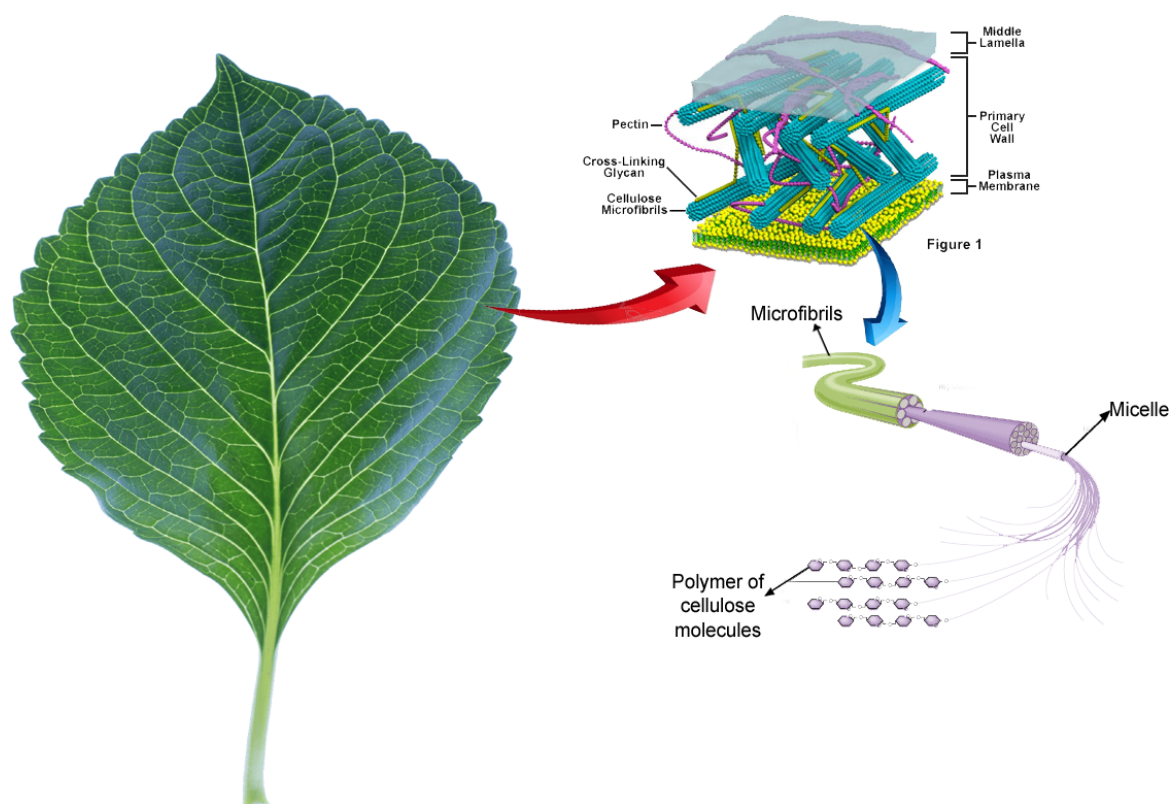
**Figure 1:** Structure of cellulose polymer.

Myriads of cellulose properties depend on its degree of polymerization ( $D_P$ ), i.e. the number of glucose units that link up one polymer molecule. The  $D_P$  of cellulose can extend to a value of 17000, even though more commonly 800-10000 units is encountered (Moser, 2009). For example, cellulose from wood pulp has a  $D_P$  between 300 and 1700. The nature of bond between the glucose molecules ( $\beta$ -1, 4 glucosidic) allows the polymer to be arranged in long straight chains. The latter arrangement of the molecule, together with the fact that the hydroxides are evenly distributed on both sides of the monomers, allows for the formation of hydrogen bonds between the molecules of cellulose. The bonds in turn result in the formation of a compound that is comprised of several parallel chains attached to each other (Figure, 2)



**Figure 2:** Demonstration of the hydrogen bonding that allows the parallel arrangement of the cellulose polymer chains (Kirk-Otmer, 2001).

Cellulose is found in both the crystalline and the non-crystalline structure. The coalescence of several polymer chains leads to the formation of micro fibrils, which in turn are united to form fibres. In this way, cellulose can obtain a crystalline structure. Figure 3 below illustrates structure as well as the placement of cellulose in the cell wall.



**Figure 3:** Plant cell wall architecture depicting cellulose microfibrils and cellulose molecules.

Cellulose is a relatively hygroscopic material absorbing 8-14% water under normal atmospheric conditions (20 °C, 60% relative humidity). Nevertheless, it is insoluble in water. The inability of cellulose to dissolve in water despite its hygroscopic nature was owed to its ability to form intra and intermolecular hydrogen bonds causing its high crystalline nature (Lindman *et al.*, 2010). Cellulose is also insoluble in dilute acid solutions at low temperature. The solubility of the polymer is strongly related to the degree of hydrolysis achieved. As a result, factors that affect the hydrolysis rate of cellulose also affect its solubility. At higher temperatures cellulose becomes soluble, as the energy provided is enough to break the hydrogen bonds that hold the crystalline structure of the molecule. Cellulose is also soluble in concentrated acids, but severe degradation of the polymer by hydrolysis is caused. In alkaline solutions extensive swelling of cellulose takes place as well as dissolution of the low molecular weight fractions of the polymer (DP < 200). Solvents of cellulose that have been applied in industrial or laboratory practice include uncommon and complex systems, such as copper ethylenediamine (CuEN) hydroxide or the cadmium complex Cadoxen as well as ionic liquids (Zhu, *et al.*, 2006). Additionally, aqueous salt solutions, such as zinc chloride, dissolve limited amounts of cellulose (Kirk-Otmer, 2002). Cellulose does not melt with temperature, but its decomposition starts at 180°C. The most widely used industrial process of dissolving cellulose is the viscose process. In this process, cellulose is first swelled using NaOH, which is then reacted with CS<sub>2</sub> to yield a cellulose derivative that more easily dissolves in alkaline aqueous solution. Thereafter, the cellulose is subsequently regenerated in sulfuric acid to shape fibrils (Lindman, *et al.*, 2010). A solvent alternative to the aqueous alkali viscose process is the N-methylmorpholine N-oxide in water (NMMO/H<sub>2</sub>O) system which dissolves cellulose at elevated temperatures ≥90°C (Perepelkin, 2007). Previously, Heinz *et al.* (2000) have advanced the dissolution of cellulose using solutions of tetrabutylammonium fluoride (TBAF) in dimethyl sulfoxide (DMSO). This system provides a good solubility but only with fluoride and not with the other halide ions. This complexity and specificity have been in agreement with deep-going NMR study by Ostlund *et al.* (2009). Cellulose solvation was also reported in other common solvent systems that are non-aqueous salt solutions, primarily LiCl in N,N-dimethyl-acetamide (DMAc) (Jing *et al.*, 2007). This system is particularly effective for generating homogeneous reaction mixtures, but requires temperatures of about 150°C to readily swell and dissolve cellulose.

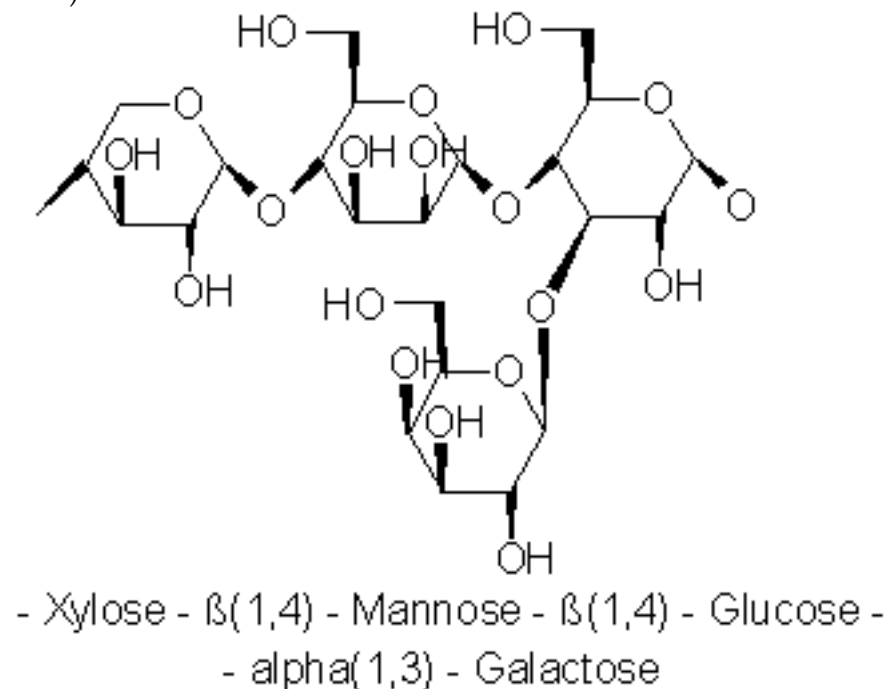
## 2.2 Hemicelluloses

The term hemicellulose is a collective term. It is used to represent a family of polysaccharides such as arabinoxylns, gluco-mannans, galactans, and others that are found in the plant cell wall and have different composition and structure depending on their source and the extraction method. The most common type of polymers that belongs to the hemicelluloses family of polysaccharides is xylan. Unlike cellulose which is an unbranched polymer, hemicellulose is a branched polymer consists of shorter chains of 500 to 3,000 sugar units as opposed to 7,000 to 15,000 glucose molecules per polymer of cellulose.

Hemicelluloses are generally found in the secondary wall and are linked with cellulose, but they can also be found in the primary cell wall. The main hemicelluloses component in hardwoods and grasses is glucuronoxylan while in softwoods it is glucomannan (Houghton, 2006). However, the hemicelluloses content can vary between different portions of the same plant, for instance, between stem and the leaf (Houghton, 2006). Hemicelluloses extracted from plants possess a high degree of polydispersity, and polymolecularity. However, the degree of

polymerization does not exceed the 200 units whereas the minimum limit can be around 150 monomers.

The xylan molecule involves  $\beta(1\rightarrow4)$  linkages of xylopyranosyl units with  $\alpha(4\text{-O})$ -methyl-D-glucuronopyranosyl units attached to anhydroxylose units (Figure 4). The result is a branched polymer chain that is mainly composed of five carbon sugar monomers, xylose, and to a lesser extent six carbon sugar monomers such as glucose. Important aspects of the structure and composition of hemicelluloses are the lack of crystalline structure, mainly due to the highly branched structure, and the presence of acetyl groups connected to the polymer chain (Yu, *et al.*, 2011).

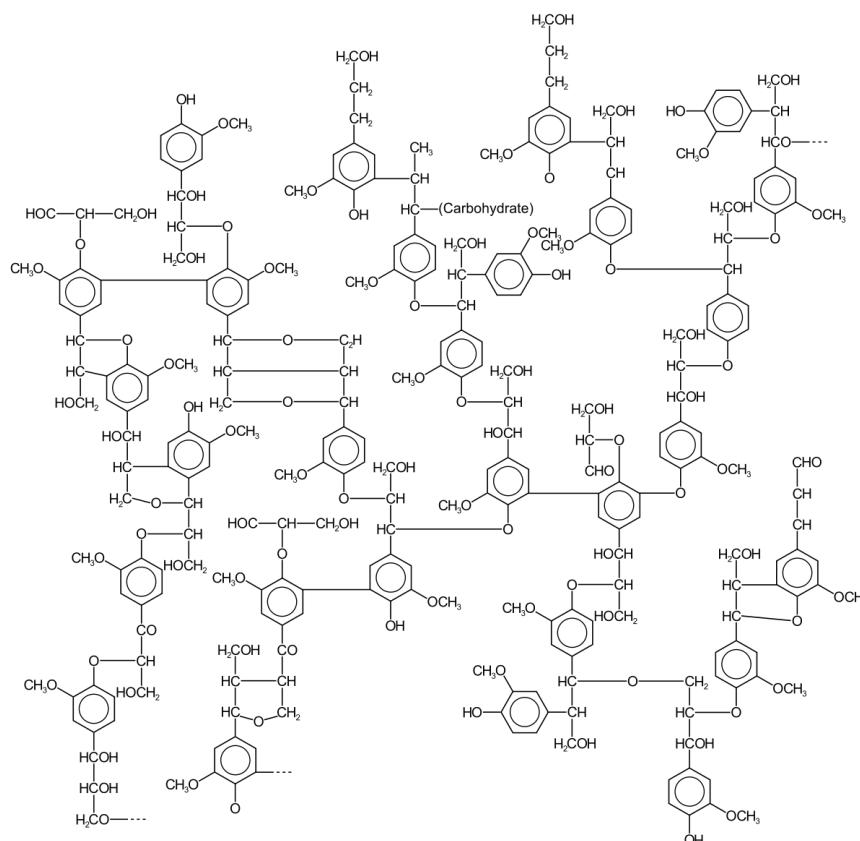


**Figure 4:** A schematic representation of the hemicelluloses backbone.

Hemicellulose is insoluble in water at low temperature. However, its hydrolysis starts at a temperature lower than that of cellulose; this renders it soluble at elevated temperatures. The presence of acid ( $\approx 0.07\%$ ) highly improves the solubility of hemicellulose in water (Jacobsen, *et al.*, 2000).

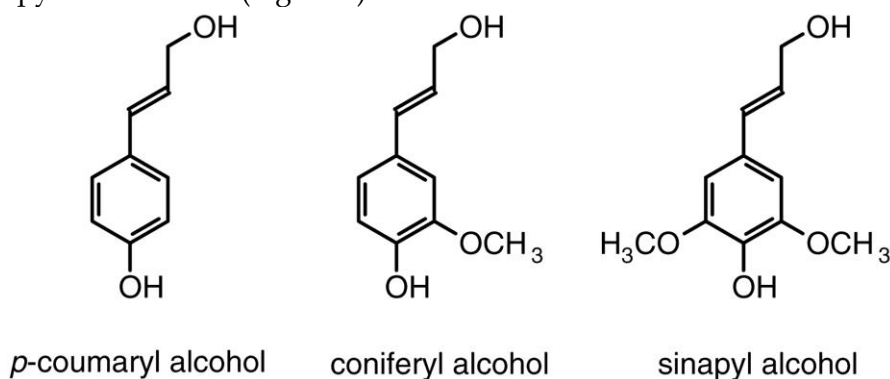
### 2.3 Lignin

Lignin is a class of complex polymers that are important in maintaining the structural support of vascular plants and some algae. Lignin is particularly important in the formation of cell walls, especially in wood and bark. They are highly crystalline, thus they lend rigidity and do not rot easily. Chemically, lignin is amorphous three-dimensional polymer of cross-linked phenolic units of mainly phenylpropane as the predominant building blocks (Figure 5). More specifically, p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol among the ones most commonly encountered (Figure 6).



**Figure 5:** A typical lignin structure (Wikipedia).

Dividing higher plants into two categories, hardwood (angiosperm) and softwood (gymnosperm), Kirk-Othmer, (2002) has opined that lignin from softwood is made up of more than 90% of coniferyl alcohol with the remaining being mainly p-coumaryl alcohol units. In contrast to softwoods, lignin contained in hardwood is made up of varying ratios of coniferyl and sinapyl alcohol units (Figure 6).



**Figure 6:** Typical alcohols found in lignin

Lignin in wood behaves as an insoluble three-dimensional network. It plays an important role in the cell's endurance and development, as it affects the transport of water, nutrients and metabolites in the plant cell (Abdulkareem, *et al.*, 2014). It acts as binder between cells creating a

composite material that has a remarkable resistance to impact, compression and bending. Solvents that have been identified to significantly dissolve lignin include low molecular alcohols, di-oxane, acetone, pyridine, and dimethyl sulfoxide (Binder & Raines, 2010). Furthermore, it has been observed that at elevated temperatures, thermal softening of lignin takes place, which allows depolymerisation reactions of acidic or alkaline nature to accelerate (Silva *et al.*, 2015).

## 2.4 Lignocelluloses

As mentioned above, lignocellulose is a class of biomass that consists of three major compounds cellulose, hemicelluloses made up of carbohydrate polymers and the lignin which is composed of aromatic polymers (Figure 7). It also includes water and a small amount of proteins and other compounds, which do not participate significantly in forming the structure of the material. It is the most abundant biomass on earth for the production of biofuels mainly bioethanol. In the lignocellulose complex, cellulose retains the crystalline fibrous structure and it appears to be the core of the complex. Hemicellulose is positioned both between the micro- and the macro fibrils of cellulose (Kobayashi, & Fukuoka, 2013). Lignin provides a structural role of the matrix in which cellulose and hemicelluloses-lose is embedded (Dombek & Ingram, 1987). Considering that cellulose is the main material of the plant cell walls, most of the lignin is found in the inter fibrous area, whereas a smaller part can also be located on the cell surface (Kirk-Otmer, 2002).

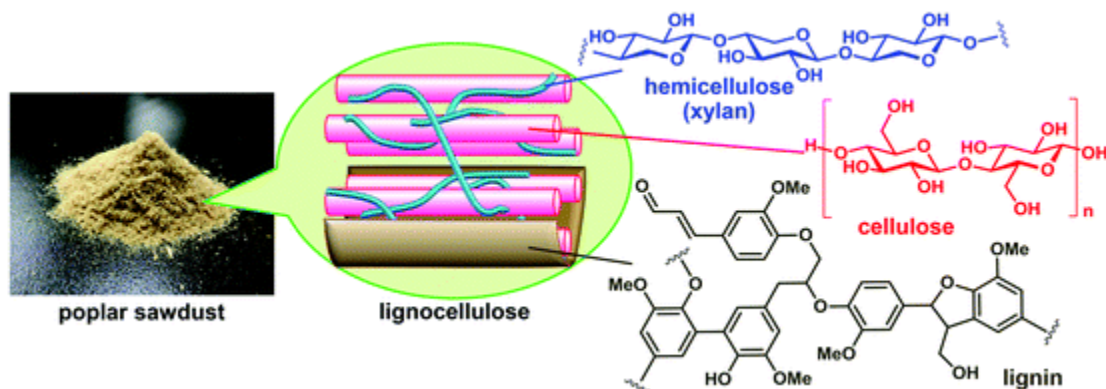


Figure 7: Lignocellulose and its components (Kobayashi, & Fukuoka, 2013).

Recently, Van den Bosch *et al.* (2015) have used simultaneous solvolysis and catalytic hydrogenolysis to valorize both polysaccharide and lignin components into a handful of chemicals in the presence of a Ru on carbon catalyst (Ru/C) in methanol under a H<sub>2</sub> atmosphere at elevated temperature, resulting in a carbohydrate pulp and lignin oil. Along this line, ionic liquids were also employed in dissolution of lignocellulosic biomass (Brandsdt, *et al.*, 2013). Similarly, enzymatic hydrolysis of lignocelluloses was also reported (Hodge, *et al.*, 2008). Again in 2004, Katelnikova *et al.* have demonstrated the use of N, N-dimethylacetamide/lithium chloride (DMAc/LiCl) system to solvate lignocelluloses biomass.

## 2.5 BIOETHANOL

Ethanol or ethyl alcohol has existed since time immemorial. 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 (Abebe, 2008, Boullanger, 1924). 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 repealed 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 (Yaliwal *et al.*, 2015).

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 remerge when ethanol was used as a fuel extender during gasoline shortages caused by the OPEC oil embargoes (John, 2006).

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 (John, 2006).

As the production increases, the effect of biofuels on agricultural markets and the environment became 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 feedstocks, 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 at that time (Shaibani *et al.*, 2012).

### **2.5.1 First generation bioethanol**

First generation ethanol has been produced primarily from starch based feedstock such as corn (Figure 8), wheat or sugarcane based feedstock's including sugarcane juice and molasses. As at 2016, first generation bioethanol which is based on based on corn and sugarcane still contributes to the majority of the 25 billion of gallons' produced worldwide. The United States and Brazil are still the major producers with approximately 85 % of the global production, respectively (Bertrand, *et al.*, 2016). For example, in 2005, approximately 11 billion bushels of corn were produced in the United States. Indiana corn production in 2005 was approximately 889 million bushels (Mosier & Ileleji, 2015). Ethanol production in the United States topped 4 billion gallons in 2005 and consumed 1.4 billion bushels of corn, valued at \$2.9 billion (Mosier & Ileleji, 2015).



**Figure 8:** A 1933 United State picture showing corn produced bioethanol been sold by the Earl Coryell & Co gas station (Abebe, 2008).

Both starch and sucrose are readily hydrolyzed into simple hexose sugars that can be fermented at high efficiency using conventional fermentation organisms (Boopathy, 2008). Unfortunately, the starch and sucrose based feedstock, are also used for both human consumption and for livestock feeds, and as a result, the price of these feedstock may be impacted by their relative value as a food. The impact of the diversion of food crops, such as corn, into ethanol has already been linked to higher food prices in some countries including Mexico and the United States of America (Sainz and Dale, 2009). Although other reports suggest that the increased use of biofuels accounted for only 10 - 30 % of the food price, increase evidence during 2007 and 2008 have point other factors such as the effects of drought, higher oil prices and economic growth increasing global demand for wheat, dairy and protein in Asia and Africa, along with market speculation and trade barriers, to impact the price of grain (Rosegrant, 2008). As the cost of first generation feedstock is typically 60 - 80 % of the ethanol production cost, factors that act to increase the price of feedstock's used for both ethanol and food production will have a significant impact on bioethanol viability during these periods of high feedstock prices.

Concerns over the long-term sustainability of first generation bioethanol, such as the impacts on land use, water resource, the potential contamination of soils with the distillation residues, and the competition for food and feed production is frequently highlighted as the setbacks of first generation bioethanol. However, current fuel ethanol research and development strives to minimize these negative externalities. The fundamental role that process design plays during the development of cost-effective technologies is evaluated through the modification of the major pathways in first generation ethanol synthesis. In this context, the central role that better performing enzymes and microorganisms play in the intensity and integration of the process, such as the typical example of simultaneous saccharification and fermentation from starchy material in first generation facilities is highly avail. A limitation to continuous fermentation is the difficulty of maintaining high cell concentration in the fermenter. The use of immobilized cells circumvents this difficulty.

Compensating ethanol production costs by the integrated valorization of energy and by-products for feed and green chemistry in a typical biorefinery concept are striking outputs of the first generation ethanol real scale experiment. Finally, rather than a mistake, first generation bioethanol should be considered as the first step that made it possible to gain the necessary experience for the successful implementation of the future greener generations biofuels from the field to the tank, starting with second generation lignocellulosic that is now coming on the market.

### 2.5.2 Second generation Bioethanol

In contrast, second generation biofuels utilize lower value lignocelluloses materials from forestry, agricultural residues or dedicated energy crops for ethanol production. Materials considered for second generation biofuel production are generally low value feedstocks that are often excess to that required in the farming system. Both the cellulose and hemicelluloses can be pretreated, hydrolyzed and fermented with varying efficiencies into ethanol. While considerable research has been undertaken on lignocelluloses ethanol since the early 20th century, there remains some significant challenge. Lignocellulose from rice straw was used as biomass to produced bioethanol (Binod *et al.*, 2010). The use of agricultural wastes as biomass for bioethanol production was extensively reviewed somewhere else (Sarkar *et al.*, 2012). The use of enzymes in the hydrolysis of cellulose is more effective than the use of inorganic catalysts, because enzymes are highly specific and can work at mild process conditions. In spite of these advantages, the use of enzymes in industrial processes is still limited by several factors: most enzymes are relatively unstable at high temperatures, the costs of enzyme isolation and purification are high and it is quite difficult to recover them from the reaction mixtures. Currently, extensive research is being carried out on cellulases with improved thermostability. These enzymes have high specific activity and increased flexibility. For these reasons they could work at low dosages and the higher working temperatures could speed up the hydrolysis reaction time. As consequence, the overall process costs could be reduced. Thermostable enzymes could play an important role in assisting the liquefaction of concentrated biomass suspensions necessary to achieve ethanol concentrations in the range 4-5 wt% (Macedo *et al.*, 2008).

Bioethanol can be produced from lignocellulosic biomass in various ways characterized by common steps: hydrolysis of cellulose and hemicellulose to monomeric sugars, fermentation and product recovery. The main differences lie in the hydrolysis phase, which can either be performed by enzymatically, dilute or concentrated acid. Several types of acids, concentrated or diluted, can be used. For example, sulphuric, hydrochloric, hydrofluoric, phosphoric, nitric and formic acid were all used with reported appreciative results (Galbe & Zacchi, 2002). Sulphuric and hydrochloric acids are the most commonly used catalysts for hydrolysis of lignocellulosic biomass (Lenihan *et al.*, 2010). The acid concentration used in the concentrated acid hydrolysis process is in the range of 10-30%. The process occurs at low temperatures, producing high hydrolysis yields of cellulose about 90% glucose of theoretical yield (Galbe & Zacchi, 2002).

### 2.6 Pretreatment in Bioethanol production

Feedstock pretreatment has been recognized as a necessary upstream process to remove lignin and enhance the porosity of the lignocellulosic materials prior to the hydrolysis process, which

is the act of converting the biomass biopolymers to fermentable sugars. There are two major methods employed in hydrolysis. The first and older method uses chemicals as catalysts, while the second uses enzymatic catalysis such as the use of cellulases.

Pretreatment is important because hydrolysis, which is the next step, can be affected by porosity of lignocelluloses biomass, cellulose fiber crystallinity, lignin and hemicelluloses content. Depending on the biomass material, chemical hydrolysis involves the use acids such as dilute acid, alkali, organic solvent, ammonia, sulphur dioxide, carbon dioxide or other chemicals to make the biomass more digestible by the microbial enzymes (Karimi & Taherzadeh, 2006).

### 2.6.1 Acid pretreatment

Acid pretreatment method was derived from the concentrated acid hydrolysis such as concentrated  $H_2SO_4$  and HCl hydrolysis, which had been a major technology for hydrolyzing lignocelluloses biomass for fermentable sugar production (Pradhan & Nag, 2007). Concentrated acid has been initially applied to remove hemicelluloses either in combination with hydrolysis of cellulose to glucose or prior to dilute acid hydrolysis of cellulose. Hydrolysis using concentrated acid has an advantage of energy efficiency as it involves low operational temperature. The concentrated acid hydrolysis had been temporarily commercialized in the World War II, even though it is powerful and effective for cellulose hydrolysis, concentrated acid is toxic, corrosive and hazardous and requires reactors that need expensive construction material which are resistant to corrosion. Additionally, the concentrated acid must be recovered and recycled after hydrolysis to render the process economically feasible. Therefore, it has phased out gradually. An alternative to this method is the use of dilute acid pretreatment which has received numerous numerous attentions recently. It has an advantage of less corrosion over the concentrated acid process but with a setback of high elevated operational temperature, which results in the degradation and cremation of the sugar monomers. Several different acids, including dilute sulfuric acid, dilute nitric acid, dilute hydrochloric acid, dilute phosphoric acid, and per-acetic acid were reported to be employed in the process (Huber, 2006).

The main principle behind the use of dilute acid is to solubilize hemicelluloses and retain lignin and cellulose intact so that the enzymatic digestibility of cellulose is enhanced. The oligomeric hemicelluloses could be completely hydrolyzed into monosaccharide's by adjusting pretreatment conditions, but also the sugar degradation products will be generated during oligomeric hydrolysis. The major advantage of dilute acid pretreatment over steam-explosion is significantly higher xylose yield. Using batch dilute sulfuric acid pretreatment process, xylose yield was showed to approach 80%-90% of theoretical value (Huber, 2006). Dilute acid pretreatment has been applied to a wide range of feed stocks, including softwood, hardwood, herbaceous crops, agricultural residues, wastepaper, and municipal solid waste. It performed well on most biomass materials. Of all acid-based pretreatment methods, sulfuric acid has been most extensively studied since it is inexpensive and effective.

### 2.6.2 Alkaline pretreatment

Alkaline pretreatment is one of major chemical pretreatment technologies receiving numerous studies. It employs various bases, including sodium hydroxide, calcium hydroxide (lime), potassium hydroxide, aqueous ammonia, ammonia hydroxide, and sodium hydroxide in combination with hydrogen peroxide or others (Banerjee *et al.*, 2012). Alkaline pretreatment is basically a delignification process, in which a significant amount of hemicelluloses is solubilized as well. The action mechanism is believed to be saponification of intermolecular ester bonds

cross linking xylan hemicelluloses and other components, for example, lignin and other hemicelluloses. Alkaline pretreatment also removes acetyl and various uronic acid substitutions on hemicelluloses that reduce the accessibility of hemicelluloses and cellulose to enzymes. Alkaline pretreatment of lignocelluloses materials causes swelling, leading to decreased DP and crystallinity, increased internal surface area, disruption of the lignin structure, and separation of structural linkages between lignin and carbohydrates (Hara, 2011). The effectiveness of alkaline pretreatment varies, depending on the substrate and treatment conditions. In general, alkaline pretreatment is more effective on hardwood, herbaceous crops, and agricultural residues with low lignin content than on softwood with high lignin content. Banerjee, *et al.* (2012) observed that the digestibility of NaOH-treated hardwood increased from 14% to 55% with the decrease of lignin content from 55% to 20%. However, slight effect of dilute NaOH pretreatment was found for softwoods with lignin content greater than 26%. Leonowicz *et al.*, (1999) used lime to pretreat corn Stover and obtained maximum lignin removal of 87.5% at 55°C for four weeks with aeration. Using lime pretreatment at ambient conditions for up to 192 h, (Huber, 2006) enhanced the enzyme digestibility of the sugarcane bagasse from 20% to 72%. He also concluded that lime would be the choice chemical based on the cost of chemicals. Using alkali chemicals (NaOH,  $\text{Ca}(\text{OH})_2$  and KOH) to pretreated rice straw in 24 h at 25°C, it was found that NaOH (6% chemical loading, g/g dry rice straw) was the best alkali chemical to achieve 85% increase of glucose yield by enzymatic hydrolysis. Aqueous ammonia is also a common alkali chemical for alkaline pretreatment. The delignification efficiencies of corn cob and switch grass were improved by 80% and 85%, respectively by ammonia recycled percolation (ARP) pretreatment. ARP was somewhat less efficient in pretreatment of softwood-based pulp mill sludge. A recent study achieved the enzymatic digestibility of the ARP treated corn Stover of 90% with an enzyme loading of 10 FPU/g-glucan. In comparison with other pretreatment technologies, alkali pretreatment usually uses lower temperatures and pressures, even ambient conditions. Pretreatment time, however, is recorded in terms of hours or days which are much longer than other pretreatment processes. A significant disadvantage of alkaline pretreatment is the conversion of alkali into irrecoverable salts and/or the incorporation of salts into the biomass during the pretreatment reactions so that the treatment of a large amount of salts becomes a challenging issue for alkaline pretreatment (Leonowicz *et al.*, 1999).

## 2.7 Enzymatic Hydrolysis

The idea of performing the enzymatic hydrolysis and fermentation simultaneously a term described as simultaneous saccharification and fermentation (SSF) was patented in 1976 by Gauss and coworkers (Gauss *et al.*, 1976). Bioprocessing of agro-industrial residues using SSF has often been found very efficient. There has been a wide-spread resurgence of SSF all over the world due to its several advantages, mainly on engineering grounds. Numerous SSF processes have been developed in which bagasse has been used as the solid substrate. While in most of the processes, it has been used as the carbon source for energy requirement (Monte *et al.*, 2011).

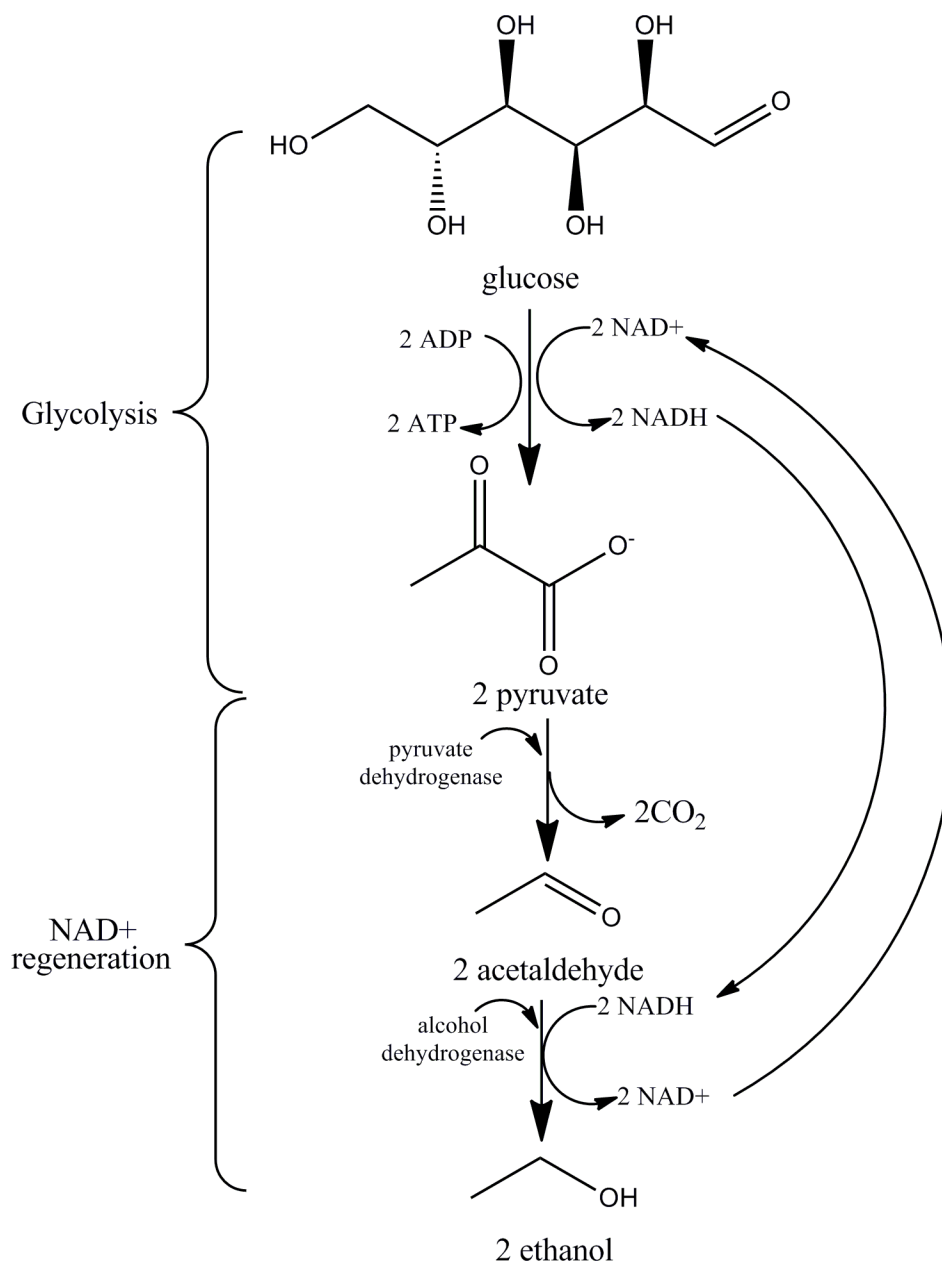
In SSF, hydrolysis and fermentation are performed in a single process unit allowing reducing sugars produced to be immediately consumed by the fermenting organism. Thus, neutralizing the effect of end-product inhibition (Sanchez and Cardona, 2008). SSF also seems to decrease the inhibition of enzymes by toxic byproducts present in pre-hydrolyses after pretreatment (Tengborg *et al.*, 2001). This improves the overall ethanol yield and productivity.

Despite its advantages, however, SSF suffered some drawbacks, one of which is the difficulty encountered with yeast recirculation due to the presence of lignin residues in the hydrolysate (Ohgren et al., 2007). A major disadvantage of SSF is that the optimum temperature condition for enzyme hydrolysis (45-50°C) is much higher than what is required for fermentation (30°C for *S. cerevisiae*). Therefore, a compromise temperature of around 38°C is employed thus making hydrolysis the rate limiting process in SSF (Sun and Cheng, 2002).

Several enzymes were known to be employed in enzymatic hydrolysis (Ballesteros, 2010). They include cellulases that are proteins that have been conventionally divided into three major groups: endoglucanase, which attacks low crystallinity regions in the cellulose fibers by endoaction, creating free chain-ends; exoglucanases or cellobiohydrolases which hydrolyze the 1, 4-glycosidyl linkages to form cellobiose; and  $\beta$ -glucosidase which converts cellobiosaccharides and disaccharide cellobiose into glucose residues (Verardi, *et al.*, 2012). In addition to the three major groups of cellulose enzymes, there are also a number of other enzymes that attack hemicelluloses, such enzymes include: glucuronide, acetylsterase, xylanase,  $\beta$ -xylosidase, galactomannase and glucomannase. These enzymes work together synergistically to attack cellulose and hemicellulose. Cellulases are produced by various bacteria and fungi that can have cellulolytic mechanisms significantly different.

## 2.8 Fermentation Process

A variety of microorganisms, generally bacteria, yeast or fungi were known to ferment carbohydrates thereby producing ethanol under oxygen-free conditions. They do so through metabolic processes of obtaining energy for normal growth. According to the reactions' stoichiometry, two molecules of ethanol are produced by each molecule of glucose (Figure 9). Methods for C6 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 lignocelluloses crops (end of the 19th century), conversion of the C5 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 did 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 pentose and hexoses, or suffer the toxicity of ethanol in high concentration, thus the low yields of ethanol. Furthermore, the oxygen free condition of fermentation slowly exterminates the microorganism population.



**Figure 9:** Glucose metabolic pathway to ethanol

Therefore, in early processes, the different sugars were fermented in different sequential reactors. However, there is a tendency towards combining reaction steps in fewer reactors. When hydrolysis and fermentation reactions are connected directly, intermediate inhibitive products are avoided, and the yield is potentially higher. Advancement in genetic and metabolic engineering coupled with new screening technologies have led to the creation of robust strains of bacteria and yeast that are capable of fermenting both glucose and xylose, as well as tolerant to higher ethanol concentration. 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% of the cleaned hydrolysate), and some protein nutrients. The consolidation of conversions in fewer reactors should have an impact on the total process integration (Pradhan & Nag, 2007).

### **Conclusion**

It is not a hidden knowledge that for bioethanol production from lignocellulosic biomass to be cost effective and sustainable, cheaper and easier ways for the release of the sugars should be devised. Although it is shown that concentrated acid hydrolysis has the advantage of solvating the biomass at lower temperature, the high acidity of the reaction mixture has posed setbacks that made the researchers to look the other way. Dilute acid as well as alkaline hydrolyses could be used but under energy intensive process, which again is not advisable. Thus making a combination of enzymatic hydrolysis coupled with alkaline pretreatment to be a method of choice for high yield of ethanol in the fermentation process.



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