

## **Review Article**

### **A review of Potential of Lignocellulosic Biomass for Bioethanol Production in Kenya**

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#### **ABSTRACT**

Lignocellulosic biomass is the earth's most abundant and renewable resource, and, lignin is its strongest component. The lignocellulosic biomass has a potential to produce bioethanol for both domestic and industrial use. The presence of lignin in the biomass, however, hinders the processing and production of bioethanol from the biomass. Hence, to enhance the chances of bioethanol production from the lignocellulosic biomass, lignin has to be pre-treated. The pre-treatment process efficiently separates the interlinked complex components. During the pre-treatment process, the strong lignin component that is highly resistant and a major barrier to solubilization is broken down by hydrolysis of cellulose and hemicellulose. Pre-treatment of lignocellulosic biomass is therefore, necessary to make it more susceptible to microorganisms, enzymes, and pathogens. The initial pre-treatment approaches include physical, physicochemical, and biological methods. The major drawback of this pre-treatment process is its cost implications, as it's very costly. Studies suggest that even though it's a costly affair, the pre-treatment methods, however, have a significant impact on the efficient production of ethanol from biomass.

Situation analysis: Bioethanol production from lignocellulosic biomass has mostly been undertaken in Brazil, USA, China, and India. In Kenya, however, little research on bioethanol production from lignocellulosic biomass has been done and adopted. The present review paper seeks to outlay the benefits of bioethanol production from lignocellulosic biomass, the composition of lignocellulosic biomass, its properties, different pre-treatment methods alongside advantages, and, disadvantages, and challenges encountered during bioethanol production. This review eventually will be of great assistance to researchers while developing bioethanol from different lignocellulosic biomass.

*Keywords:* Lignocellulosic biomass, lignin, pre-treatment, Kenya

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## 1. INTRODUCTION

Energy is a vital component of development and human life, energy sustainability is one of the challenges facing and will continue to face mankind over the coming decades, particularly due to the need to ensure sustainability [1]. Biomass is a promising raw material for energy supply as it can be used for production of heat, power, transport fuels, and bio-products [2]. Production and use of biofuels are growing at a very rapid pace. With sugar cane-based ethanol already a competitive biofuel in some countries in the tropical regions, in the near future, ethanol and high-quality synthetic fuels from woody biomass are expected to compete with crude oil on pricing [2]. Biofuels are products that can be processed into liquid fuels for either transport or heating purposes. Bioethanol is derived from agro-products including starchy and cereal crops such as sugarcane, corn, beets, wheat, and sorghum. Biodiesel is generated from oil- or tree-seeds such as rapeseed, sunflower, soya, palm, coconut or jatropha [3].

Lignocellulosic biomass is one of the most promising sources of alternative energy which if well harnessed can be used as an alternative to fossil fuels so as to bridge the challenges of energy security. According to most studies, biomass has been identified as a significant contributor to achieving sustainable development goals [4]. Bioethanol research in the last few decades have come out with new novel ideas on how to generate energy from biomass and its related materials [4–6]. This success can be attributed to the abundance and availability of biomass for biofuel production and the low cost of biomass and processing costs into biofuel [5,7]. Biodiesel, for instance, is a product derived from the biomass through a chemical process involving plants and animals having long chain of fatty acid esters through the transesterification process. Studies on plants have yielded products and consequently, these plants and their products have been used to not only generate energy, but also develop biodiesel from them. Some of the products from plants that have been utilized to develop biodiesel are rubber seed [5], Rubber seed oil [2], oil palm biomass [2, 6], Wilson's Dogwood [7], Brassica napus seed oil [2], Koelreuteria integrifolia oil [2], jatropha oil [2], castor oil [2], Eruca sativa [2] and Pongamia [2]. Lignocellulosic biomass is, however, low in oil content, and is therefore, not suitable for biodiesel production. [5,7,8].

Due to the abundance of lignocellulosic biomass, the EU, for example, is targeting to replace 10% of their automobile fuel with biofuels by 2020 [5]. They have as a consequent committed a substantial amount of money for the project in support of biofuels development from lignocellulosic biomass under the seventh framework program [9,10]. The United States of America, is targeting to produce 60.5 10<sup>9</sup>L of lignocellulosic bio-ethanol by the year 2022 [5,11]. China is targeting to produce 12.7 10<sup>9</sup> L non-grain fuel ethanol by 2020 [12,13], and, India also announced her intentions to replace 20% of fossil fuel consumption with bioethanol and biodiesel by the same year [5,14].

In Kenya, the Ministry of Energy is promoting the development of bioethanol and biodiesel. Through biodiesel production, the Ministry aimed to reduce the number of households using fuel wood by 25% by 2015, and those using kerosene by 50% by 2050. A draft Strategy for the development of biodiesel industry has been developed [15]. Currently, ethanol production in Kenya, is undertaken by Agro-Chemical and Food Company (ACFC), and Kibos Sugar and Allied Industries (KISAIL). The two companies have a combined production capacity of 125,000 litres per day, and are heavily reliant on molasses as a feedstock. The molasses produced by the Kenyan sugar companies is however not sufficient, and, is therefore, inadequate to sustain ethanol production for both industrial and domestic use. The molasses produced is sold for human consumption, and, a sizeable quantity is used in the baking industry as well in the brewing of ale [16,17]. Additionally, molasses is used mainly in the manufacture of industrial/ potable alcohol, yeast and cattle feed. The distilled alcohol in turn is used to produce ethanol, rectified spirit and various value added chemicals. These factors makes ethanol production from molasses very much costly and unsustainable and, thus an alternative source of feedstock has to be considered [18].

Lignocellulosic biomass offers the greatest alternative to sugar cane feedstock for biofuel development. The lignocellulosic plants are abundant and readily available and some of the plants can thrive in drier, more marginal agricultural areas than sugar cane, and this can help maximize the economic potential of lignocellulosic biomass for bioethanol production in Kenya. The studies by governmental agencies, NGOs, and the private sector have indicated that the lignocellulosic plants have a great potential for biofuel production. These studies have been centered mostly on biodiesel, and, have jatropha as the main feedstock, although other studies involving other feed stocks like castor, croton, and coconut are underway [16].

Kenya, produces an abundant quantity of primary sources of lignocellulosic biomass, also known as field based crop residue, which are commonly considered useless. It is estimated that the four major crops produce 5,158,119 tons of residue annually including 1,247,000 tons of sugarcane bagasse. From the estimates, approximately, 10.942 million tons of resources are available from four crops i.e. wheat, rice, maize, and cotton, and, have no commercial and domestic utilization [5]. Lignocellulosic feedstock from residue crops such as cotton straws, sugarcane tops, rice straw, maize stalks, and wheat straw can enhance the potential of bioethanol production [19,20]. This review critically appraises lignocellulosic biomass for bioethanol production, and, will be of great help while selecting and developing bioethanol from lignocellulosic biomass.

## **Methodology**

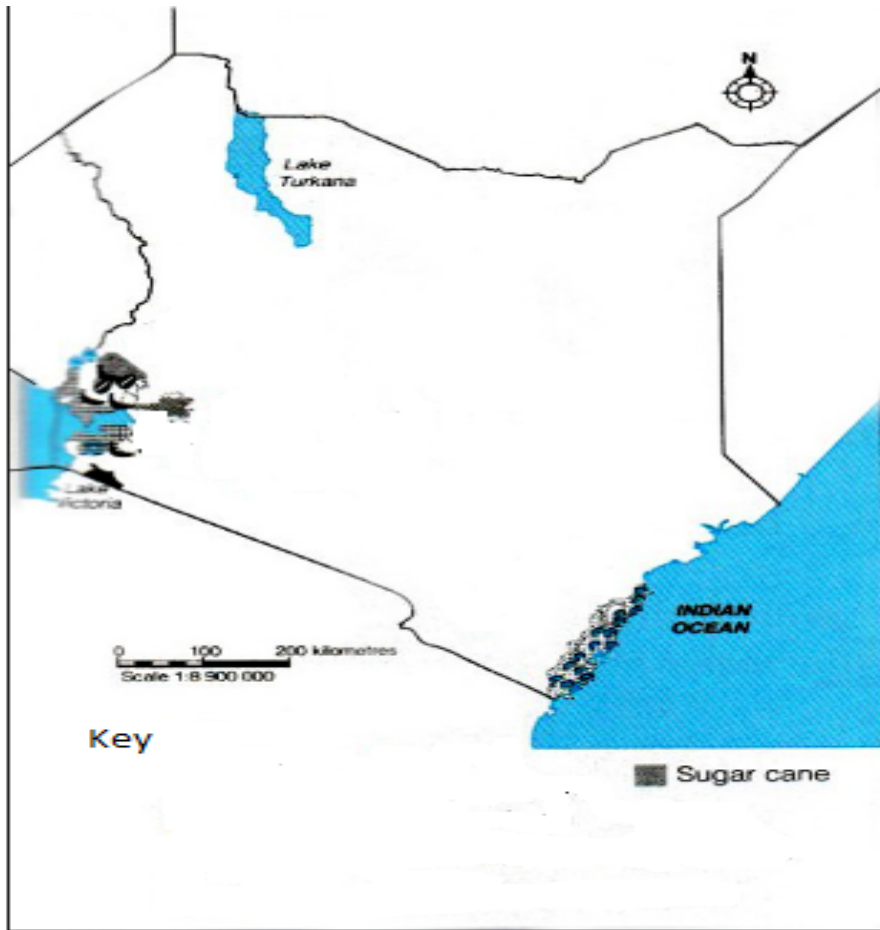
The review took a systematic approach with the keywords “lignocellulosic”, “Lignocellulose AND Kenya”, pretreatment AND lignocellulosic “Bioethanol AND production”, Bioethanol AND Kenya, Bioethanol AND global

## **Kenyan Bioethanol Situation**

Kenya's attempts on bioethanol production can be traced back to 1977 with the construction of the Kenya Chemical and Food Corp (KCFC) which was aimed at producing ethanol for blending [16]. The blended ethanol was to be substituted for premium gasoline (93 octane) with a volume composition of 65% super petrol, 10% alcohol and 25% ordinary or regular petrol. In 1983, another power alcohol plant, Agro Chemical and Food Corp (ACFC) was constructed to support the national blending programme. The fuel blending programme was however abandoned in 1995 after the liberalization of the industry mostly due to unsustainable commercial arrangements as well as an inadequate policy framework [16].

Ethanol is currently produced by Agro-Chemical and Food Company Limited and Kibos sugar and allied industries. These two companies have a combined production capacity of 125,000 litres per day. The capacity is expected to increase with Mumias Sugar Company having built a distillery plant with the capacity to produce 22M litres of ethanol annually. Kwale International sugar company (KISCOL) is also planning a 30,000-litre per day ethanol plant. As such, the ethanol industry in Kenya is expected to grow rapidly in the next decade [16].

The sugar industry supports the livelihoods of about six million Kenyans directly or indirectly, contributing to both urban and rural household economies. Sugarcane is mainly grown in western and coastal parts of Kenya, particularly around Nyando, Migori, Homabay, Transmara, Mumias, Busia, Kwale and Nandi areas of Kenya. There are about 250,000 small- scale sugarcane farmers who supply most of the cane milled in Kenya. The area under sugarcane in Kenya is about 202,000 ha, with total production averaging 5.262 million tonnes of cane supplied to factories per year [21].



*Figure 1. Map of Kenya showing sugarcane growing areas. Mainly located in Western and Coastal Kenya*

### **Bioethanol feedstock**

Lignocellulosic biomass feedstock is categorized as primary, secondary or tertiary. The primary sources include sugar cane, cotton, and other lignocellulosic plants or key by product such as bagasse, rice husks, and straw [19,22]. The secondary sources are forestry residues. These include biomass, not harvested or removed during the commercial harvesting of trees, such as thinning and removal of dead and dying trees. Forestry waste also includes wood chips, sawdust, and bark [19].

The tertiary sources includes agro industrial residues and dried manure. Though it's hard to estimate the total production of agro industrial residues worldwide, it is approximated at between 5 to 55 EJ/year, with 5EJ/year being the lower estimate due to its use as fertilizer, while the 55EJ/year being the total higher estimate with a considerable technical potential [20,23].

According to the International Union for Conservation of Nature (IUCN), there is an estimated total of 7500 plant species in Kenya. Among these are wild species of

vegetables, fruits, forage grasses, legumes, browse plants, cereals, pulses, oil crops, forest species and medicinal plants [18]. These plant species can thus be utilized as lignocellulosic biomass feedstocks for bioethanol production.

### Composition of Lignocellulosic biomass

Lignocellulosic biomass is the most abundant renewable resource on earth, and is the main primary building block of plant cell walls. [8,9,24]. Lignocellulosic biomass is hence, a complex mixture of cellulose, hemicellulose, and lignin, with minor amounts of ash, proteins, lipids, and other lipophilic compounds. The composition of lignocellulosic biomass is, however, not uniform and therefore, varies from one plant species to the other [25]. The agricultural residues such as sugarcane bagasse and forestry residues such as eucalyptus wood typically comprises 35-55% of cellulose, 25-35% of hemicellulose and 15-30% of lignin, with minor amounts of proteins and lipids and ash [7,9].

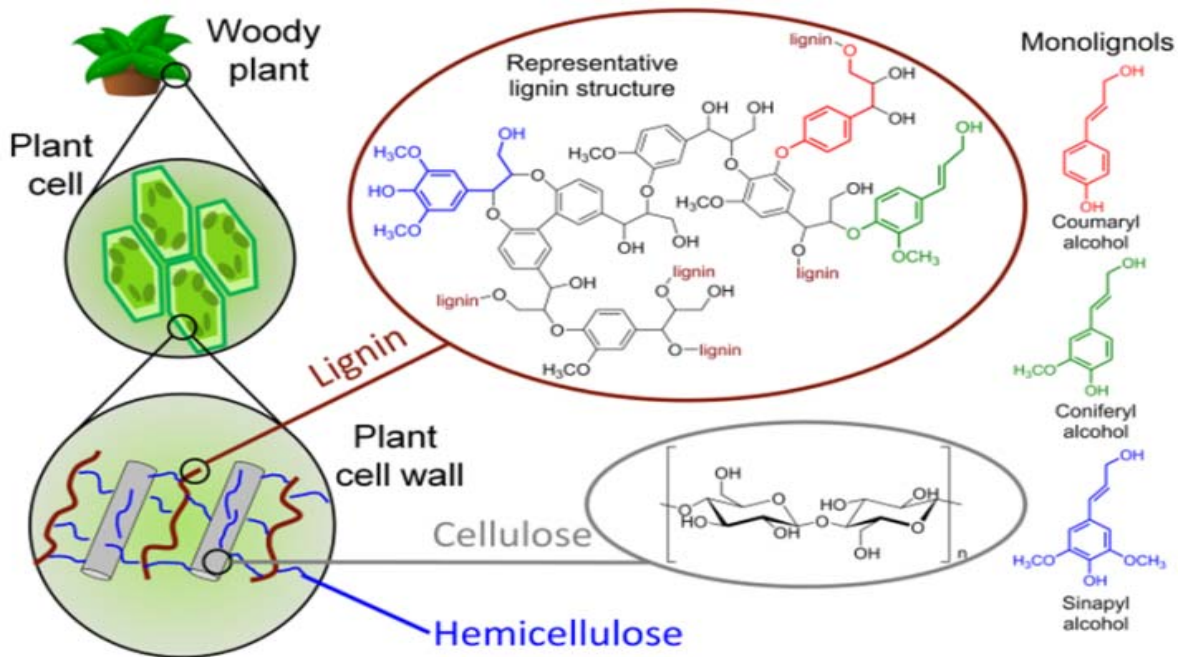


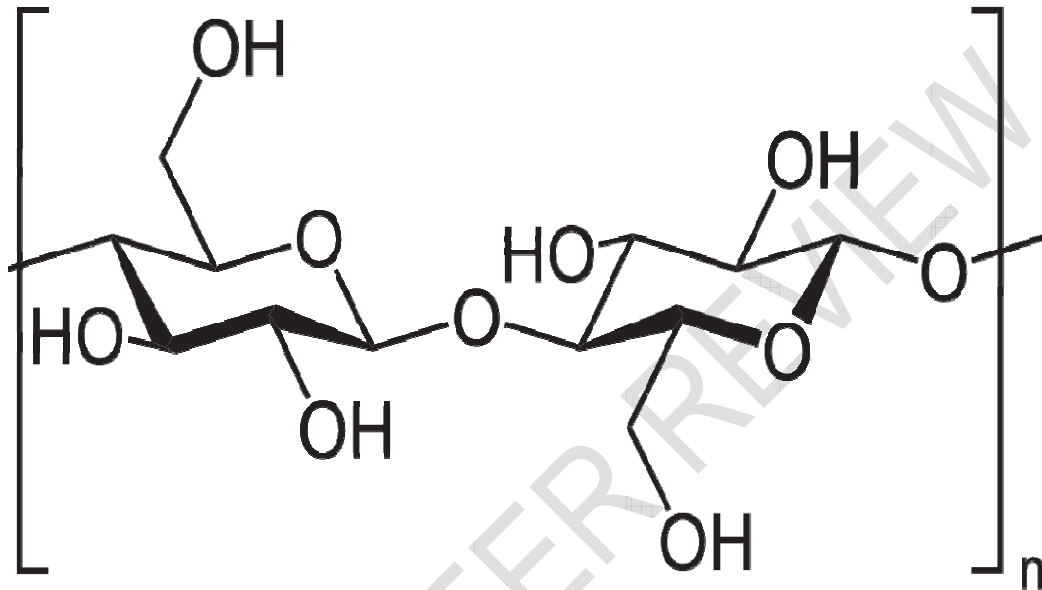
Figure 2. Structure of lignocellulose in plant biomass.

The lignocellulosic biomass is mainly made up of cellulose, hemicellulose and lignin.

### The Cellulose

Cellulose is a linear homopolymer of D-glucopyranose units linked at the 1 and 4 carbon atoms by  $\beta$ -glucosidic bonds, with hydroxyl groups at C-2, C-3 and C-6 [7]. Owing to the structure of cellulose, along with the intermolecular hydrogen bonds, the cellulose has a high tensile strength, that makes it insoluble in most solvents, and is partly responsible for the resistance of cellulose to microbial degradation [26].

The cellulose properties such as cellulose content, pH, moisture content, crystallinity index, moisture sorption capacity of the cellulose are dependent on the degree of polymerization (DP) i.e. the number of glucose units that makes up one polymer molecule [27]. Cellulose from wood pulp, for instance, has a DP of between 300 and 1700 units. The DP values of cellulose can however, extend up to 17000 units, even though 800-10000 units is the most commonly encountered [25].

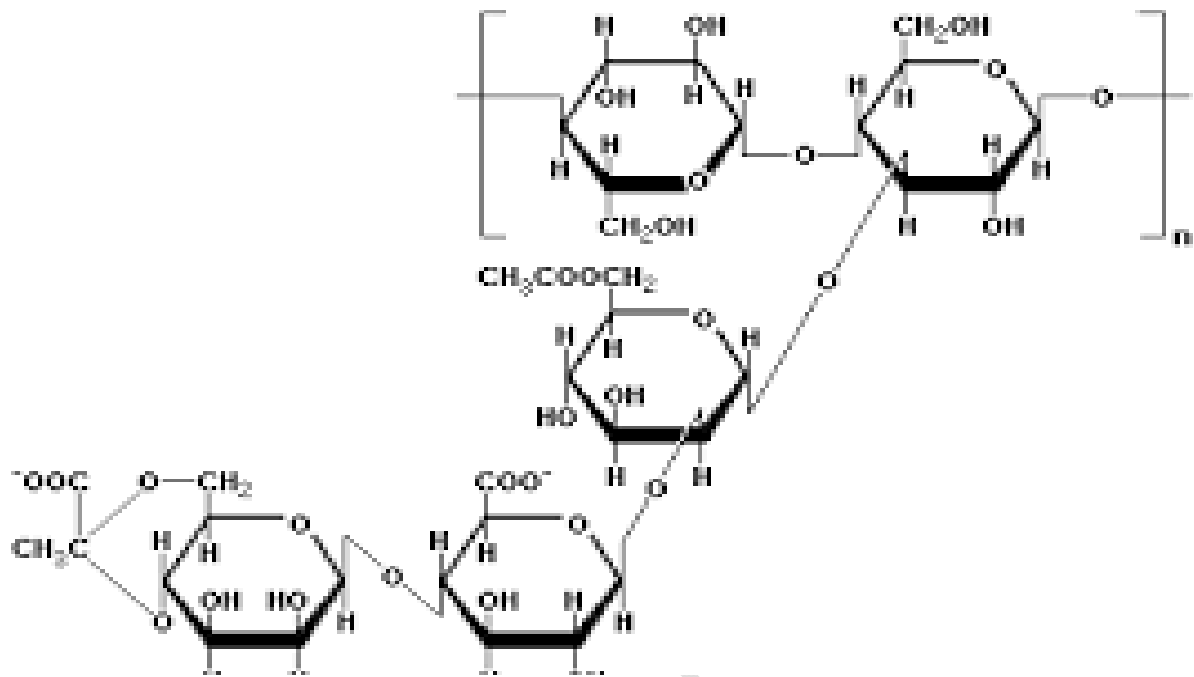


*Figure 3. The Structure of cellulose*

### ***The Hemicellulose***

It is the second major constituent of lignocellulose material and is heterogeneous, consisting of C5 sugars (xylose, arabinose), C6 sugars (mannose, glucose and galactose), and uronic acids [22]. Hemicellulose serves as the link between the lignin and the cellulose fibres and therefore, gives the cellulose-hemicellulose-lignin network a rigidity [8].

Hemicellulose unlike cellulose, has a random and amorphous structure, and usually provides little structural support to the cell wall, and is less resistant to hydrolysis [9]. Due to hemicellulose resistance to hydrolysis, it undergoes thermal decomposition at a lower temperatures of 220-315 °C [19].



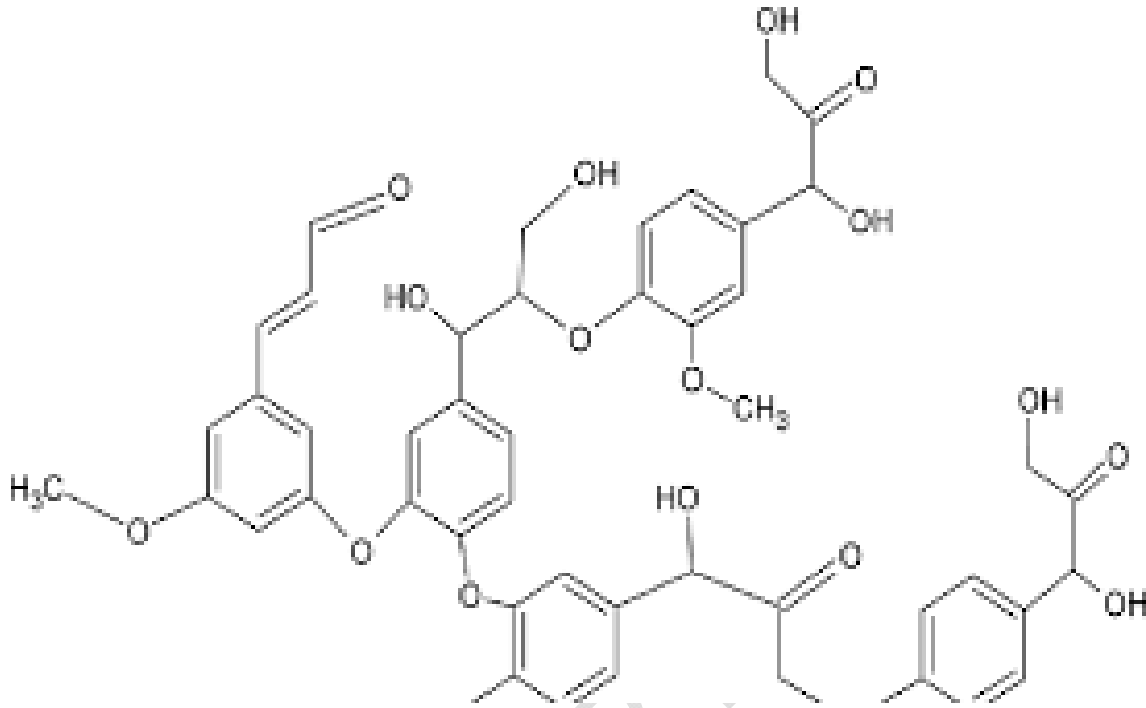
*Figure 4. The structure of hemicellulose*

### The Lignin

Lignin is a three dimensional amorphous polymer consisting of methoxylated phenylpropane structures, and involves the polymerization of three primary monomers: p-coumaryl alcohol, coniferyl and sinapyl alcohol [7]. Lignin fills the space between cellulose and hemicellulose in plant cell walls, cross-linking it with the carbohydrate polymers to confer strength and rigidity to the system [28].

Due to the rigid network, lignin has a considerable impact on the other existing links. The most important being the enhancement of the strength of hydrogen-bonds between polysaccharides, which in turn increases the stability and rigidity of the cellulose hemicellulose structure. This arrangement, however, reduces the chances of penetration by wall-degrading enzymes, and effectively locks out pests and diseases thereby, protecting the plant body [29].



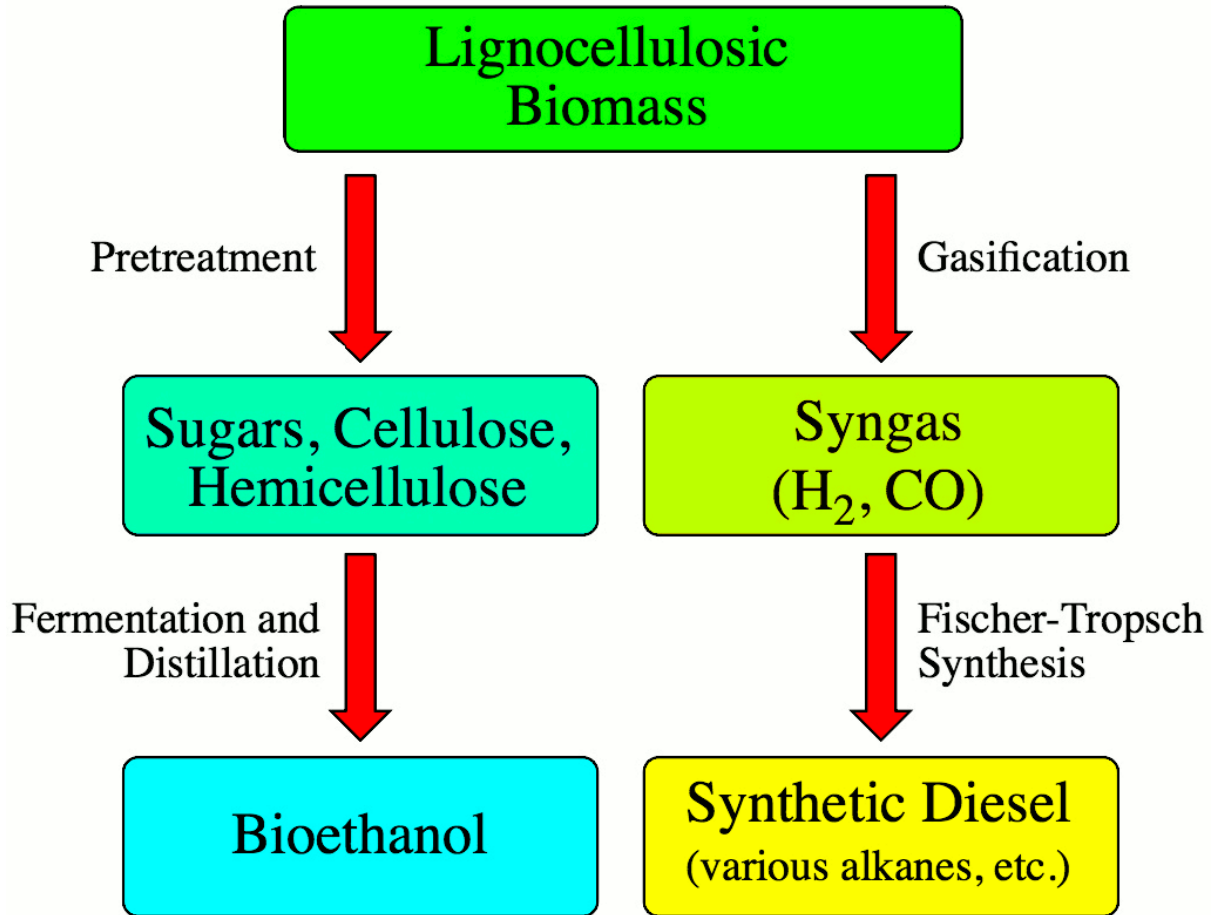


*Figure 5. The structure of Lignin*

## **BIOETHANOL PRODUCTION FROM LIGNOCELLULOSIC BIOMASS**

Bioethanol production typically consists of four main steps i.e. pretreatment of lignocellulosic materials, enzymatic hydrolysis of cellulose, and fermentation of glucose to bioethanol and the recovery of ethanol by distillation method [22,30].

Lignin, the primary organic component of the silage has the greatest potential of producing aromatic compounds through depolymerisation when the cellulose and hemicellulose contents of lignocellulosic biomass are degraded to ethanol [30].



*Figure 6 .Flow chart for biofuel production*

### **Pretreatment**

The pretreatment methods are classified into four mainly, biological, physical, chemical and physicochemical. Each pretreatment method has its own advantages and disadvantages and no single pretreatment approach is suitable for all the biomass species. Consequently, the pretreatment process roughly represents 33% of the total cost of the process [23].

During the pretreatment process, the crystallinity and degree of polymerization of the lignocellulosic biomass is reduced, while the surface area of the biomass is increased. The lignocellulose then easily hydrolyses and becomes much more efficient to break ,thereby making it more accessible for enzymatic or microbial attacks [26,31].

Harsh conditions are however, at times encountered during the pretreatment process thereby leading to the partial degradation of the hemicellulose and lignin, and the

generation of toxic compounds. The partial degradation of the hemicellulose and lignin can be avoided if the selected pretreatment methods is determined and configured well for further hydrolysis and fermentation [5,32].

Pretreatment method is considered the most expensive processing step in the conversion of lignocellulosic biomass to fermentable sugars. However, research is underway to make the pretreatment methods cost-effective.

Therefore, to achieve higher sugar yields, an effective pretreatment method should be able to preserve maximum hemicellulose fractions that could be converted into fermentable sugars for further conversion to ethanol, the pretreatment method should be able to minimize the formation of inhibitors due to the degradation of products, as it limits the loss of the carbohydrate. The pretreatment method should be able to minimize energy input and the process should be economically efficient as well as cost effective [33].

The pretreatment methods are classified as biological, physical, chemical or physicochemical pretreatments.

### **Biological pretreatment Process**

Biological pretreatment process involves the use of microorganisms, mainly brown, white and soft-rot fungi to digest lignin and hemicellulose at relatively mild environmental conditions[5,20]. The biological pretreatment utilizes *white-rot fungi microorganism* since white rot fungi easily mineralises lignin to CO<sub>2</sub> and water [17]. Other *white-rot fungi* such as *Phanerochaete chrysosporium*, *Ceriporiopsis subvermispora*, *Phlebia subserialis* and *Pleurotus ostreatus* have similarly been examined for use on different lignocellulosic biomass [34].The *white-rot fungi* can be applied in bio pulping, bio bleaching, ruminant feed, and xylose, ethanol, biogas and enzymes production [35].

The biological pretreatment process offers the greatest potential and advantage over physical or chemical pretreatments. Some of the biological advantages include its lower energy requirements, lower pollution output and high product yields [13,20].

The biological pretreatment has a major drawback as its hydrolysis rate is lower in comparison to other techniques. Moreover, the carbohydrates for fungal growth are consumed by some species. These disadvantages can be corrected if the biological pretreatment is carried out alongside other pretreatment methods such as a mild chemical pretreatment to enhance the saccharification yields [5] .

### **Physical pretreatment Process**

Physical pretreatment methods primarily reduces the particle size and this in return, results in an increase in the biomass surface area, and a decrease in its degree of

polymerization and crystallinity [11]. The physical pretreatment methods makes the subsequent processes much more effective and easier [5].

The most commonly used physical pretreatment methods are mechanical comminution, extrusion, microwave treatment, and ultrasonication. These pretreatment methods are eco-friendly and seldom produce any toxic material [35].

The major disadvantage of physical pretreatment method is its high energy consumption [11].

The physical methods are classified into mechanical comminution, extrusion, microwave treatment and ultrasonication.

### **a) Mechanical comminution**

Mechanical comminution involves chipping, grinding or milling [5]. During comminution, both the lignocellulosic biomass size is reduced and the degree of crystallinity. Different milling procedures for lignocellulosic biomass have been developed so as to suit different biomass compositions. The ball milling for example, can be used for both dry and wet materials. The ball milling improves the optimal number of ball heads for enzymatic hydrolysis for pretreatment prior to the enzymatic hydrolysis [32]. Other mechanical comminution processes like extrusion, roller mill, cryogenic mill and hammer mill are applicable to the dry material [24].

During the milling process the energy generated is dependent on the final particles size [36]. For example, the energy required to mill herbaceous biomass to size smaller than 2 mm usually corresponds to the quantity of ethanol produced, while the particle sizes that are below the pretreatment methods shows no significant improvement and are dependent on specific technology i.e. steam explosion, liquid hot water, dilute acid and base pre-treatments [35].

Mechanical comminution process inability to remove lignin and its high energy consumption tendencies are the major drawbacks [37].

### **b) Extrusion**

The lignocellulosic biomass is passed through a defined cross section die, and at the end of the die, the biomass is extruded out with a fixed definite profile. The extrusion process is majorly used in the sugar industry for the sugar recovery from biomass. The extrusion process is mainly used due to its adaptability to modifications, non-degradation of products, controllable environment, and the high throughput [33,36,38,39].

During the extrusion process, the lignocellulosic material can either be treated with an alkaline or acidic solution in order to increase its sugar recovery. Acidic treatment is less preferred to alkali due to the corrosion caused by the acids. The corrosion can

be solved by the use of AL6XN alloy for barrel fabrication and screws of extruder [39]. Alkali treatment is most suitable during the extrusion process as it degrades less of the carbohydrate as it degrades the side chains of esters and glycosides leading to the structural modification of lignin [5,8,40,41]. Sodium hydroxide is the most commonly used in alkaline pretreatment as it breaks the ester linkages and solubilizes the lignins and hemicelluloses [42].

#### **c) Microwave treatment**

The microwave treatment is commonly used for plant biomass. Microwave treatment was first reported in 1984 by a team of researchers from Kyoto University, Japan. They treated sugarcane bagasse, rice straw, and rice hulls with microwaves in the presence of water [36]. The microwave treatment is combined with mild alkali treatment for an effective degradation [5]. The microwave pretreatment exhibits three properties, namely, penetration, reflection, and absorbance. The microwave passes through glass and plastic, absorbed by water and biomass, whereas microwaves are reflected by metals. Microwave reactors can be divided into two types, one that allows the passage of microwaves, and the other that reflects the microwaves [7,33,39].

The advantages of microwave treatment are its ease of pretreatment, increased heating capacity, short processing time, minimal generation of inhibitors, and less energy requirement [11].

#### **d) Ultrasonication**

Ultrasonication is relatively a new technique used for the pretreatment of lignocellulosic biomass. The ultrasound waves produce both physical and chemical effects which alter the morphology of the lignocellulosic biomass. Ultrasonication treatment leads to the formation of small cavitation bubbles which rupture the cellulose and hemicellulose fractions thereby increasing the accessibility to cellulose degrading enzymes for effective breakdown into simpler reducing sugars [41].

### **Chemical pretreatment**

Chemical pretreatment methods are used more often than biological or physical pretreatment methods because of their effectiveness and ability to biodegrade even the complex materials [43]. Some of the most widely used chemical pretreatment are liquid hot water, weak acid hydrolysis, strong acid hydrolysis, and alkaline hydrolysis [41].

#### **a). Liquid hot water**

The biomass is pretreated with water at high temperature and pressure. The liquid hot water pretreatment is also known as hydrothermolysis, hydrothermal pretreatment, aqueous fractionation, solvolysis or aquasolv [5,8]. When the biomass

is pretreated by the solvolysis method at higher temperatures of 200-300°C, 40% to 60% of the total biomass is dissolved in the process, and 4–22% of the cellulose, 35–60% of the lignin and all of the hemicellulose is removed. In addition, acetic acid is formed during the treatment period and it acts as a catalyst for polysaccharide hydrolysis. The resultant monomeric sugars may further decompose to furfural, an inhibitor of fermentation [39].

#### **b.) Weak acid hydrolysis**

Weak acid hydrolysis is one of the most effective pretreatment methods for the lignocellulosic biomass. There are two types of weak acid hydrolysis i.e. the high temperature and continuous flow process for low-solids loading and the Low temperature and batch process for high-solids loading.

During the weak acid hydrolysis process, the biomass is sprayed with the dilute sulphuric acid, and the mixture is held at 160-220 °C for few minutes. Organic acids such as maleic acid, fumaric acid can also be used as alternative to the inorganic acids used during the dilute acid pretreatment [5].

The weak acid hydrolysis offers good performance when recovering the hemicellulose sugars. The hemicellulose sugars however might be degraded further to furfural and hydroxymethylfurfural during the hydrolysis process. Furfural and hydroxymethylfurfural are strong inhibitors to microbial fermentation[36].

As the acids used during the hydrolysis process can be corrosive they are neutralized and their neutralization results in the formation of solid waste. The weak acid hydrolysis therefore, is suitable for biomass with low lignin content, where almost no lignin is removed from the biomass [10].

#### **c.) Strong acid hydrolysis**

Strong acid hydrolysis has been widely used to treat lignocellulosic biomass due to their strong and powerful agents, and the non-requirement of enzymes in the subsequent treatment processes. Some of the widely used acids are concentrated H<sub>2</sub>SO<sub>4</sub> and concentrated HCL [36].

Strong acid hydrolysis is commonly used due to its flexibility in terms of feedstock choice, high monomeric sugar yield as well as mild temperature conditions that are needed [9].

Strong acid hydrolysis however, has its own share of drawbacks as well. Due to the corrosive nature of the strong acids, they must be recycled after every reaction, and this has an impact on the process cost [9,26].

#### **d.) Alkaline hydrolysis**

Alkaline hydrolysis removes the lignin from the biomass, thereby, improving the reactivity of the remaining polysaccharides. In addition, the process removes acetyl and other uronic acid substitutions on the hemicellulose that may hamper the accessibility of the enzyme to the hemicellulose and cellulose surface [39]. The alkaline hydrolysis mechanism is based on saponification of intermolecular ester bonds crosslinking xylan hemicelluloses and other components such as lignin [5].

The most commonly used alkaline hydrolysis are calcium or sodium hydroxide and ammonia.

#### **i) Calcium or sodium hydroxide**

In this alkaline hydrolysis process, lime (calcium hydroxide) or sodium hydroxide is usually employed. During the lime or sodium hydroxide hydrolysis the conditions are relatively mild and the reaction takes a while to complete, resulting in the formation of salts that may be incorporated in the biomass. The salts formed are eventually removed or recycled [5]. The mild conditions during alkaline hydrolysis, prevents condensation of the lignin, thereby resulting in high lignin solubility, especially for the biomass with low lignin content such as softwood and grasses, and the degradation of sugars to furfural, HMF and organic acids is limited [5].

#### **ii) Ammonia**

This alkaline hydrolysis process employs techniques that include the use of ammonia fibre explosion-method (AFEX), ammonia recycle percolation (ARP) and soaking in aqueous ammonia (SAA). When the lignocellulosic biomass is for instance, pretreated with aqueous ammonia at an elevated temperature, the lignin content is reduced and some of the hemicellulose is removed as the cellulose is decrystallised, and when the biomass is soaked in aqueous ammonia (SAA) at low temperatures, the lignin is removed efficiently through the minimal interaction with the hemicellulose [42].

When the lignocellulosic biomass is pretreated with ARP in a flow-through column reactor, the liquid flows at a high temperature through the reactor column packed with biomass, causing flash evaporation to occur. The flash evaporation can be prevented by slightly pressurizing the reactor system [5].

When the ammonia reaction is complete, the solid fraction that is rich in cellulose and hemicellulose is separated from the liquid and the liquid fraction is then sent into a steam-heated evaporator for the recovery of ammonia and lignin and the separation of other sugars [5,33,42].

The ammonia pretreatment is not sustainable due to the high cost of ammonia and its recovery [9].

#### **e.) Organosolv**

Organosolv processes employ the use of organic solvent or mixtures of organic solvents with water for lignin removal before the enzymatic hydrolysis of the cellulose fraction. In addition to lignin removal, the hemicellulose hydrolysis occurs leading to improved enzymatic digestibility of the cellulose fraction [5]. Organic and inorganic acids are the possible catalysts employed in this process [30]. The most commonly used solvents for this process are ethanol, methanol, acetone, and ethylene glycol.

The benefits of organosolv pretreatment include, the production of a high-quality lignin, which might facilitate higher-value applications of lignin such as production of chemicals and the potential lowering of the enzyme costs by separation of lignin before the enzymatic hydrolysis of the cellulose fraction. Additionally, organosolv pretreatment improves the accessibility of the cellulose fibres, and the absorption of cellulase enzymes to lignin is minimized by actual removal of lignin beforehand [44].

### **Oxidative delignification**

During oxidative delignification, the biomass is treated with an oxidizing agent such as hydrogen peroxide, ozone, oxygen or air for the delignification of lignocellulose. The oxidative treatment affects the hemicellulose fraction of the lignocellulose complex in addition to its effects on lignin. The effectiveness of oxidative delignification can be attributed to the high reactivity of oxidizing chemicals with the aromatic ring [22].

Oxidative delignification pretreatment process cannot however, sustain the sugar production due to the substantial degradation of the hemicellulose. The most commonly used oxidizing agents for the delignification of the lignocellulose are hydrogen peroxide, ozone and wet oxidation processes [22].

### **Hydrogen peroxide**

A solution of 2% H<sub>2</sub>O<sub>2</sub> at 30 °C is used for dissolution of about 50% of lignin to achieve most of the hemicellulose. The enzymatic hydrolysis yield afterwards can be as high as 95% [45].

### **Ozonolysis**

Ozonolysis focuses on lignin degradation by attacking and cleaving on the aromatic rings structures, as the hemicellulose and cellulose remains intact. Ozonolysis process can be used to disrupt the structure of many different lignocellulosic biomass such as wheat straw, bagasse, pine, peanut, cotton straw and poplar sawdust [42].

### **Wet oxidation**



Wet oxidation pretreatment method, is an alternative to steam explosion method and operates when oxygen or air is combined with water at an elevated temperature and pressure[39]. Wet air oxidation has been used industrially for the treatment of wastes with a high organic matter by oxidizing the soluble or suspended materials by using oxygen in aqueous phase at high temperatures [9]. This has been successfully applied for the treatment of wheat straw and hardwoods [9,10,39].

Wet oxidation method unlike other pretreatment methods, has been proved to be the most efficient in the treatment of lignocellulosic biomass treatment because of cellulose crystalline structure [31]. Wet oxidation process is advantageous due to its ability in lowering the production of furfural and 5-hydroxymethylfurfural, which are potential inhibitors in the fermentation step [36].

### **Room Temperature Ionic Liquids (RTIL)**

Room Temperature Ionic Liquids (RTIL) are salts composed of inorganic anion and an organic cation of the very heterogeneous molecular structure, and are in liquid phase at room temperature [10].

Due to the polarity of the salts and their unique properties there are possibilities that they can function as selective solvents of lignin or cellulose [39]. However, there is no industrial application employing the use of RTIL currently. Moreover, there is limited literature describing their actions with lignocellulose biomass [10]. The room temperature ionic liquids pretreatment is however, not able to recover the RTIL salts used, and the toxicity of the compounds and its combination with water render it inappropriate as a treatment method [39,46].

### **Enzymatic hydrolysis**

Enzymatic hydrolysis is a multi-step catalytic decomposition of the cellulosic biomass into fermentable sugars by the addition of specific enzymes, thereby breaking down the proteins into amino acids[26]. Thus the starch and cellulose present in the lignocellulosic biomass are converted into glucose by the addition of cellulase enzymes [47]. Cellulase enzymes are specialized and includes, glycosyl hydrolases that catalyses the enzymatic hydrolysis of the 1, 4- $\beta$ -glycosidic bonds of the lignocellulosic biomass [9].

The cellulase enzymes are aided by three cellulase activities that are carried out by endoglucanases, cellobiohydrolases, and  $\beta$ -glucosidases. The endoglucanases catalyses the random cleavage of the cellulose chains especially those in the amorphous regions thereby causing the rapid reduction in the cellulose DP while liberating cello-oligomers in the process. The cellobiohydrolases or exoglucanases, catalyses the cleavage of cellobiose from the cellulose chain ends, and the  $\beta$ -glucosidase catalyses the hydrolysis of the liberated cello-oligomers to glucose [8].

Enzymatic hydrolysis process, therefore, involves several key steps including, the transfer of enzymes from the bulk aqueous phase to the surface of the cellulose, the adsorption of the enzymes and formation of enzyme-substrate complexes, the hydrolysis of the cellulose, the transfer of the hydrolysis products from the surface of the cellulosic particles to the bulk aqueous phase, and the hydrolysis of cellodextrins and cellobiose to glucose in the aqueous phase[31].

### **Factors affecting enzymatic hydrolysis**

There are two major groups affecting the enzymatic hydrolysis, the substrate related factors and enzyme-related factors.

The substrate specific factors affecting the cellulose hydrolysis are porosity, cellulose fibre, crystallinity and lignin and hemicellulose content[48]. The crystallinity of the cellulose is particularly important as it makes the accessible amorphous parts of the cellulose more prone to degradation by the cellulolytic enzymes while the least accessible parts of the cellulose-the crystalline region is not attacked by the cellulolytic enzyme. And as the crystallinity level of the cellulose increases, the cellulose becomes more resistant to further hydrolysis [19].

The enzyme related factors are the optimization of different enzyme types, the enzyme dosages required to achieve optimal sugar yields and the end-product inhibition of the cellulase activity [5]. Several methods have been developed to reduce inhibition, including the use of high concentrations of enzymes, the supplementation of  $\beta$ -glucosidases during hydrolysis and the removal of sugars during hydrolysis by ultrafiltration or simultaneous saccharification and fermentation (SSF) [31].

Surfactants also affects the enzymatic hydrolysis [36]. Surfactants are amphiphilic compounds that are capable of self-assembling into micelles. They adsorb onto surfaces depending on the surfactant structure and the polarity of the surface. Additionally surfactants can cause the surface structure modification or disruption of the lignocellulose, they can affect the enzyme substrate interaction by preventing non-productive adsorption of the enzymes, and, surfactants can act as enzyme stabilizers preventing enzyme denaturation. Some of the surfactants commonly used in enzymatic hydrolysis include Tween 20, Tween 80, Emulgen 147, and Tween 81 among others [9,11].

### **Fermentation**

Fermentation process biologically converts sugars to ethanol by the application of a wide range of microorganisms. Other hexoses such as fructose and galactose, may *also be converted to ethanol in a similar manner [16]. The baker's yeast and saccharomyces cerevisiae are the most commonly used fermenting microorganisms [48]. The bacterium Zymomonas mobilis, first isolated from a glass of Capirinhas in*

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*the city of Recife, Brazil*. is another fermenting organism that produces ethanol at high yields [44]. The *bacterium zymomonas*, can be applied in the alternative pathway of the catabolic production of pyruvate from glucose [49].

### **Mixed sugar fermentation**

The mixed sugar fermentation process is employed in the presence of several inhibiting compounds, such as low molecular weight organic acids, furan derivatives, phenolic and inorganic compounds that are formed during the pretreatment of the lignocellulosic biomass[9].

The lignocellulosic biomass is made up of cellulose and hemicellulose, and the hemicellulose are heteropolymers made up of many different monosaccharides that contain both hexoses and pentoses. The pentoses can be handled by, using a naturally occurring microorganism for pentose fermentation, genetically engineering a suitable host organism for conversion of pentoses, or, fermenting only the hexoses and using the remaining pentoses for other purposes [9]. The pentoses cannot be fermented to ethanol by *Saccharomyces cerevisiae*, since xylose is the most dominant of the pentose sugars [48]. However, some bacteria can convert xylose to bioethanol under strict anaerobic conditions. These bacteria include *Bacillus macerans*, *Bacillus polymyxa*, *Klebsiella pneumoniae*, *Clostridium acetobutylicum*, *Aeromonas hydrophila*, *Aerobacter sp.*, *Erwinia sp.*, *Leuconostoc sp.*, *Lactobacillus sp.*, *Escherichia coli*, *Klebsiella*, *Erwinia*, *Lactobacillus*, *Bacillus* and *Clostridia* [50].

### **Recovery and Distillation**

On completion of the fermentation process, the fermentation broth is usually a mixture of bioethanol, water, cell mass and other components such as residual sugars, non-fermentable sugars and the hydrolysis by-products. [14,36,51].

The bioethanol is then recovered from the fermented media through the distillation process whereby bioethanol is separated from the binary alcohol-water mixture based on their different boiling points [7,36,48].

### **Conclusion and future prospects**

Lignocellulosic materials are the most abundant feedstock available for bioethanol production and hence, tremendous research efforts have been made to enhance the various aspects of the processes involved. There are however, several major challenges hindering the large scale and cost-effective production of cellulosic biofuels. The capital investment requirement is the biggest challenge towards the establishment of a bioethanol plant. In some instances, there arises a need for reactors capable of resisting high pressures, high temperatures, and/or corrosive catalysts. So as to reduce the cost of the pretreatment process, different researchers

have studied the economic features of different pretreatment approaches considering both the capital and operating costs as well as glucose and xylose sugar yields.

In addition to cost, there are several other factors such as toxicity and waste disposal that need to be addressed prior to the selection of a pretreatment process. Many types of pretreatment require water and organic solvents in at least one stage of the process. This results in the generation of wastewater which must be remediated to prevent contamination of the environment, while many organic solvents are highly flammable, volatile, and potentially toxic requiring additional safety and storage considerations; all adding to the total cost of the process. Besides toxicity and waste disposal, some pretreatment methods involve additional equipment or have high energy requirements which should also be factored into the cost and feasibility of the overall process.

Based on a study, it was predicted that simultaneous saccharification process accounted for 15% of the overall production cost, whereas pretreatment accounted for 17% of the production cost. Currently, most of the reports available in the literature were performed at lab scale and there is limited information on the real-world production cost arising from all the stages involved in biofuel production from lignocellulosic biomass, i.e., delignification, hydrolysis, and fermentation. Therefore, future research works should be devoted to the optimization of operating parameters and assessment of total cost of biofuel production from lignocellulose biomass at large scale by using different pretreatment methods. Such information would pave the way for industrial-scale lignocellulosic biofuels production. Lastly, more research should be directed toward the identification/isolation of more effective lignin hydrolyzing microbes by taking advantage of advanced molecular techniques.

## **ETHICAL APPROVAL**

No ethical approval was required in this study

## **REFERENCES**

1. WEC. World Energy Resources Bioenergy: Strategic insight. World Energy Council. 2013;60.
2. IEA Bioenergy. Potential Contribution of Bioenergy to the World's Future Energy Demand. 2007;1–11.
3. Dufey A. Biofuels production, trade and sustainable development: emerging issues, International Institute for Environment and Development, London. 2006;
4. Triwahyuni E, Muryanto, Sudiyani Y, Abimanyu H. The effect of substrate loading on simultaneous saccharification and fermentation process for bioethanol production from oil palm empty fruit bunches. *Energy Procedia* [Internet]. Elsevier B.V.; 2015;68:138–46. Available from: <http://dx.doi.org/10.1016/j.egypro.2015.03.242>

5. Tayyab M, Noman A, Islam W, Waheed S, Arafat Y, Ali F, et al. Bioethanol production from lignocellulosic biomass by environment-friendly pretreatment methods: A review. *Appl Ecol Environ Res*. 2018;16:225–49.
6. Dias MOS, Ensinas A V., Nebra SA, Maciel Filho R, Rossell CEV, Maciel MRW. Production of bioethanol and other bio-based materials from sugarcane bagasse: Integration to conventional bioethanol production process. *Chem Eng Res Des*. 2009;87:1206–16.
7. Branco RHR, Serafim LS, Xavier AMRB. Second generation bioethanol production: On the use of pulp and paper industry wastes as feedstock. *Fermentation*. 2019;5:1–30.
8. Mahalaxmi S, Williford C. Biochemical conversion of biomass to fuels. *Handb. Clim. Chang. Mitig. Adapt*. Second Ed. 2016.
9. Kumar R, Tabatabaei M, Karimi K, Horváth IS. Recent updates on lignocellulosic biomass derived ethanol - A review. *Biofuel Res J*. 2016;3:347–56.
10. Bensah EC, Mensah M. Chemical pretreatment methods for the production of cellulosic ethanol: Technologies and innovations. *Int J Chem Eng*. 2013;2013.
11. Ahorsu R, Medina F, Constantí M. Significance and challenges of biomass as a suitable feedstock for bioenergy and biochemical production: A review. *Energies*. 2018;11.
12. Love J, Bryant JA, Lynch JM. The Sustainability of Biofuels. *Biofuels and Bioenergy*. 2017;261–72.
13. Elder M, Prabhakar S, Romero J, Matsumoto N. Prospects and challenges of biofuels in Asia: policy implications. *Clim Chang Policies Asia- Pacific Re-Uniting Clim Chang Sustain Dev* [Internet]. 2008;105–31. Available from: [https://pub.iges.or.jp/system/files/publication\\_documents/pub/book/796/07\\_chapter5.pdf](https://pub.iges.or.jp/system/files/publication_documents/pub/book/796/07_chapter5.pdf)
14. Europe IE. Assessment of Sustainable Lignocellulosic Biomass Potentials from Kenya for export to the European Union 2015 to 2030. 2016;
15. Kenya Ministry of Energy. Sessional Paper No . 4 on Energy: National Energy Policy. *Natl energy policy*. 2004;
16. Ndegwa G, Moraa V, Jamnadass R, Mowo J. Potential for biofuel feedstock in Kenya. Available from: <http://dx.doi.org/10.5716/WP11272.PDF>
17. Deenanath ED, Iyuke S, Rumbold K. The bioethanol industry in sub-Saharan Africa: History, challenges, and prospects. *J Biomed Biotechnol*. 2012;2012.
18. Gomez Jimenez I. Feasibility Study on the use of Sustainable Aviation Fuels. 2017;
19. Kang Q, Appels L, Tan T, Dewil R. Bioethanol from lignocellulosic biomass: Current findings determine research priorities. *Sci World J*. 2014;2014.
20. Mohd Azhar SH, Abdulla R, Jambo SA, Marbawi H, Gansau JA, Mohd Faik AA, et al. Yeasts in sustainable bioethanol production: A review. *Biochem Biophys Reports*. 2017;10:52–61.
21. Mati BM, Thomas MK. Overview of Sugar Industry in Kenya and Prospects for Production at the Coast. 2019;1477–85.
22. Ioelovich M. Recent findings and the energetic potential of plant biomass as a

- renewable source of biofuels - A review. *BioResources*. 2015;10:1879–914.
23. Mtui GYS. Recent advances in pretreatment of lignocellulosic wastes and production of value added products. *African J Biotechnol*. 2009;8:1398–415.
24. IRENA. Bioethanol in Africa: The case for technology transfer and South-South co-operation [Internet]. Int. Renew. Energy Agency. 2016. Available from: [https://www.irena.org/-/media/Files/IRENA/Agency/Publication/2016/IRENA\\_Bioethanol\\_in\\_Africa\\_2016.pdf](https://www.irena.org/-/media/Files/IRENA/Agency/Publication/2016/IRENA_Bioethanol_in_Africa_2016.pdf)
25. Chatel G, Rogers RD. Review: Oxidation of lignin using ionic liquids-an innovative strategy to produce renewable chemicals. *ACS Sustain Chem Eng*. 2014;2:322–39.
26. Cannella D, Hsieh CWC, Felby C, Jørgensen H. Production and effect of aldonic acids during enzymatic hydrolysis of lignocellulose at high dry matter content. *Biotechnol Biofuels*. 2012;5:1–10.
27. Li M, He B, Li J, Zhao L. Physico-chemical Characterization and Comparison of Microcrystalline Cellulose from Several Lignocellulosic Sources. *BioResources*. 2019;14:7886–900.
28. Li TC, Universit T, Pr KH, Lercher JA, Rieger B, Haller GL, et al. Selective Cleavage of C-O Bonds and Hydrodeoxygenation of Lignin Fragment Molecules *Jiayue He*. 2014;
29. Purohit P, Fischer DG. Second-generation biofuel potential in India: Sustainability and Cost Considerations. Report from UNEP [Internet]. 2014. Available from: [www.unep.org/transport/lowcarbon/PDFs/Biofuels\\_Report.pdf](http://www.unep.org/transport/lowcarbon/PDFs/Biofuels_Report.pdf)
30. Swann WFG. Mass and energy. *J Franklin Inst*. 1932;213:63–74.
31. Nikolić S, Pejin J, Mojović L. Izazovi u proizvodnji bioetanol: Korišćenje pamučnih tkanina kao sirovine. *Chem Ind Chem Eng Q*. 2016;22:375–90.
32. Rocha-Meneses L, Raud M, Orupöld K, Kikas T. Second-generation bioethanol production: A review of strategies for waste valorisation. *Agron Res*. 2017;15:830–47.
33. Dahiya S, Goyal S. Pretreatment of Lignocellulosic Biomass for Bioethanol Production: A Brief Review. *Res Rev J Agric Sci Technol*. 2018;5:1–7.
34. Bioenergy IEA. Contribution of Bioenergy to the World ' s Future Energy. 2007;
35. Shirkavand E, Baroutian S, Gapes DJ, Young BR. Pretreatment of radiata pine using two white rot fungal strains *Stereum hirsutum* and *Trametes versicolor*. *Energy Convers Manag* [Internet]. Elsevier Ltd; 2017;142:13–9. Available from: <http://dx.doi.org/10.1016/j.enconman.2017.03.021>
36. -Galvan, R. F, Barranco V, Galvan JC, Batlle, Sebastian FeliuFajardo S, García. We are IntechOpen , the world ' s leading publisher of Open Access books Built by scientists , for scientists TOP 1 %. Intech [Internet]. 2016;i:13. Available from: <https://www.intechopen.com/books/advanced-biometric-technologies/liveness-detection-in-biometrics>
37. Barakat A, Mayer-Laigle C, Solhy A, Arancon RAD, De Vries H, Luque R. Mechanical pretreatments of lignocellulosic biomass: Towards facile and environmentally sound technologies for biofuels production. *RSC Adv* [Internet].

Royal Society of Chemistry; 2014;4:48109–27. Available from:  
<http://dx.doi.org/10.1039/C4RA07568D>

38. Jurisic V, Julson JL, Kricka T, Curic D, Voca N, Karunanithy C. Effect of Extrusion Pretreatment on Enzymatic Hydrolysis of Miscanthus for the Purpose of Ethanol Production. *J Agric Sci.* 2015;7:132–42.
39. Harmsen P, Bermudez L, Bakker R. Literature Review of Physical and Chemical Pretreatment Processes for Lignocellulosic Biomass. , 49 P. 2010;1–49.
40. Chiaramonti D, Giovannini A, Janssen R, Mergner R. Lignocellulosic ethanol production plant by Biochemtex in Italy Lignocellulosic Ethanol Process and Demonstration A Handbook Part I [Internet]. 2013. Available from: [www.wip-munich.de](http://www.wip-munich.de)
41. Kumar AK, Sharma S. Recent updates on different methods of pretreatment of lignocellulosic feedstocks: a review. *Bioresour Bioprocess.* Springer Berlin Heidelberg; 2017;4.
42. Cheah WY, Sankaran R, Show PL, Tg. Ibrahim TNB, Chew KW, Culaba A, et al. Pretreatment methods for lignocellulosic biofuels production: current advances, challenges and future prospects. *Biofuel Res J.* 2020;7:1115–27.
43. Zhou S, Zhang Y, Dong Y. Pretreatment for biogas production by anaerobic fermentation of mixed corn stover and cow dung. *Energy* [Internet]. Elsevier Ltd; 2012;46:644–8. Available from: <http://dx.doi.org/10.1016/j.energy.2012.07.017>
44. Pereira N, Couto M, Anna LS. Biomass of lignocellulosic composition for fuel ethanol production and the context of biorefinery [Internet]. *Ser. Biotechnol.* 2008. Available from: <http://www.ladebio.org.br/download/series-em-biotecnologia-vol-ii-lignocellulosic-biomass.pdf>  
<http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:Biomass+of+Lignocellulosic+Compostion+for+Fuel+Ethanol+Production+and+the+Context+of+Biorefinery#>
45. Zhao W, Zhao F, Zhang S, Gong Q, Chen G. Ethanol production by simultaneous saccharification and cofermentation of pretreated corn stalk. *J Basic Microbiol.* 2019;59.
46. Matsutani A, Harada T, Ozaki S, Takaoka T. Inhibitory effects of combination of CDDP and cepharanthin on the cultured cells from rat ascites hepatoma. *J. Japan Soc. Cancer Ther.* 1993.
47. Fan Z. *Enzymatic Hydrolysis Consolidated Bioprocessing for Ethanol Production Phospholipid-Based Surfactants.* 2018;
48. Kang Q, Appels L, Tan T, Dewil R. Bioethanol from lignocellulosic biomass: Current findings determine research priorities. *Sci. World J.* 2014.
49. Ren H, Zong MH, Wu H, Li N. Utilization of Seawater for the Biorefinery of Lignocellulosic Biomass: Ionic Liquid Pretreatment, Enzymatic Hydrolysis, and Microbial Lipid Production. *ACS Sustain Chem Eng.* 2016;4:5659–66.
50. Horn SJ, Vaaje-Kolstad G, Westereng B, Eijsink VGH. Novel enzymes for the degradation of cellulose. *Biotechnol Biofuels.* 2012;5.
51. Play SOF, Perspectives C. *Second Generation Biofuel Markets* : 2016;

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