



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

**John Odhiambo Otieno<sup>1\*</sup> and Fredrick Onyango Ogutu<sup>2</sup>**

<sup>1</sup>*Natural Products Division, Kenya Industrial Research and Development Institute, Western Region Centre (KWRC), P.O. Box 6017-40103, Kisumu, Kenya.*

<sup>2</sup>*Food Technology Division, Kenya Industrial Research and Development Institute (KIRDI), P.O. Box 30650-00100, Nairobi, Kenya.*

## **Authors' contributions**

*This work was carried out in collaboration between both authors. Author JOO did the literature search and drafted the manuscript. Author FOO designed and revised the manuscript. Both authors approved the manuscript for submission to the journal. Both authors read and approved the final manuscript.*

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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.

\*Corresponding author: E-mail: [odhiambo.john2@gmail.com](mailto:odhiambo.john2@gmail.com);

**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. Research, technology adaption/adaptation, and policy targeted at growing bioethanol industry, could enable Kenya to grow her bioethanol industry.

*Keywords: Lignocellulosic biomass; lignin; pre-treatment; Kenya.*

## 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 and transport fuels. Production and use of biofuels are growing at a very rapid pace. Sugar cane-based ethanol is 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 biofuel energy, which can be used as an alternative to fossil fuels, so as to bridge the challenges of energy security. Biomass derived from corn has become one of the primary feedstocks for bioethanol production for the past several years in the U.S.A and there is an ongoing industrial research efforts focusing on low-cost large-scale processes for lignocellulosic feedstocks originating mainly from agricultural and forest residues along with herbaceous materials and municipal wastes [4], fireweed, common broom, hay and goldenrod [5] pineapple fruit peel waste [6], wheat straw, corn Stover, sugarcane and agave biomasses [7] winter rye, oilseed rape and faba bean [8] watermelon waste[9], sweet lime peel [10], banana bulbs [11].

Accordingly, biomass has been identified as a significant contributor to achieving sustainable development goals [12], moreover, it is cost effective, renewable and abundant [13]. 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 [12,14,15]. 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 trans-esterification process. Research on some plants have yielded products and consequently, these plants and their products have been used to generate renewable energy, and, in the development of biodiesel. Some of the products from plants that have been utilized in biodiesel development are; rubber seed, Rubber seed oil , oil palm biomass , Wilson's Dogwood , Brassica napus seed oil,, Koelreuteria integrifolia oil , jatropha oil , castor oil , Eruca sativa , and Pongamia [2,6]. Lignocellulosic biomass is, however, low in oil content, and is therefore, not suitable for biodiesel production [14,16,17], hence has largely not been considered in this respect.

### 1.1 Global Bioethanol Outlook

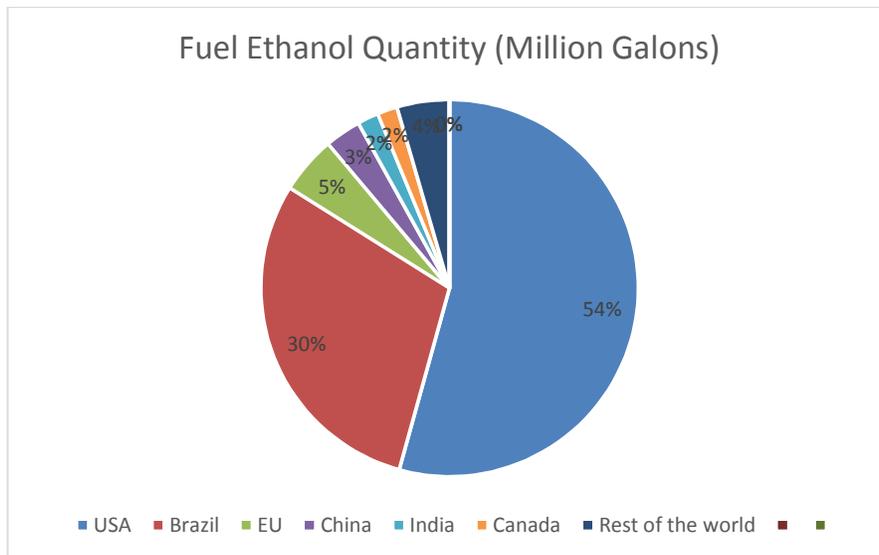
The United States, Brazil and China are both the world's biggest producers and consumers of ethanol, collectively accounting for around 80% of world output and consumption, and the most important raw materials from which ethanol is made in these countries are corn and sugarcane [18]. The EU is targeting to replace 10% of their automobile fuel with biofuels by 2020 [14]. The main crops used in the production of bio-ethanol in the EU are starch rich crops such as common wheat and sugar beet [19]. Furthermore, the EU countries committed a substantial amount of money for biofuels development from lignocellulosic biomass under the seventh framework program [20,21]. India, has

abundance of various agro residues such as; wheat straw, Cotton stalks banana stem, sugarcane bagasse, sunflower stalk, sweet sorghum, weeds like *Saccharum spontaneum*, *Typha latifolia*, *Eicchornia crassipes*, *Prosopis juliflora*, and Lantana camara that can be utilized for bioethanol production. [22,23]. Consequently, the Indian government intends to replace 20% of fossil fuel consumption with bioethanol and biodiesel by 2020 [14,24].

In Kenya, the promotion of biofuels started in the early 1980s with the gasohol program, which was later abandoned in the 1990s [25]. Under this programme, ethanol was to be blended with petrol at 10% to form E10 fuel. The blend did not require any modification of engines to be used as fuel. At present, 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 sugar cane molasses as a feedstock. The molasses produced by the Kenyan sugar companies is not sufficient, and, is therefore, inadequate to sustain ethanol production for both industrial and domestic use as the molasses produced is either sold for human consumption, or used in the baking industry and in the brewing of ale [26,27]. In addition, molasses produced 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 sugar cane molasses very much costly and unsustainable and, thus an alternative source of feedstock has to be considered [28].

Lignocellulosic biomass offers the greatest alternative to sugar cane feedstock for biofuel development. These lignocellulosic resources 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 bioethanol production in Kenya [24]. The Kenya Agricultural and Livestock Research Organization (KALRO) identified cane tops as a potential source of biomass. At harvest time, the sugarcane biomass includes stalks that can be milled, tops, dead and dying leaves, stubble and roots .In addition, studies by ICAO focusing on lipid producing feedstocks found out that oil-rich feedstocks including castor, coconut, rapeseed, sunflower, jatropha and croton nut, as well as sugar/starch feedstocks can be fermented and converted into ethanol [28]. Similarly, Onifade et.al found out that agricultural residues have the potential to generate renewable energy [29]. In 2008, an NGO in Kieni, Nyeri county,-the Help Self Help Centre (HSHC) reportedly bought croton seeds from farmers for processing into biodiesel. The project is still ongoing and, with the support of Solarix Netherlands and Kenya School Project (USA) among others. Studies by stakeholders like



**Fig. 1. The global outlook for fuel bioethanol production**  
 Source Adapted from Stastica, 2020

governmental agencies, NGOs, and the private sector indicated that the lignocellulosic plants have a great potential for biofuel production [26,30] ,and, jatropha has been utilized as the main feedstock, although other studies involving other feed stocks like castor, croton, and coconut are underway [26,30,31]

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; sugarcane, maize, wheat, rice and cotton generates 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 [14]. Lignocellulosic feedstock from residue crops such as cotton straws, sugarcane tops, rice straw, maize stalks, and wheat straw can therefore enhance the potential of bioethanol production [32,33]. This review critically appraises lignocellulosic biomass for bioethanol production, and, will be of great help while selecting and developing bioethanol from lignocellulosic biomass.

## 2. 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

### 2.1 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 [26]. 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 [26]. Since then Kenya has continued to rely entirely on petroleum fuel imports [34]. A strategy to develop

ways of the introduction of biofuels blends in Kenyan market was developed [35]. Blending was expected to begin at the Kenya Pipeline Company depots in Kisumu, Eldoret and Nakuru due to their proximity to the sugar belt. However, the programme is yet to begin [26].

In the recent past, Kenya has identified energy as one of the key enablers upon which the economic pillar of the Kenya's Vision 2030 development blueprint is built on under the Third Medium Term Plan 2018-2022 of the vision, the government intends to improve the energy infrastructure, promote the development and use of renewable energy source to create a cost-effective energy supply regime that is reliable and adequate to support industrialization, food security and job creation for economic growth [36]. In pursuit of this, the government established regulations Energy Act of 2019, the act also created an agency, the Rural Electrification and Renewable Energy Corporation mandated to perform the following roles; development of the renewable energy master plan, the development, promotion and management in collaboration with other agencies, the use of renewable energy and technologies, including but not limited to biomass (biodiesel, bio-ethanol, charcoal, fuel-wood, biogas) municipal waste, solar, wind, tidal waves, small hydropower and co-generation but excluding geothermal, and, the formulation of a national strategy for coordinating research in renewable energy; among other functions [37].

The Kenyan energy mix currently consists of geothermal 45%, hydro power 28%, wind 13%, diesel run generators 11%, and the remaining percentage taken up by solar amongst others. Of this, diesel run generators are the most expensive, and it normally increases the cost of power more so during the dry spell [38]. Therefore there is need to explore alternative source of renewable energy, of which biofuel is one of the promising options .Biofuels have a potential use in transport sector and households for cooking, lighting, and heating. Biodiesel is specifically, well-suited to certain uses, such as underground mining where workers are exposed to high levels of diesel exhaust. Using biodiesel therefore, can reduce as much as 90 percent of air toxins. One of the advantages of using biofuels for transportation is that they can be blended with fossil fuels using simple splash-blending techniques [36].The Kenyan bio-ethanol industry is however, negatively impacted by poor planning and strategy, and by the aging infrastructure [28,39].

Presently, ethanol is 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 [31]. The capacity is expected to increase with Mumias Sugar Company having built a bio refinery plant with the capacity to produce 22M litres of ethanol annually [40]. Mumias Sugar Company has taken advantage of the concept of bio-refinery or integrated sugar production which utilises the entire crop for a variety of environmentally favourable outcomes. The effective use of the by-products in an integrated sugar plant diversifies the income stream by adding new intermediate-value and high-value products. The efficient utilization of by-products of the sugar industry provides a significant impact on the profitability of sugar industry [26]. A new entrant in the sugar industry, Kwale International sugar company (KISCOL) is also planning a 30,000-litre per day ethanol plant [41]. As such, the ethanol industry in Kenya is expected to grow rapidly in the next decade [26].

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 [42]. A status analysis of feedstock production has established that enough sugarcane is currently produced for 49 million liters of ethanol if only molasses is used and 345 million liters if all cane went into ethanol instead of sugar. Moreover, the existing production level of castor is enough to produce approximately 1.3 million liters of biodiesel. Besides, coconuts and croton seeds remains largely unexploited, and if exploited for biofuels processors could produce millions more [31].

## 2.2 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 [32,43]. 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 [32].

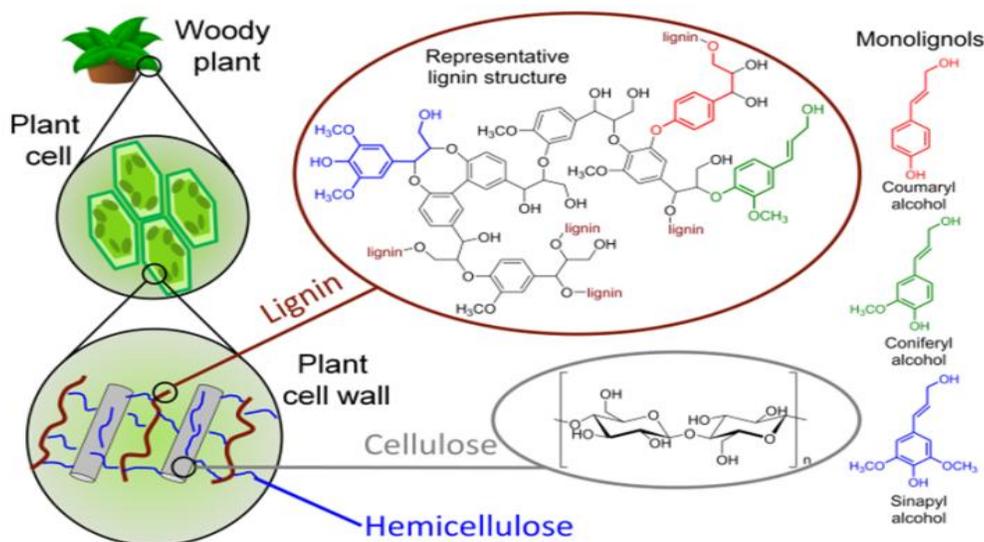
The tertiary sources includes agro industrial residues and dried manure. Though it's hard to estimate the total production of agro industrial residues worldwide, it is approximated at between 5 to 55 EJ/year, with 5EJ/year being the lower estimate due to its use as fertilizer, while the 55EJ/year being the total higher estimate with a considerable technical potential [33,44]. 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.

## 2.3 Composition of Lignocellulosic Biomass

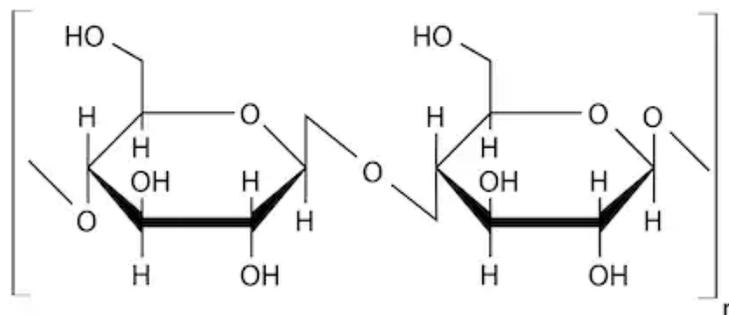
Lignocellulosic biomass is the most abundant renewable resource on earth, and is the main primary building block of plant cell walls [16,20,45]. Lignocellulosic biomass is hence, a complex mixture of cellulose, hemicellulose, and lignin, with minor amounts of ash, proteins, lipids, and other lipophilic compounds. The composition of lignocellulosic biomass is, however, not uniform and therefore, varies from one plant species to the other [46]. 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 [17,20].

## 2.4 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 [17]. Beta glucose is the monomer unit in cellulose with alpha being the building block for starch. 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 [47].



**Fig. 2. Structure of lignocellulose in plant biomass. Indicating cell wall polymers lignin, hemicellulose and The lignocellulosic biomass is mainly made up of cellulose, hemicellulose and lignin**



**Fig. 3. The chemical structure of cellulose showing D-glucopyranose linked at the 1 and 4 carbon atoms by  $\beta$ -glycosidic bonds**

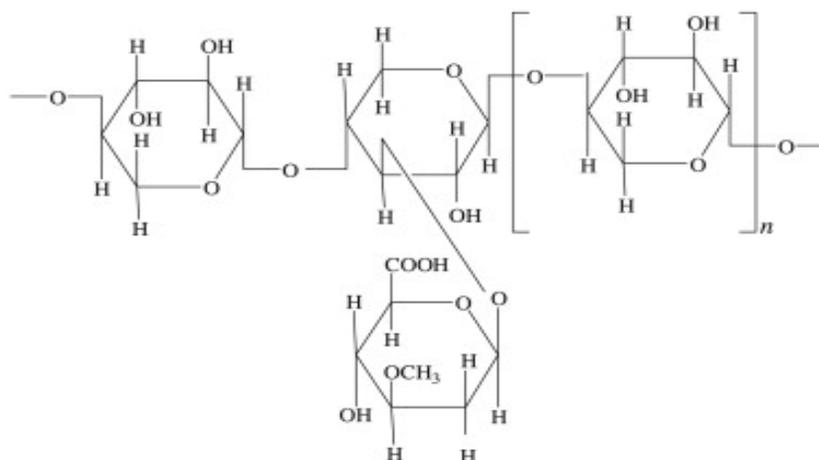
The cellulose properties such as cellulose content, pH, moisture content, crystallinity index, moisture sorption capacity of the cellulose are dependent on the degree of polymerization (DP) i.e. the number of glucose units that makes up one polymer molecule [48]. Cellulose from wood pulp, for instance, 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 [46].

## 2.5 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. Hemicelluloses can account for up to 20-40% of the total dry weight of lignocellulosic materials cell wall [43]. Hemicelluloses molecules contain 50-200 monomer units, it serves as the link between the lignin and the cellulose fibres and therefore, gives the cellulose-hemicellulose-lignin network rigidity [16].

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 [20]. Due to hemicellulose resistance to hydrolysis, it undergoes thermal decomposition at a lower temperatures of 220-315°C [32].



**Fig. 4. The structure of hemicellulose showing the repeating monomer unit and side chain**

## 2.6 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 [17]. 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 [49].

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 [50].

## 3. BIOETHANOL PRODUCTION FROM LIGNOCELLULOSIC BIOMASS

Bioethanol production typically consists of four main steps i.e. pretreatment of lignocellulosic materials, enzymatic hydrolysis of cellulose, fermentation of glucose to bioethanol and the recovery of ethanol by distillation method [43,51].

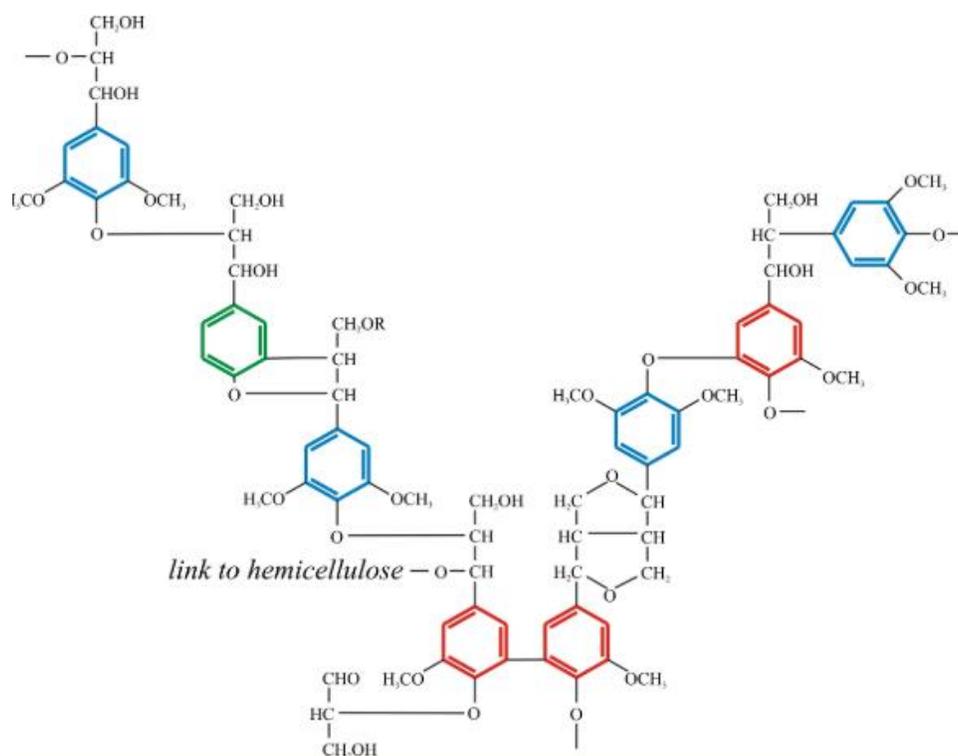
Lignin, the primary organic component of the silage has the greatest potential of producing aromatic compounds through depolymerisation when the cellulose and hemicellulose contents of

lignocellulosic biomass are degraded to ethanol [30]. The different steps in bioethanol production can be integrated for economic reasons as separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) among others.

## 4. PRE-TREATMENT

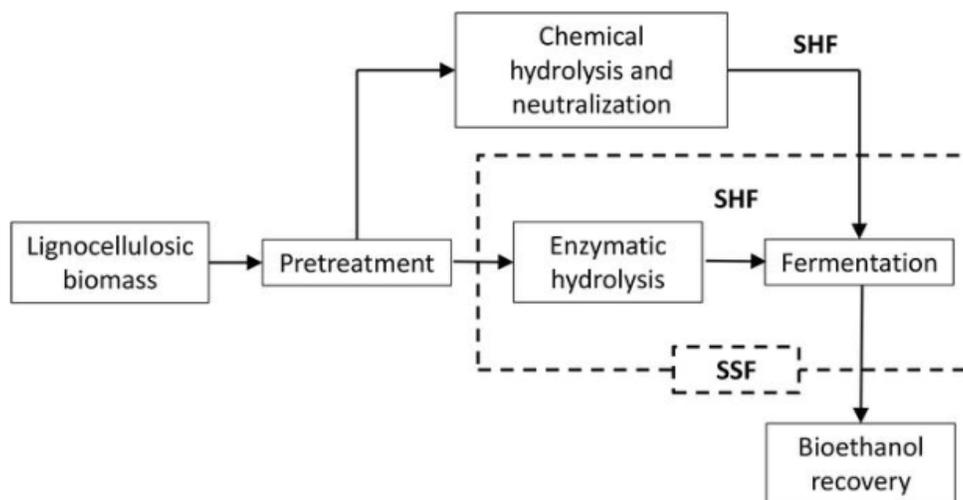
The pretreatment methods are classified into four mainly, biological, physical, chemical and physicochemical. Each pretreatment method has its own advantages and disadvantages and no single pretreatment approach is suitable for all the biomass species. Consequently, the pretreatment process roughly represents 33% of the total cost of the process [44]. Thus pretreatment method is considered the most expensive processing step in the conversion of lignocellulosic biomass to fermentable sugars.

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 [47,52]. Harsh conditions are however, at times encountered during the pretreatment process hereby 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 [14,53].



**Fig. 5. Schematic formula of the polymer structure of lignin**

Chemical structures of the phenylpropanoid alcohols (coniferyl, sinapyl, and coumaryl alcohols, red, blue and green respectively) used to construct the lignin polymer. These are also called monolignols. They are colour coded here to indicate how they probably contribute to the lignin polymer. The structure shows the predominance of ether linkages and carbon-carbon bonding, and the presence of a few hydroxyls that can take part in cross links to other polymers (polysaccharides like hemicellulose and proteins). Besides the predominance of benzene rings. Source Vanholme et al. (2010)



**Fig. 6. Bioethanol production from lignocellulose steps indicating the key steps in bioethanol production from lignocellulose**

SHF-Separate hydrolysis and fermentation. SSF- and simultaneous saccharification and fermentation

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 [54].

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

#### 4.1 Biological Pre-treatment 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 [14,33]. 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 subvermispota*, *Phlebia subserialis* and *Pleurotus ostreatus* have similarly been examined for use on different lignocellulosic biomass [55]. The white-rot fungi can be applied in bio pulping, bio bleaching, ruminant feed, and xylose, ethanol, biogas and enzymes production [56].

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 [33,57]. 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 [14].

#### 4.2 Physical Pre-treatment 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 [58]. The physical pretreatment methods makes the subsequent processes much more effective and easier [14].

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 [56]. The major disadvantage of physical pretreatment method is its high energy consumption [58]. The physical methods are classified into mechanical comminution, extrusion, microwave treatment and ultrasonication.

##### 4.2.1 Mechanical comminution

Mechanical comminution involves chipping, grinding or milling [14]. 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 [53]. 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 [59]. 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 [60].

##### 4.2.2 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 [54,59,61,62].

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 [62]. 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 [14,16,63,64]. Sodium hydroxide is the most commonly used in alkaline pretreatment as it breaks the ester linkages and solubilizes the lignins and hemicelluloses [65].

#### 4.2.3 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 [59]. The microwave treatment is combined with mild alkali treatment for an effective degradation [14]. 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 [17,54,62]. The advantages of microwave treatment are its ease of pretreatment, increased heating capacity, short processing time, minimal generation of inhibitors, and less energy requirement [58].

#### 4.2.4 Ultrasonication

Ultrasonication is relatively a new technique used for the pretreatment of lignocellulosic biomass. The ultrasound waves produce both physical and chemical effects which alters the morphology of the lignocellulosic biomass. Ultrasonication treatment leads to the formation of small cavitation bubbles which ruptures the cellulose and hemicellulose fractions thereby increasing the accessibility to cellulose degrading enzymes for effective breakdown into simpler reducing sugars [64].

### 4.3 Chemical Pre-treatment

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 [66]. Some of the most widely used chemical pretreatment are liquid hot water, weak acid hydrolysis, strong acid hydrolysis, and alkaline hydrolysis [64].

#### 4.3.1 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 [14,16]. 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 [62].

#### 4.3.2 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 [59]. 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 [21].

### 4.3.3 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 [59]. 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 [20]. 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].

### 4.3.4 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 [62]. The alkaline hydrolysis mechanism is based on saponification of intermolecular ester bonds crosslinking xylan hemicelluloses and other components such as lignin [14]. The most commonly used alkaline hydrolysis is calcium or sodium hydroxide and ammonia.

#### 4.3.4.1 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 [14]. 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 [14].

#### 4.3.4.2 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 [65].

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 [14].

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 [14,54,65]. The ammonia pretreatment is not sustainable due to the high cost of ammonia and its recovery [20].

## 4.4 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 [14]. 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 [67].

## 4.5 Oxidative Delignification

During oxidative delignification, the biomass is treated with an oxidizing agents 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 [43].

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 [43].

#### 4.5.1 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% [68].

#### 4.5.2 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 [65].

#### 4.5.3 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 [62]. 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 [20]. This has been successfully applied for the treatment of wheat straw and hardwoods [20,21,62].

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 [52]. 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 [59].

#### 4.6 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 [21].

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 [62]. However, there is no industrial application employing the use of RTIL currently. Moreover, there is limited literature describing their actions with lignocellulose biomass [21]. 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 [62,69].

#### 4.7 Physico-chemical Pretreatment

The pretreatments that combine both chemical and physical processes are known as physicochemical processes. Example of physicochemical pretreatment include; steam explosion, steam explosion with addition of SO<sub>2</sub>, CO<sub>2</sub> explosion among others.

##### 4.7.1 Steam explosion (auto hydrolysis)

Steaming with or without explosion (auto hydrolysis) has received substantial attention in the pretreatment process of lignocellulosic biomass for bioethanol production. The process removes most of the hemicellulose, thus improving the enzymatic digestion. In steam explosion, the pressure is suddenly reduced and this makes the biomass undergo an explosive decompression at high pressure and high temperature, between 160 and 260°C, for a few seconds like 30 s to several minutes (e.g. 20 min) [44]. Previous studies have found out that the steam explosion process energy cost is relatively moderate, and satisfies all the requirements of the pretreatment process. Increase in temperature up to a certain level can effectively release hemicellulosic sugars. However, when the temperature is increased further, there is a steady sugar loss, resulting in a decrease in the total sugar recovery. For instance, when sunflower stalks is pretreated

with steam explosion before enzymatic hydrolysis at a temperature range of 180–230°C, the highest glucose yield of sunflower stalks is obtained at 220°C, while the highest hemicellulose recovery is obtained at 210°C of the pre-treatment temperature. Additionally, when steam explosion process is employed for the pretreatment of poplar (*Populus nigra*) biomass, at 210°C and 4 min, the cellulose recovery is above 95%, the enzymatic hydrolysis yielded about 60%, and xylose recovery is 41% in the liquid fraction [70]. Steam explosion has been employed in the production of ethanol from several lignocellulosic materials. The steam explosion extensively solubilizes the hemicellulosic sugars and decreases 75–90% of xylose content, depending on the substrate. The steam and mechanical treatments can be combined to effectively disrupt the cellulosic structure. Studies combining “thermal” pretreatment with the addition of bases such as NaOH other than individual thermal or chemical pretreatment yields better results [71–73]. Special care should, however, be taken in selecting the steam explosion conditions in order to avoid excessive degradation of the physical and chemical properties of the cellulose. In very harsh conditions, the lower enzymatic digestibility of lignocelluloses may also be observed after steam explosion [74].

#### 4.7.2 Steam explosion with addition of SO<sub>2</sub>

Steam explosion with addition of sulfur dioxide (SO<sub>2</sub>). The aim of adding SO<sub>2</sub> is to improve the recovery of both the cellulose and hemicellulose fractions. The treatment is accrued at 1-4% SO<sub>2</sub> (w/w substrate) at elevated temperatures, e.g. 160-230 °C, for a period of 10 min [72]. For instance, when willow is pretreated with steam with the addition of SO<sub>2</sub> or H<sub>2</sub>SO<sub>4</sub> the glucose yield is at 95% on the addition of 1% SO<sub>2</sub> at 200°C [75]. However, the yield of xylose recovery by SO<sub>2</sub> is not as high as with the pretreatment with dilute sulfuric acid.

#### 4.7.3 CO<sub>2</sub> explosion

Supercritical carbon dioxide is solvent extraction process, it displays gas-like mass transfer properties, besides a liquid-like solvating power. In the presence of water, supercritical CO<sub>2</sub> can efficiently improve the enzymatic digestibility of both hard and soft woods [64]. The delignification with carbon dioxide at high pressures can be improved by co-solvents such as ethanol–water or acetic acid– water, thus, efficiently increase

the lignin removal. The process possesses several advantages such as availability at relatively low cost, non-toxicity, nonflammability, easy recovery after extraction, and environmental acceptability [59].

The simultaneous pretreatment by CO<sub>2</sub> explosion and enzymatic hydrolysis in one step has been also considered. Studies shows that a glucose yield of 100% is obtained when supercritical CO<sub>2</sub> and enzymatic hydrolysis of cellulose is applied simultaneously [62]. The cellulase is sustained at pressures of up to 160 bar for 90 min at 50°C under supercritical carbon dioxide. Under supercritical conditions, the kinetic constants are increased in comparison to those under atmospheric conditions. In addition, the cellulase enzyme are stable in supercritical CO<sub>2</sub> at temperature of 35°C [44,76]. Treatment of biomass with carbon dioxide pressure, causes the disruption of the cellulosic structure thereby increasing the accessible surface area of the substrate for enzymatic hydrolysis. Under this process, the temperature is an important factor in the cellulosic hydrolysis [77,78]. The hydrolysis process can be carried out at either supercritical or subcritical temperature (respectively above and below 31.1°C). At subcritical temperatures, the carbon dioxide is less effective than at supercritical temperatures, as the subcritical carbon dioxide is likely to lowly diffuse in liquid carbon dioxide [16]. At supercritical temperatures, the carbon dioxide molecules finds it relatively hard to penetrate the pores in the cellulosic structures, to disrupt them when the carbon dioxide pressure is suddenly released. The higher pressure is desirable for faster penetration of the carbon dioxide molecules into the cellulosic pores that results in a higher glucose yield [77]. However, the supercritical CO<sub>2</sub> process might be too expensive for industrial application.

#### 4.8 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 [47]. Thus the starch and cellulose present in the lignocellulosic biomass are converted into glucose by the addition of cellulase enzymes [79]. Cellulase enzymes are specialized and includes, glycosyl hydrolases that catalyses the enzymatic hydrolysis of the 1, 4-β-glycosidic bonds of the lignocellulosic biomass [20].

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 [16].

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 [52].

#### 4.8.1 Factors affecting enzymatic hydrolysis

There are two major factors 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 [80]. 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 [32].

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 [14]. 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) [52].

Surfactants also affects the enzymatic hydrolysis [59]. 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 [20,58].

## 5. 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 [26]. While cellulose in a homopolymer made up of beta glucose, lignin and 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 [20].

The baker's yeast and *saccharomyces cerevisiae*, The bacterium *Zymomonas mobilis* and some genetically engineered microbes are the most commonly used fermenting microorganisms [80]. However, most of these microbes use face several obstacles like high cost, inability to ferment pentoses sugars, low tolerant to high sugar concentration, some can't work at extreme temperatures and high sugar concentration. Therefore to overcome these obstacles recent fermentation studies have been carried out using extremophiles. Some of the thermophilic microorganisms are *Clostridium acetobutylicum*, *C. thermo sulfurogenes*, *C. thermo saccharolyticum*, *C. thermohydrosulfurium*, *C. tetani*, *Kluyveromyces marxianus*, *Thermoanaerobacterium saccharolyticum*, *Aeromonas hydrophila*, *Thermoanaerobacter ethanolicus*, *Geobacillus* sp., *Erwinia* sp. [22].

Fermentation of hexose sugars (glucose, galactose, and mannose) are metabolized by glycolytic pathway, while, pentose sugars (xylose, arabinose) goes through pentose phosphate pathway (PPP) [22,81]. However, bacteria utilize isomerase pathway, for converting xylose to xylitol whereas yeast and fungi undergo reductase pathway where xylose is reduced to xylitol and subsequently oxidized to xylulose [2]. The intermediate products of PPP [glyceraldehyde-3-P (G3P) and fructose-6-P (F6P)] enter into the glycolytic pathway for the eventual production of ethanol [16]. Pyruvate is a key intermediate in the metabolism of sugars to ethanol. However, in case of thermophilic/thermotolerant microorganisms, there is formation of acetyl CoA by pyruvate ferridoxin oxidoreductase or pyruvate formate lyase, which is further reduced to acetaldehyde [81].

The pentoses cannot be fermented to ethanol by *Saccharomyces cerevisiae*, since xylose is the most dominant of the pentose sugars [80]. 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*, and *Clostridia* [82].

There are two distinct types of fermentation processes based on fermentative sugar types broken by microorganism, namely:

## 6. SIMULTANEOUS SACCHARIFICATION AND FERMENTATION (SSF)

This is the most commonly used fermentation process. It consists of two stages of enzymatic hydrolysis and ethanol fermentation in the same reactor and at the same time. The process has several advantages such as; lower investment costs in comparison to the single reactor, the sugars are fermented immediately after their production, the reduced risks of inhibition of enzymes by the glucose and cellobiose, as well as the reduced risks of contamination [65]. The major drawback of the SSF process is the enzymatic yield that is not maximal since the temperature used in this process is less than the optimum working temperature (<37°C) of the enzymes that destroy the yeasts [13]. This can be solved by the use of thermophilic yeasts and introducing substrate gradually. Gradual

introduction of substrate enabled obtaining a concentration of 83.40 g/L of ethanol using a substrate concentration loading of 25% (g/mL) [12]. Overall the process of SSF is considered to be better than the SHF process for both the yield and the ethanol concentration obtained.

## 7. SEPARATE HYDROLYSIS AND FERMENTATION (SHF)

In this process, hydrolysis and fermentation reactions are performed in two separate two-stroke reactors. The optimum temperatures for the two stages are different, it is 45–50°C for cellulase (hydrolysis) and 30–37°C for microorganisms that produce ethanol (fermentation). The process has the advantage of having optimum working conditions for hydrolysis and for fermentation. However, it has many drawbacks, like the inhibition of cellulase by cellulose and glucose. Studies have shown that increased glucose content in the hydrolysate leads to an increase in the degrees of inhibition of both  $\beta$ -glucosidase and cellulase. Moreover, the increase in the investment required for this process is made steep by the use of two reactors, although this second reactor is not always necessary and this by working in batch mode. However, the SHF process offers the possibility to recycle yeast fermentation which is not always possible with SSF processes.

## 8. DISTILLATION AND RECOVERY

The fermented biomass, referred to as wash, is filtered to remove solid waste then stored in a wash tank before distillation [83]. The ethanol recovery rate for different lignocellulosic biomass was reported by Mishra & Ghosh for various lignocellulosic biomass as follows: oil palm frond Maximum bioethanol concentration (18.2 g/L) and yield (57.0%), Pretreated wheat straw Sugar yield increased from 33 to 54% , Sugarcane bagasse, *Lantana camara* 87.2% lignin removal , 80.0% saccharification and ethanol yield of - 17.7 g/L of ethanol with corresponding yields of 0.48 g/g , Sugarcane bagasse highest energy efficiency (Steam Explosion Pretreatment + SSF + Dehydration) reaching 79.58%, Rice straw Maximum ethanol concentration was 25.1 g/L and - Yield of product/substrate (Yp/s) 0.4 g/g, Rapeseed straw Yp/s 0.29 g/g - Ethanol concentration 39.9 g/L representing 57.9% of theoretical ethanol yield, Kans grass + Wheat straw + Sugarcane bagasse Maximum (saccharification 84.88%, Yp/s 0.44 g/g - 82.45% of the maximum theoretical ethanol - Maximum

ethanol concentration: 67.28 g/L) [84]. These values demonstrate that lignocellulosic biomass is such great resource more so with relevant process steps.

Ethanol distillation and heat recovery system employs various types of heat exchangers; heat exchangers can be simple or more complex, with simplest design requiring one heat exchanger, which heats the wash up to the boiling point, so as to separate water and ethanol through a fractionating column [85]. More complex ones encompasses ethanol condenser, a wash preheater and a main heat exchanger which exploits the waste heat rejected from the power plant [83]. 95.7% ethanol is assumed to be the upper limit for a single distillation process, single heat exchanger design usually leads to a very low ethanol production rate, multi-heat exchanger system have been designed to achieve a higher ethanol production rate with increased energy recovery efficiency [80].

## 9. ECONOMIC ASSESSMENT

The feedstock and the capital costs are the main cost contributors. The feedstock and the capital cost constitutes between 23-28% and 40-49% of the total cost, respectively. The cost of utilities (process and cooling water) is negligible, since steam and electricity demands of the process are covered by on-site steam and power generation. Other factors that contributes to cost includes enzymes, chemicals, and utilities among others [86]. This study factored in all the production parameters and their costs to draw different scenarios. Enzymes cost is high also due to diverse enzymes used such as hexoses, pentoses, Hexoses, pentosans etc. However, the enzyme cost is based on assumptions made regarding the cost of commercial enzymes whose cost as per the literature values for enzyme cost vary considerably, leading to some literature concluding that enzymes cost is negligible in overall costs [87]. Improvement of commercial enzymes used in the processes has been ongoing o reduce overall enzymes cost [43,82]. It is worth acknowledging ha experimental costs tend to be higher than commercial scale production.

## 10. CONCLUSION AND FUTURE PROSPECTS

Lignocellulosic biomass offers the greatest potential to develop biofuels, slow down eliminate global warming and eliminate

dependence on fossil fuels. Lignocellulosic biomass for bioethanol is a promising path in the roadmap to the future world of renewable energy and sustainable energy supply [31]. In Kenya lignocellulosic ethanol industry is still in its infancy and its survival is relying on heavy policy support, increased research in interest and financial investment in the industry. Moreover, Kenya needs to develop her industries so as to increase the consumption of biofuels. In the meantime sugarcane industries growth is bound to lead to increased biomass for biofuel production.

Quick technology adoption and adaption is also bound to enable Kenya to make a leap in the industry. Overall, having a vibrant lignocellulosic ethanol industry calls for substantial improvement in the following areas: use of effective and low-cost biomass pretreatment methods, steps improvement of enzymes to enhance their tolerance to extreme conditions and inhibitors hence ensure enhanced fermentation of all sugars and reduced process steps. Genetic engineering of microorganisms holds a key to enzymatic and microorganisms improvement [22].

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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