



PRODUCTION OF BIOETHANOL FROM AGRICULTURAL WASTE

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ABSTRACT:

This chapter argues that because it emits fewer greenhouse gases and is produced using renewable resources, bioethanol has drawn the most attention relative to other fuels. It is mostly made from feedstocks that contain sugar. Feedstocks are used to make bioethanol, but they are also used to provide food for people. The globe faces a food crisis as a result of production. Agricultural waste is used to make bioethanol, which is the greatest choice is the one that is most plentiful globally. Lignin, cellulose, and hemicelluloses are present in agricultural wastes, which makes the conversion of these materials into ethanol more difficult. During the pretreatment process, lignin and hemicelluloses from agricultural waste are removed, and then the sugars are hydrolyzed enzymatically. Bioethanol is produced by fermenting the sugars pentose and hexose. The resistance to deterioration of the agricultural material is a significant obstacle to the development of a technological solution that is commercially viable. For ethanol production to be economically viable, it is possible to develop the utilisation of two or more delignification pretreatment techniques as well as agricultural biomass that has undergone genetic modification.

Key words: agriculture waste, pretreatment, fermentation, bioethanol, conventional.

INTRODUCTION:

The production of fuels, chemicals, materials, and power throughout the 20th century was mostly dependent on fossil fuels including petroleum, coal, and natural gas. The transportation and agricultural sectors are the two principal uses of fossil fuels. However, it also adds to global warming and environmental damage. The detrimental effects of fossil fuels on the environment, including the rise in global warming caused by greenhouse gas (GHG) emissions (such as CO, CO₂, CH₄, and NO₂), constant energy demand, reduction in the sources of energy supply, and the lack of a stable oil market, have drawn attention to the need for alternative fuels over the past few decades (Sarkar *et al.*, 2011). Due to the rapid growth in GHG emissions, other health conditions are also on the rise. Around 22% of all GHG emissions worldwide come from the transportation sector. The International Energy Agency (IEA) predicts a 92% increase in worldwide GHG emissions between 1990 and 2020. Additionally, it is anticipated that between 2020 and 2035, these GHG emissions in the atmosphere will total 8.6 billion metric tonnes. This increase in GHG emissions will raise the temperature by 2°C, which might result in the death of hundreds of millions of people around the world (Pimentel & Patzek, 2005).

Biofuels are an alternative energy source that can help us become less reliant on fossil fuels. Due to their potential to reduce GHG emissions by more than 80%, many nations have set their sights on developing biofuels (Walker, 2011). With the advancement of technology and scientific research, it is anticipated that these alternative fuels, which currently account for 2% of the entire transportation sector, will be promoted and used more widely in the near future. The use of alternative fuels has a number of advantages, including a decrease in environmental pollution and greenhouse gas emissions as well as a large supply of raw materials (Du *et al.*, 2016).

Following the challenges with the oil crisis, several nations have begun looking for a plentiful and affordable fuel. The development of alternative fuels has been made possible by extensive research activities and the search for renewable energy sources, both of which have drawn a lot of attention in the ongoing effort to address the energy supply problem and the environmental effects of the transportation and agricultural sectors. The main alternatives to non-renewable energy (such as petroleum, coal, or natural gas to manufacture fuels, chemicals, materials, and power), rising environmental pollution, and rising greenhouse gas emissions are renewable energy sources like biofuels. Vegetable oils, bio-oil, bio-char, biogas, and bio-synthetic gas are all types of biofuel, along with biomethanol, bioethanol, biohydrogen, and biodiesel (Ayadi *et al.*, 2016).

Due to the rapidly diminishing global petroleum supplies, ethanol is thought to be the most promising replacement fuel (Prasad *et al.*, 2007b). The most significant alcohol that can be made by turning starchy material into alcohol and creating carbon dioxide (CO₂) is ethanol (Duff & Murray, 1996). Enzymes made by bacteria and fungus catalyse the anaerobic fermentation process. This process converts the starch and sugar-containing components into fermentable sugars and ethanol by combining yeast and heat. The following equation shows how to turn glucose into ethanol:



The yeast cells ferment the sugar during fermentation, producing ethanol and carbon dioxide as a result (Demirbas, 2005).

DEMAND FOR ETHANOL:

In 2007, transportation sector had an energy consumption which was greater than in prior years. In general, bio-refineries should be situated near the supply source of raw materials, such as palm oil mills, or in a heavy industrial zone. It is crucial to construct the plant close to the plantation since oil palm waste accounts for 82 percent of lignocellulosic biomass. Heavy industrial areas are also appropriate as locations for the production of bioethanol. The outskirts of large cities are thought to have considerable energy demand. The distribution of storage facilities on grid squares should reflect this. Additionally, in order to position the storage facilities in a useful location, transportation considerations are also taken into account. A biorefinery is proposed to have a production capacity of 100 tonnes per day of bio-ethanol. If demand increases by 20–30% over the next ten years, 100 bio-refineries would be needed to meet the demand, and each grid square would need to have a storage facility to ensure that customers had access to bio-ethanol. (Alvo and Belkacemi, 1997).

CONVENTIONAL METHODS FOR ETHANOL PRODUCTION:

The two main categories of ethanol production are chemical and microbiological. These techniques are based on how ethanol is typically produced. The first chemical synthesis of ethanol from ethylene and fermentation of carbohydrates are the two methods of producing ethanol.

CHEMICALLY SYNTHESIZED ETHANOL:

Ethylene, a waste product of the oil industry, is hydrated to create chemically manufactured ethanol. This is a type of industrial alcohol with many uses. Qualities of matter. Absolute

alcohol, or pure ethanol, is a colourless liquid. Water, ether, acetone, benzene, and a few other organic solvents are all miscible with it in all quantities. Anhydrous alcohol is hygroscopic; a certain level of stability does develop with a water absorption of (0.3-0.4). (Ullmann, 1990b). 95.57 percent ethanol and 4.43 percent water make up the isotropic combination. Therefore, 95.57 percent ethanol by volume can be produced during regular distillation. A membrane method, tertiary solvent, molecular sieves, or other technique can be used to further remove water from an isotropic combination.

Chemical attributes. The functional OH group, which can undergo a variety of chemical processes including oxidation, esterification, dehydration, and halogenation, dominates the chemical makeup of ethanol (Ullmann, 1990b).

SYNTHETIC ETHANOL:

There are two main methods for industrial production of synthetic ethanol (Ullmann, 1990c):

1. Direct catalytic hydration of ethylene:



Numerous writers have researched the kinetics of reactions with phosphoric acid and various catalysts. Examples include H_3PO_4 and blue lead oxide in the presence of silica gel catalyst. Pressure, temperature, and the molar ratio of water to ethylene have all been adjusted according to a monogram. The molar ratio of ethylene to water in a reaction is 1:1 under normal conditions. Ethanol conversion achieves 7 percent and 22 percent at temperatures between 250 and 300°C and a pressure of 5e8 MP. Lower temperatures improve conversion efficiency (reaction 1), however a secondary reaction also produces diethyl ether in this case:

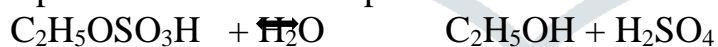


Ethylene is polymerized at increased pressure, producing higher olefins and butylenes. A variety of ethylene hydration catalysts are described in technical and patented literature. Zeolite and diatomite loams are used.

2. Indirect ethylene hydration, esterification hydrolysis or H_2SO_4 process, based absorption of large amounts of ethylene in concentrated sulfuric acid (Ullmann, 1990d). Using diluted H_2SO_4 , diethyl ether and ethanol are produced.



Hydrolysis is performed in three steps:



Ethanol and diethyl ether with a 5:10 ratio are products. Changing the reaction parameters, particularly the ethylene to sulfuric acid ratio, allows for the verification of ether yield. Methanol homologation, methanol and methyl acetate carboxylation, and syngas heterogeneous and homogeneous catalysis are further techniques used in industrial manufacturing. We won't go into more detail about synthetic processes in this chapter because we're concentrating on using biologically derived feedstock to make motor gasoline.

ETHANOL RECOVERY FROM FERMENTATION OF CARBOHYDRATES

First-generation ethanol is biochemically created from plant feedstock that contains a lot of carbs. Yeasts are used in fermentation. The most prevalent yeast species are extremely productive ones like *Candida utilis*, *Saccharomyces varoom* (formerly *Saccharomyces carlsbergensis*), and *Saccharomyces cerevisiae*. Additionally, *Schizosaccharomyces pombe* and *Saccharomyces anamnesis* species have been employed. Making ethanol from whey is possible when the species *Kluyveromyces* ferments lactose (Ullmann, 1990d).

Using yeast throughout the ethanol manufacturing process guarantees a high rate of fermentation and a high yield of ethanol. Low byproduct accumulation, good substrate concentration tolerance, and lower pH values define this process. Yeast cell viability assures that the procedure will occur.

Currently, the main method for producing bioethanol as a fuel involves fermenting plant sugars with yeast. Sugars, such as sucrose from sugar cane, sugar sorghum, or sugar beet, are classified as soluble carbohydrates. Storage carbohydrates include starch from grains and tubers, while structural carbohydrates include cellulose, hemicelluloses, and pectin. The main source of carbohydrates is starch, which is also found in other plant foods like potatoes, Jerusalem artichokes, and oats in addition to grains like corn, wheat, barley, and oats. There are two main types of starch: amylopectin and d-amylose (BeMiller and Whistler, 1996). A (1e4) glycosidic linkages hold the glucose molecules in amylose's straight-chain polymer together (Fig. 3.2). Long polymers wrap into a helical conformation as a result of this fundamental structure (BeMiller and Whistler, 1996).

Additionally, the main component of amylopectin is a straight chain of glucose molecules connected by a (1e4) glycosidic linkages. Additionally, it has branches made up of alpha (1e6) bonds that appear every 24e30 glucose units. When water molecules destroy the hydrogen bonds inside and between starch molecules during the gelatinization process, starch transforms from a semi crystalline to an amorphous state (a gel) at 60 to 70 degrees Celsius. It is simple to hydrolyze starch, particularly gelatinized starch, to produce the individual glucose molecules.

The following scheme describes how starch (polysaccharides) is hydrolyzed into sugars by enzymatic activity and fermented into ethanol by yeast enzymes.

$(C_6H_{10}O_5)_n$ / hydrolysis of starch / $C_6H_{12}O_6$ / sugar fermentation / $CH_3CH_2OH + CO_2$

Summary reaction: $C_{12}H_{22}O_{11}$ disaccharides / $2C_6H_{12}O_6$ / monosaccharides $4C_3H_3O_3$ pyruvate / $4C_2H_6O$ ethanol + $4CO_2$ carbon dioxide

BIOETHANOL – AN ALTERNATE SOLUTION TO CONVENTIONAL METHOD:

The most popular processes for producing bioethanol from lignocellulosic biomass include separate hydrolysis and fermentation (SHF), pre-saccharification followed by simultaneous saccharification and fermentation (PSSF), SSF, SSCF, and CBP.(Olofsson, K. *et al.* , 2008). And (Chiaramonti, D. *et al.* 2011). The treated lignocellulosic biomass is hydrolyzed enzymatically to yield glucose during SHF. Fermentation of sugar is then carried out to create bioethanol. The primary benefit of this procedure is that both of these steps are carried out at the ideal temperature for yeast and cellulose enzymes. But one of the main downsides is the production of glucose, which prevents the cellulase enzymes from working.(Rastogi, M. and ; Shrivastava, S. , 2017). In contrast, hemicellulose and cellulose hydrolysis occurs in separate vessels in the case of SHCF. Hexose and pentose are combined and fermented.

During the co-fermentation process of hexoses and pentoses, the microorganisms that may create a high percentage of ethanol are not available on a big scale.(Carrillo-Nieves, D.*et al.* , 2019). The rate of sugar buildup in the overall process is reduced by the creation of glucose using enzymes, which is then converted to ethanol with the aid of yeast, preventing sugar-induced cellulose inhibition. One of the most important downsides is that yeast and cellulase enzymes work best at different temperatures. Some altered strains, such *Zymomonas mobilis*, have been developed recently.(Kim, T.H. *et al.* , 2008). , recombinant *E. coli* (KO11).(Morales-Rodriguez, *et al.* 2011). *Saccharomyces cerevisiae* 1400 (pLNH33). (Taherzadeh, 2007 *et al.*) have been created to use a microbe to ferment both pentose and hexose. In the PSSF scheme, enzymes are added to pretreated biomass to start the saccharification process. After a few hours, glucose starts to develop. The solution is then combined with the fermentative bacteria to prevent the buildup of glucose and create ethanol. The viscosity in the

solution remains at a lower level throughout the initial hours of the operation, and the enzymes can carry out a reaction at their ideal working temperature. Enzymes can account for up to 22% of the overall cost to make bioethanol, which is a significant fraction (Carrillo-Nieves *et al.*)

Because all the necessary procedures may be completed in a reactor to make ethanol, consolidated bioprocessing (CBP) is regarded as the most environmentally friendly method. Through CBP, the cost and different interruptions connected with converting lignocellulosic biomass to biofuel production are reduced to a greater extent. (Carrillo-Nieves *et al.*). Various microorganisms ranging from bacteria. (Carrillo-Nieves *et al.* and Amoah *et al.*) Finding a microbe (bacteria or yeasts) capable of creating efficient enzymes that will convert hemicellulose and cellulose into sugars, removing lignin enzymatically to reduce chemical use, tolerating high inhibitory concentrations, and converting sugar to ethanol is the main goal of CBP. (Food and Agriculture Organization of United Nations).

CHARACTERISTICS OF BIOETHANOL (PHYSICOCHEMICAL):

Standard techniques were used to measure the physical and chemical properties of the sewage sludge. According to CEN/TS 14 774 methodology, the moisture content was measured, the ash content was measured, the volatile solids content was determined using CEN/TS 14 774 methodology, and the volatile solids were determined using CEN/TS 14 780 methodology. The final analysis for ash content, fixed carbon, and the carbon to nitrogen ratio was carried out in accordance with CEN/TS 15 104. An HI 9124 pH probe was used to determine the pH. Liquid lime was used to keep the pH consistent.

EXISTING SOURCES FOR PRODUCTION OF BIOETHANOL:

First generation bioethanol is defined as bioethanol made from natural sources, such as sugarcane, sugarbeet, corn, wheat, etc. Due to their straightforward structures, first-generation bioethanol does not require any pretreatment prior to fermentation. It is a less expensive technique of producing bioethanol (Sun & Cheng, 2002).

Sources for First Generation Bioethanol Production:

New and affordable carbohydrate sources are needed for the production of biofuels. For usage as renewable energy sources, biofuels derived from plants like corn, sugar cane, wheat, and soybeans are already available. Despite it using renewable plant materials for biofuels may appear advantageous, but doing so leads to a number of issues, such as food shortages and the depletion of essential soil resources (Balat *et al.*, 2009).

The three main categories of bioethanol feedstocks include sucrose-containing feedstocks (such as sugar beet, sweet sorghum, cane sugar, and fruits), starch materials (such as wheat, rice, potatoes, and corn), and lignocellulosic biomass (e.g. grasses and wood etc).

Sugar Containing Plant Crops:

1. Sugarcane:

Around the world, tropical and subtropical regions are used to grow sugarcane (*Saccharum* spp.). Due to its extraordinary ability to produce dry matter, it is a crucial crop not only for the manufacture of sugar but also increasingly for the generation of bioenergy. According to Dufey *et al.* (2006), sugarcane accounts for the majority of the world's bioethanol production, with the remainder coming from other crops such sugarbeet, sorghum, wheat, and rice. Brazil is the world's largest producer of sugarcane, which accounts for around 27% of global production and is used to make the majority of the nation's bioethanol. Starch from corn, wheat, and barley are used mostly in the US and Europe, respectively (Linde *et al.*, 2008).

2. Sweet Sorghum:

A perennial member of the Poaceae family, sweet sorghum (Ratnavathi *et al.*, 2010). Carbon emissions are decreased by sweet sorghum. In temperate, subtropical, and tropical areas, sorghum is grown. All parts of the plant have a purpose for money; the grain from sweet sorghum can be consumed as food, the leaves can be used as forage, the stalk can be burned together with the grain, and the cellulose fibre can be utilised as mulch or as animal feed. It takes 3-5 months for it to reach maturity, which is less time than sugarcane (10-12 months). Salinity tolerance is another aspect of it. The juice made from sorghum stalks has a significant potential for ethanol production (Figure 2). Sweet sorghum cultivars have high quantities of carbohydrates in the stalk (15-23 percent). There are three major sugars that make up all fermentable carbohydrates: sucrose (70 percent), glucose (20 percent), and fructose (10 percent) (Prasad *et al.*, 2007a).

3. Sugarbeet:

Beet molasses is the most commonly used sucrose-containing feedstock for bioethanol production in European nations. More bioethanol is produced from the sugar beet crop than from sweet sorghum or wheat. Shorter duration of crop production, high yield, excellent tolerance to a wide variety of climatic fluctuations (such as drought and flood, etc.), and low water and fertiliser requirements are all advantages of sugar beet (Balat *et al.*, 2009).

Starch containing plants crops:

Corn, wheat, and barley are a few examples of materials that contain starch and are used as biofuel feedstocks (Balat *et al.*, 2008). To achieve a high sugar concentration for biofuel generation, starch components are processed with acid and/or enzyme (Figure 3). D-glucose units make up the polymer that it is. Hydrolysis breaks fermentable sugars by breaking the starch down. To hydrolyze the polysaccharide chains and produce glucose syrup, which yeasts can turn into bioethanol, bioethanol synthesis from starch is necessary. The most popular feedstock for bioethanol synthesis that contains starch is corn, followed by wheat (Cardona *et al.*, 2007). Compared to other nations, the United States produced 14% more bioethanol made from corn.

TYPES OF AGRICULTURAL WASTE AND POTENTIAL FOR BIOETHANOL PRODUCTION:

Globally, the use of bioenergy to satisfy the rising need for energy is rising day by day. With a share of about 10% in worldwide primary energy consumption in 2014, bioenergy was significant. By 2050, this percentage is expected to rise to between 15% and 50%, according to the Renewable Global Status Report 2015. (Renewables 2015 Global Status Report). About 70% of people rely on biomass energy for daily household needs like heating and cooking, either directly or indirectly. The most common sources of biomass are municipal garbage, animal manure, and agricultural leftovers, with agricultural residues constituting the majority of biomass generation. Rice husk, rice straw, sugarcane bagasse, and jute sticks make up around 46% of total bioenergy. The majority of agricultural land is used by rice fields, which during the fiscal year 2010–2011 produced about 58.503 million tonnes of agricultural wastes. Around the nation, there are more than 100 rice mills. Additionally, rice left over from dining establishments has been used to create biogas. One experiment used leftover rice to create biogas that is 69 percent methane-enriched. (Rofiqul Islam *et al.*), (Baky, *et al.*) Rice husk is obtained after paddy processing, be used to generate heat while the boilers generate electricity. Approximately 9 million tonnes of rice husk was produced in 2011. (Food and Agriculture Organization of United Nations). The amount of surplus husk and the annual processing capacity was 455,366 tonnes and 3.62 million tonnes, respectively. Islam & Ahiduzzaman

(2013) estimated the electricity production from steam turbines and gasification plants using rice husk as a feedstock to be 41.45 and 29.05 MW, respectively (Islam, *et al.*) The production of biofuels from biomass is being considered positively. Renowned companies such as are becoming increasingly interested in the production of biofuels from ethanol molasses (Islam, *et al.*). Sugarcane bagasse is also a good resource to be considered for green energy production, because has many sugar mills installed at various locations in the country. (Huda *et al.*)

PRODUCTION OF BIOETHANOL FROM AGRICULTURAL WASTE

Sugarcane bagasse, sugarcane bark, corncob, cornhusk, and corn husk are among the agricultural waste products used in the production of ethanol from sugarcane (*Saccharum officinarum*) and maize (*Zea mays*) plants. Acid hydrolysis is used to pretreat the agricultural waste before exposing the simple sugars that yeast can use. (Das, H. and Singh, 2004). Without a pretreatment, lignin in plant cell walls forms a barrier against enzymatic attack, preventing lignocellulosic biomass from being saccharified by enzymes to higher yields. (Sheoran, *et al.*) Following this pretreatment process, *Saccharomyces cerevisiae* carried out a five-day alcoholic fermentation using the sugar content of the agro-waste as nutrients, ultimately converting the sugar to ethanol under anaerobic conditions. (Wyman, C.E 1996). Throughout the course of fermentation, the yeast goes through a number of physiological changes. At the beginning of fermentation, sterols and unsaturated fatty acids, which are essential nutrients for the yeast, begin to accumulate. As the fermentation develops, the yeast eats these nutrients and reduces the sugar content. The process of fermentation was deemed finished when practically all of the sugar supply had been transformed to ethanol. (Vereen, *ET al.* 2009). Based on their colour, bitterness, moisture content, and pH levels, the raw materials were evaluated prior to fermentation to see if they are ideal substrates for fermentation. Throughout the five-day fermentation period, the pH of the broth for each of the raw materials generally fell, with the ideal pH for maximal ethanol production falling between 3.6 and 4.0 at 72 hours of incubation (Nester, *ET al.* 2001). Were saccharification increased from pH 3.5 to a maximum of 4.0. The reduced pH favours *Saccharomyces cerevisiae* which converts the sugar present in the medium to ethanol (Braide, W. and Nwaoguikpe, R.N., 2011). Also offers an acidic environment that stops bacterial contamination during fermentation. The fermenting broth grew more acidic as the pH dropped, modifying the metabolic processes of the yeast to boost the generation of ethanol.

PRETREATMENT OF AGRICULTURAL WASTE

Separation and solubilisation of the components of agricultural commodities are steps in pretreatment procedures. Agriculture biomass mostly consists of cellulose and lignin. For the reasons listed below, pretreatment is crucial: In order to: (i) break down hemicelluloses and remove the lignin barrier from agricultural materials, (ii) disrupt the cellulose's crystalline structure, (iii) increase the substrate's available surface area and pore volume, (iv) prevent the loss of formed sugars, (v) prevent the formation of inhibitors that impede the fermentation process, (vi) reduce energy requirements, and (vii) lower costs. These modifications to agricultural biomass facilitate enzymatic hydrolysis, increase the amount of fermentable sugars (glucose, xylose, arabinose, mannose, and GA lactose), and will significantly affect the process as a whole. (Sun & Cheng, 2002; Yang & Wyman, 2008). The following requirements should be addressed by a pretreatment technique that is effective and affordable. Simple operation, minimum energy, chemical, and chemical-processing consumption, low equipment corrosion, excellent recovery of important hemicelluloses and lignin-derived products,

decreased formation of undesirable degradation products, and reduced waste creation are all benefits. (Taherzadeh & Karimi, 2008).

For the production of bioethanol from agricultural waste, four different pretreatment methods have been used: (1) physical (such as milling, grinding, pyrolysis, and irradiation); (2) chemical (such as alkali, dilute, concentrated acid, oxidising agents, and organic solvents); (3) physicochemical (such as steam explosion pretreatment, ozonolysis, liquid hot water, and wet oxidation); and (4) biological (fungi and bacteria) (Taherzadeh & Karimi, 2008).

Physical Methods

Agricultural waste can be physically pretreated using a variety of mechanical and non-mechanical techniques. Agriculture materials can be milled, ground, or chipped using mechanical processes. The agricultural waste is hydrolyzed using non-mechanical processes such as irradiation with gamma rays, electron beams, and microwaves. (Zheng *et al.*, 2009). Physical pretreatment has the advantages of not using chemicals, not producing fermentation inhibitors, and being environmentally benign. However, these techniques take a lot of time and demand both mechanical prowess and good abilities. (Hendriks *et al.*, 2009).

Mechanical Methods:

The combination of chipping, grinding, shearing, or milling used in this approach reduces particle size and increases surface area, making it easier for cellulases to attack the surface of the biomass and improves the conversion of cellulose to ethanol.

1. Milling

A form of mechanical preparation for agricultural biomass is milling. This pretreatment reduces the size of the agricultural material's particles and increases its surface area, making cellulose more accessible for enzymatic hydrolysis (Harmsen *et al.*, 2010). For the generation of bioethanol from agricultural biomass, a variety of milling techniques are utilised, including ball milling, hammer milling, two-roll milling, disc milling, and colloid milling. The size of the final particle varies on the type of physical treatment method, for example, the size of the particle obtained following chipping is up to 10-30 mm and the size obtained through milling is 0.2-2 mm, respectively (Sun & Cheng, 2002). The primary drawback of milling is the high energy required.

On sugarcane bagasse and straw, wet disc milling (WDM) and ball milling (BM) pretreatment were compared. It has been observed that BM reduces crystallinity, enhancing enzymatic hydrolysis, and WDM removes waste fibres, favouring enzymatic conversion (da Silva *et al.*, 2010). Corn straw underwent centrifugal roller mill pretreatment, which increased the surface area by 4.8 times, decreased the crystallinity of the cellulose, and increased the output of low molecular carbohydrates by 2 times (Bychkov *et al.*, 2014).

2. Pyrolysis or Thermal Cracking

Waste is processed in this process at a high temperature of 300°C. It degrades cellulose and releases gaseous byproducts such residual char, carbon monoxide gas, and hydrogen. The cellulose degradation is slower and less volatile chemicals are formed at lower temperatures (300°C) (Prasad *et al.*, 2007b; Mtui, 2009). However, because it uses high temperatures, the pyrolysis pretreatment approach is more expensive.

In a study, pyrolysis transformed 80 weight percent of the dry lignin in wheat straw into 30-40 percent biochar and 40-60 percent bio-oil (Wild *et al.*, 2012).

Non Mechanical Methods

Irradiation Pretreatment

In order to improve the enzymatic hydrolysis of agricultural waste, this pretreatment uses high energy radiation, such as x-rays (Chunping *et al.*, 2008), ultrasounds (Rehman *et al.*, 2013), electron beams (Bak *et al.*, 2009; Henniges *et al.*, 2013), as well as microwave heating (Keshwani *et al.*, 2007; Ma *et al.*, 2009). These intense radiations also alter the structure of

lignin, decrease the crystallinity of cellulose, increase the specific surface area, and hydrolyze hemicellulose. However, using these techniques on a big scale is expensive (Taherzadeh & Karimi, 2008).

The most popular processing techniques for agricultural waste is microwave irradiation. Its benefits include (i) straightforward operation, (ii) low energy requirements, (iii) low levels of inhibitor production, and (iv) degradation or disruption of cellulose's structural integrity (Kumar & Sharma, 2017).

At varying lime concentrations (0, 0.1, 0.15, and 0.2 g/g sorghum bagasse), water content of 10 or 20 ml/g sorghum bagasse, and exposure periods of 2, 4, and 6 min, the pretreatment of sweet sorghum bagasse using microwave radiation was investigated. A sugar yield of 32.2g/100g sorghum bagasse was obtained under ideal circumstances of 0.1 g lime and 10 ml water/g sorghum bagasse in 4 min (Choudhary *et al.*, 2012).

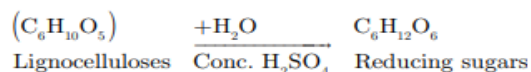
Chemical Methods

Different chemical agents, such as oxidising agents (hydrogen peroxide and ozone), acids (H₂O₂), and other substances are used in chemical pretreatment.

Alkalis (NaOH, Na₂HPO₄), organic acids (SO₄, HCl, etc. CO₃, Ca(OH)₂ & NH₃ organic solvents, etc.), SO₂, CO₂ & additional substances that remove the hemicellulose from the cellulose lignin derived from lignocellulosic waste (Nwosu-Obieogu *et al.*, 2016).

Acid Pretreatment

A technique called acid pretreatment involves treating agricultural waste with weak or strong acids (e.g. sulphuric acid, hydrochloric acid, peracetic acid, or nitric acid). By hydrolyzing polysaccharides, particularly hemicelluloses, to monosaccharides, this technique makes cellulose more accessible for enzymatic hydrolysis. It can be carried out either with low acid concentration and high temperature or with high acid concentration and low temperature (Taherzadeh & Karimi, 2008).



Many agricultural wastes have been subjected to acid pretreatment. For instance, pretreatment with (0.5–4%) H₂ SO₄ was applied to sorghum. After pretreatment and enzymatic hydrolysis, 0.408g reducing sugars are created (Akansha *et al.*, 2014). Another study found that pretreating durian seed waste with 0.6 percent sulphuric acid and then enzymatic hydrolysis produced 50.0944g/L of glucose concentration. The drawback of utilising acid is that it corrodes machinery and produces inhibitors that prevent fermentation, such as furans, carboxylic acids, and phenolic compounds (Singh & Trivedi, 2013).

Alkaline Pretreatment

Agricultural waste can be processed using bases like sodium, potassium, calcium, and ammonium hydroxide during an alkaline pretreatment procedure. According to Singh *et al.* (2013), alkali pretreatment (i) causes the ester linkages between lignin, hemicellulose, and cellulose to dissolve; ii) disrupts the structure of lignin; iii) slows down the crystallisation of cellulose; and iv) increases the accessibility of enzymes to cellulose. Alkali technique provides various benefits, including low temperature and pressure requirements, removal of lignin and hemicelluloses, and increased accessible surface area. Long residence times and the need for a high alkali concentration are two of this method's limitations. Ca(OH)₂ pretreatment is preferred over NaOH because it is more affordable, safer, and can be easily removed from the hydrolysate by reaction with CO₂ as compared to NaOH (Mosier *et al.*, 2005b). Alkaline pretreatment has been used in a variety of research. Enzymatic hydrolysis was performed after

pretreatment with 0.18 g of NaOH and 0.06 g of lime on wheat straw. Enzymatic hydrolysis of cellulose resulted in a 62.5 percent delignification and a 93.1 percent conversion of cellulose to glucose. From the cellulose and hemicellulose of wheat straw, a yield of monosaccharide sugars (glucose + xylose and arabinose) totaling 80.3 1.2 percent was attained (Jaisamut *et al.*, 2013).

4 percent (w/v) NaOH was used to pre-treat coffee pulp for 25 minutes. After NaOH treatment, delignification was accomplished to a 25.19 percent level, and 38.13 g/L of reducing sugars were generated (Menezes *et al.*, 2013).

Organosolv Pretreatment

This procedure involves pretreating agricultural waste at temperatures between 150 and 200 °C using organic or aqueous solvents like methanol, ethanol, acetone, ethyleneglycol, triethylene glycol, and tetrahydrofurfuryl alcohol. The use of catalysts such oxalic, salicylic, acetylsalicylic, hydrochloric, and sulfuric acid is optional for this pretreatment (Taherzadeh & Karimi, 2008). The lignin and hemicellulose linkages are broken by these solvents (Joshi *et al.*, 2011). For the purpose of pretreating wheat straw with organosolvs, five solvents—ethanol, methanol, diethylene glycol, acetone, and butanol—were investigated. Sulphuric acid was used to catalyse the biorefinery process for 20 min at 160 °C. The most lignin was removed by butanol among these solvents (Sdiras *et al.*, 2015). Utilizing ionic liquids and solvents, such as formic acid (formalin process), concentrated phosphoric acid (CPA), N-methylmorpholine-N-oxide (NMMO), and 1-allyl-3-methylimidazolium chloride, wheat straw was successfully pretreated ([AMIM]Cl). The most effective treatment was CPA after treatment. The most effective of these solvents, CPA, achieved an ethanol concentration of 41.6g/L with a yield of 91.2 percent (Li *et al.*, 2017). Due to the straightforward distillation procedure used in this treatment, organic solvents can be recovered and recycled (Harmsen *et al.*, 2010). The creation of harmful inhibitors, corrosion due to the need for organic acids, and the high cost of the process due to the usage of high pressure and temperature are the drawbacks (Bensah & Mensah, 2013).

Ozonolysis Pretreatment

Ozonolysis is the term used to describe the process of using ozone to detoxify agricultural waste. A potent oxidant that is readily available, soluble in water, and ozone. This pretreatment effectively breaks down lignin and improves the digestibility of the biomass. Ozone significantly degrades hemicellulose, although cellulose is unaffected (Travaini *et al.*, 2016). In feedstocks such bagasse (Eqra *et al.*, 2014), maize stover (Li *et al.*, 2015), wheat, rye straw (Garcia-Cubero *et al.*, 2009; Aljibouri *et al.*, 2015), and poplar sawdust, lignin has been degraded using ozone (Vidal & Molinier, 1988). The benefits of ozone pretreatment include the absence of hazardous inhibitors and the ability to conduct reactions at room temperature and pressure (Vidal & Molinier, 1988). Costly because of the huge amount of ozone that is required. As a result, at the industry level, this method is inefficient. (Kumar *et al.*, 2009).

Wet Oxidation Pretreatment

Agricultural waste is treated with oxygen-containing water or air at a high temperature (120°C for 30 minutes) (Varga *et al.*, 2003). Because the efficacy of delignification declines as lignin amount increases, this procedure is only utilised for waste with low lignin content. Several agricultural feedstocks, including rape straw (Arvaniti *et al.*, 2012), sugarcane bagasse, rice hulls, cassava stalks, and peanut shells, have undergone wet oxidation pretreatment (Banerjee *et al.*, 2012; Martin *et al.*, 2007). The following benefits of this process include: (i) hemicellulose and lignin degradation at lower temperatures; (ii) no chemical recovery is necessary; and (iii) increased enzymatic hydrolysis efficiency. However, the cost of this procedure is significant because it requires chemicals and high pressure oxygen (Biswas *et al.*, 2015).

Physicochemical Methods

This category includes a mix of physical and chemical pretreatment methods.

Pretreatment with Steam Explosion (Autohydrolysis)

One of the most common methods for pretreatment of agricultural waste is steam pretreatment. This pretreatment employs hot steam (180 to 240°C) under pressure (1 to 3.5 MPa), and the pressure is rapidly reduced, resulting in the explosive decomposition of agricultural biomass (Sun & Cheng, 2002). This method was previously known as "steam explosion" because it is based on explosive action on cellulose fibres, which allows the materials to be hydrolyzed (Mosier *et al.*, 2005). This method has been used successfully to remove cellulose fibres from meadow hay (Tutt *et al.*, 2014).

One of the benefits of steam explosion pretreatment is that it requires less energy (Sun & Cheng, 2002; Kumar *et al.*, 2009). Steam explosion has limitations such as incomplete lignin-carbohydrate matrix cleavage, destruction of a portion of the xylan fraction, and the generation of inhibitor products (e.g., furaldehyde, 5-hydroxymethyl-2-furaldehyde, and phenolic compounds) that have the potential to inhibit enzymatic hydrolysis and fermentation (Garcia-Aparicio *et al.*, 2006).

Pretreatment with Liquid Hot Water (LHW)

In this method, hot liquid water with a pressure greater than the saturation point is used to hydrolyze hemicelluloses in agricultural waste. The main advantage of this process is its high xylose recovery and lack of the use of chemicals or catalysts, making it environmentally friendly (Hamelinck *et al.*, 2005). The production of inhibitors that inhibit the growth of microorganisms during the fermentation process is one of LHW's limitations (Taherzadeh & Karimi, 2008). LHW has been successfully used to pre-treat rye straw, rice straw, corn stover, and sugarcane bagasse for hemicellulose hydrolysis (Ingram *et al.*, 2009; Imman *et al.*, 2012; Li *et al.*, 2014; Gurgel *et al.*, 2014).

Explosion of Ammonia Fibres (AFEX)

It is based on the steam explosion process and the use of liquid ammonia at medium temperature (60-120°C) and high pressure (1.72-2.06 MPa) for several minutes (>30 minutes) (Kumar *et al.*, 2009). The rapid release of ammonia gas disrupts the lignin-carbohydrate complex, increasing the enzymatic digestibility of agricultural waste (Chundawat *et al.*, 2007). AFEX has been used for corn stover pretreatment. The metabolic yield and ethanol production were increased as a result of this treatment (Lau & Dale, 2009). It's also been used to pre-treat sugarcane bagasse and cane leaf residue. The AFEX pretreatment made hemicellulose and cellulose more accessible to enzyme hydrolysis (Krishnan *et al.*, 2010). The AFEX method has the following advantages: (i) no inhibitors such as furans are produced; and (ii) high biomass depolymerisation is achieved. , (iii) less expensive and less energy required (Chundawat *et al.*, 2007; Sanchez, 2009).

Biological Pretreatment

Agricultural waste can be treated using either lignin-degrading microorganisms or enzymes (Lee, 1997). Brown-rot fungi have been discovered to attack cellulose. Ray *et al.* (2010) White-rot and soft-rot fungi, on the other hand, primarily degrade the lignin, cellulose, and hemicellulose components of agricultural waste while also enhancing enzymatic hydrolysis of waste. Endoglucanases, cellobiohydrolases (exoglucanases), and -glucosidases are extracellular cellulolytic enzymes produced by fungi such as *Trichoderma reesei* and *Aspergillus niger* that efficiently degrade cellulose residues. Basidiomycetes (for example, *Phanerochaete chrysosporium*) degrade agricultural waste (Dashtban *et al.*, 2009; Sanchez, 2009). Biological lignin removal in pulp has been studied using white rot fungi and *Streptomyces*. Lignin loss was observed in 10.5 percent of soft wood and 23.5 percent of hard wood (Saritha *et al.*, 2012).

Several studies on biological treatment of agricultural waste using enzymes produced by microorganisms have been conducted. Sawdust treated with cellulose enzyme derived from *Trichoderma/Hypocrea* achieved 85.6 percent delignification (55.2 g/L) (Saravana kumar & Kathiresan, 2014). To remove lignin, rice straw was biologically pretreated for 44 days with white rot fungi, *Pleurotus ostreatus*.

In 24 days, this pretreatment degraded 27.85 percent of the lignin and 24.30 percent of the cellulose (Balasubramaniam & Rajarathinam, 2013).

The benefits of this pretreatment include the absence of chemicals, the use of less energy, high specificity to lignin, mild operating conditions, no inhibitor generation, and environmental friendliness (Sanchez, 2009). The main disadvantage is the requirement for a long residence time due to fungi's lower hydrolysis rate (Sun & Cheng, 2002).

FERMENTATION PROCESS FOR PRODUCTION OF BIOETHANOL

The lack of microorganisms capable of efficiently fermenting (with high yield and rate) all sugars (both pentoses and hexoses) released during pretreatment and hydrolysis is a significant barrier to industrial utilisation of lignocelluloses for bioethanol production. In terms of commercial ethanol production, the ideal microorganism should have broad substrate utilisation, high ethanol yield and productivity, tolerance to inhibitors found in hydrolyzates and high ethanol concentrations, cellulolytic activity, and the ability to ferment sugar at high temperatures (Hahn-Hagerdal et al., 2007). The yeast *Saccharomyces cerevisiae* and the bacterium *Zymomonas mobilis* are the best known microorganisms for ethanol production from hexoses (Claassen et al., 1999), providing high ethanol yields (90-97 percent theoretical) and ethanol tolerance up to about 10% (w/v) in fermentation medium. Recently, a native *S. cerevisiae* strain yielded 99 percent ethanol based on initial glucose concentration (Jorgensen, 2009).

The inability of native strains of *S. cerevisiae* and *Z. mobilis* to utilise xylose, the main C5 sugar derived from hemicellulose, is the main disadvantage (Rogers et al., 2007; Talebnia and Taherzadeh, 2006). Other microorganisms known to ferment xylose to ethanol, including enteric bacteria and the yeasts *Pichia stipitis*, *Candida shehatae*, and *Pachysolen tannophilus* (Chandel et al., 2007; Lin and Tanaka, 2006), have low ethanol yields and a proclivity to re-assimilate the produced ethanol (Karakashev et al., 2007). To overcome this limitation, genetically modified strains of *S. cerevisiae* capable of fermenting both hexoses and pentoses have been developed (Karhumaa et al., 2007). However, those strains demonstrated low productivity in the conversion of xylose to ethanol (Watanabe et al., 2007). Furthermore, there is a practical disadvantage to widespread use of recombinant ethanologens because plasmids containing xylose conversion genes are frequently rejected by the host (Krishnan et al., 2000), indicating the possibility of isolating new species/strains from appropriate sources. Under extreme thermophilic conditions, a mixed bacterial culture dominated by *Thermoanaerobacter*, *Thermoanaerobacterium*, and *Caldanaerobacter* was recently found to produce ethanol and hydrogen simultaneously from glucose (Zhao et al., 2009).

Ethanol fermentation of wheat straw hydrolyzates as feedstock has been extensively studied using various microorganisms such as yeasts, bacteria, and fungi, which are typically grown as pure cultures.

The most widely studied yeasts for ethanol fermentation using wheat straw hydrolyzates as feedstock were *P. stipitis* (Nigam, 2001), *Kluyveromyces marxianus* (Tomas-Pejo et al., 2009), native (Jorgensen, 2009), and recombinant strains of *S. cerevisiae* (Panagiotou and Olsson, 2007). So far, the native non-adapted *S. cerevisiae* has produced the best results in terms of ethanol yield, final ethanol concentration, and volumetric ethanol productivity. *Pichia* sp. ethanol yields have reached up to 0.42 (g/g), but the average volumetric ethanol

productivity was roughly half that of *Kluyveromyces* sp. Zayed and Meyer demonstrated a high-yielding single-batch fermentation method (86 percent ethanol yield) using two phylogenetically distinct microorganisms (1996) The fungus *Trichoderma viride* hydrolyzed delignified wheat straw to simple sugars in this study, followed by yeast *Pachysolen tannophilus* aerotolerant fermentation of the resulting xylose and glucose to ethanol. In terms of thermophiles, ethanol productivity obtained with *Thermoanaerobacter* sp. grown on pretreated wheat straw (Georgieva et al., 2008) was significantly lower than that obtained with yeasts and fungi. We recently isolated an ethanologen from *Thermoanaerobacter* that produces primarily ethanol from pentoses with a 70% ethanol yield. Acetate and hydrogen were the only byproducts other than ethanol (unpublished results). Recombinant bacteria with by-product-producing gene deletions have also been considered for ethanol production. A recombinant bacterium (*E. coli* strain FBR5) was tested for ethanol production from pretreated wheat straw and demonstrated the highest ethanol yield among the bacteria studied for bioconversion of wheat straw derived feedstocks (Saha and Cotta, 2006). The reported ethanol concentration and volumetric productivity were comparable to the ethanol concentration obtained with yeast *P. stipitis* and productivity obtained with yeast *K. marxianus*. Efforts to research ethanol fermentation are still ongoing. Finding new wild-type ethanologens or developing new promising genetically modified organisms (GMOs) with higher ethanol tolerance, productivity, and yield will lay the groundwork for future commercial lignocellulose-based bioethanol production from wheat straw.

Methods for enzymatic hydrolysis and fermentation

Different strategies for enzymatic hydrolysis and fermentation can be used: separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF), and direct microbial conversion (DMC). Hydrolysis and fermentation are carried out in separate vessels under their own optimal conditions in SHF; however, this process is associated with end-product inhibition of enzyme activity and contamination issues. To address the shortcomings of the SHF process, SSF, which combines hydrolysis and fermentation in a single vessel, was developed. Sugars produced during hydrolysis are immediately fermented into ethanol, avoiding problems such as sugar accumulation, enzyme inhibition, and contamination (Galbe and Zacchi, 2002; Ohgren *et al.*, 2007). Another advantage of SSF over SHF is the cost savings from using only one reactor. The main disadvantage of SSF is that the optimum temperatures for the hydrolysis and fermentation processes differ. Most fermenting yeasts thrive at temperatures around 30-35 degrees Celsius, while hydrolyzing enzymes thrive at temperatures around 50 degrees Celsius (Kadar *et al.*, 2004). Saha et al. (2005) used recombinant *E. coli* to evaluate the performance of both SSF and SHF on dilute acid pretreated wheat straw (FBR5). The SHF method performed better than the SSF method, yielding more ethanol in less time. Detoxification using the overliming method significantly reduced SHF fermentation time and increased ethanol yield in SSF. With the same strain (*E. coli*) and using alkaline peroxide pretreated wheat straw, the SHF approach performed better than the SSF approach (Saha and Cotta, 2006). However, the time required for separate enzymatic hydrolysis in the SHF approach was not considered. When total time (separate hydrolysis + fermentation) in SHF is considered, the SSF approach appears to be far more advantageous and effective than SHF. Alfani et al. (2000) estimated SHF and SSF processes for steam-exploded wheat straw bioconversion. The authors reported that the time required to complete SSF and SHF was 30 and 96 hours, respectively (72 h hydrolysis + 24 h fermentation). respectively. In contrast to the significantly faster ethanol productivity in SSF, the final ethanol yield in SHF was higher (81 percent of theoretical versus 68 percent in SSF). In DMC, no external enzyme is used, and the hydrolytic process is mediated by microorganism-produced enzymes (Demirbas, 2005). To our knowledge, the DMC of alkali-

pretreated wheat straw to ethanol has only been studied with the mesophilic fungus *Fusarium oxysporum* F3, but yield and volumetric productivity have been reported to be relatively low (Christakopoulos et al., 1991).

CHALLENGES FOR BIOETHANOL PRODUCTION

Feedstocks for Bioethanol Production.

The availability of crops as feedstocks for bioethanol production complicates the fuel versus food debate. Crops are a major source of food for the human population in SSA. In Zambia, for example, 95 percent of maize grown is consumed as a staple food. (E. Chakauya, et al.2009). If maize is used as a fuel crop, as it is in the United States, food prices will rise significantly. Sugar cane and bagasse are used to generate electricity and heat in Mauritius. As a result, in addition to threatening the food supply, if sugar cane is used for bioethanol, the electricity supply will also be adversely affected (S. Karekez ,2009). Furthermore, the demand for food in SSA for basic domestic needs is increasing, and 33 percent of crops had to be imported in 2000 to meet human food consumption without using crops as a source of biofuel (B. Amigun, et al.2011).

Land Availability and Development.

The dilemma of land availability, similar to the fuel versus food debate, is whether to use the majority of the land for food crop or bioethanol crop cultivation. Smallholder farmers own agricultural land in Tanzania, for example, and farming is their way of life . If their land is converted for bioethanol crop production, the question of whether these farmers will benefit economically from bioethanol crops and whether there will be enough food to feed the growing population arises. In Burkina Faso, women typically use limited cropland for household needs and medicinal purposes . Large-scale bioethanol production necessitates the cultivation of biofuel crops on a large scale. This may result in the use of limited crop land and, as a result, a reduction in the amount of land available to African women for basic subsistence . Women perform 75 percent of agricultural work in Cameroon, but own only 10 percent of the land. The use of this land for bioethanol crops will severely disadvantage the female owners, who will be unable to obtain bank credit for bioethanol crop production . Furthermore, increasing agricultural land for biofuel crop production will result in significant deforestation, biodiversity loss, and erosion of organic soil matter. (B. Amigun, et al.2011).

Government Policies.

SSA is primarily comprised of impoverished rural areas with limited production markets, severe poverty, high child mortality rates, high HIV infection rates, women's marginalisation, and extreme reliance on agriculture for food and livelihood . These situations' vulnerability overshadows government policies and full support for sustainable bioethanol development. Bioethanol policies have been proposed in Sub-Saharan African countries including Mozambique, Nigeria, Uganda, South Africa, Malawi, and Ghana . The Mozambican government has adopted policies of blending 5-10% (v/v) bioethanol with gasoline, whereas the South African government estimates that 400 million litres of biofuel will be produced by 2013 . However, these policies are characterised by ambiguous language and a lack of financial commitments, coordinated research, and educational training to acquire necessary skills . Furthermore, because these policies prohibit international trade and investment, bioethanol production in SSA cannot be recognised on a global scale, and financial responsibility falls primarily on SSA national and regional governments. In Tanzania, the government was forced to halt any bioethanol production that could endangered food and land security, fearing farmer and environmental unrest . In order for bioethanol policies to be acceptable, rural lifestyles must be considered. As a result, Sub-Saharan governments would

prefer to fund current policies rather than implement fuel development policies. (B. Amigun,*et al.*2011),and. (C. B. L. Jumbe,*et al.* 2009).

FUTURE OF AGRICULTURAL WASTE FOR BIOETHANOL PRODUCTION

Due to the abundance and low cost of agricultural materials, the conversion of agricultural waste to bioethanol has great potential. Due to the complex structure and resistance to degradation of agricultural materials, there are still numerous challenges in developing an economically viable technology (Sun & Cheng, 2002). A single pretreatment method is insufficient for complete biomass delignification. For efficient hydrolysis, combined pretreatment methods (e.g., physical with chemical, chemical with biological) should be used (Kumar & Sharma, 2017). Components of agricultural biomass are resistant to degradation. As a result, genetically modified agricultural biomass can be used to produce ethanol. Genetic engineering produces plants with new genes that produce biomass with altered composition. Genetically modified agricultural biomass has the potential to increase ethanol yield while lowering the cost of enzyme hydrolysis (Wang & Zhu, 2010).

CONCLUSION

The society consumes fossil energy sources such as coal, gasoline, and so on to produce fuels and chemicals. These fossil fuels emit GHGs, which contribute to global warming and pollution. As a result, there is a need to find an environmentally friendly and less expensive biofuel. As an alternative fuel, bioethanol is the best option. It produces less greenhouse gases and is made from renewable resources. Bioethanol is primarily produced from feedstock such as sugarcane, sugar beet, rice, and corn grain, but these feedstock are in short supply. If these feedstock are used for bioethanol production, this limitation will result in a food crisis. Agriculture waste is a cheap and plentiful source of biomass for bioethanol production.

Various pre-treatments (physical, chemical, physico-chemical, and biological) can be used to convert agricultural waste into bioethanol. Biological pre-treatments should be used because they are safer, more environmentally friendly, and use less energy than other pre-treatments. Enzymatic hydrolysis of pre-treated biomass is followed by fermentation with microbes such as bacteria or yeast. Bioethanol production from agricultural waste will help reduce greenhouse gas emissions and pollution while also serving as a sustainable solid waste management strategy.

REFERENCE

1. Ahiduzzaman, M. Sustainability of biomass energy development technology (densification) in Bangladesh. *Int. J. Bio. Res.* 2006, 1, 40–46. 59. Huda, A.S.N.; Mekhilef, S.; Ahsan, A. Biomass energy in Bangladesh: Current status and prospects. *Renew. Sustain. Energy Rev.* 2014, 30, 504–517. [CrossRef]
2. Ahiduzzaman, M.; Islam, A.K.M.S. Greenhouse gas emission and renewable energy sources for sustainable development in Bangladesh. *Renew. Sustain. Energy Rev.* 2011, 15, 4659–4666. [CrossRef]
3. Akansha, K., Prasad, A., Sukumaran, R. K., Nampoothiri, K. M., & Pandey, A. (2014). Dilute acid pretreatment and enzymatic hydrolysis of sorghum biomass for sugar

- recovery: A statistical approach. *Indian Journal of Experimental Biology*, 52, 1082–1089. PMID:25434103
4. Alfani, F., Gallifuoco, A., Saporosi, A., Spera, A., Cantarella, M., 2000. Comparison of SHF and SSF processes for the bioconversion of steam-exploded wheat straw. *Journal of Industrial Microbiology & Biotechnology* 25, 184–192.
 5. Aljibouri, A. K., Turcotte, G., Wu, J., & Cheng, C. (2015). Ozone pretreatment of humid wheat straw for biofuel production. *Energy Science & Engineering*, 3(6), 541–548. doi:10.1002/ese3.93
 6. Alvo, P., Belkacemi, K., 1997. Enzymatic saccharification of milled timothy (*Phleum pratense* L.) and alfalfa (*Medicago sativa* L.). *Bioresource Technology* 61 (3), 185–198.
 7. Arvaniti, E., Bjerre, A. B., & Schmidt, J. E. (2012). Wet oxidation pretreatment of rape straw for ethanol production. *Biomass and Bioenergy*, 39, 94–105. doi:10.1016/j.biombioe.2011.12.040
 8. Ayadi, M., Sarma, S. J., Pachapur, V. L., Brar, S. K., & Cheikh, R. B. (2016). History and Global Policy of Biofuels. In C.R. Soccol, S.K. Brar, C. Faulds et al. (Eds.), *Green Fuels Technology*. Switzerland: Springer International Publishing. doi:10.1007/978-3-319-30205-8_1
 9. B. Amigun, J. K. Musango, and W. Stafford, “Biofuels and sustainability in Africa,” *Renewable and Sustainable Energy Reviews*, vol. 15, no. 2, pp. 1360–1372, 2011.
 10. B. Amigun, J. K. Musango, and W. Stafford, “Biofuels and sustainability in Africa,” *Renewable and Sustainable Energy Reviews*, vol. 15, no. 2, pp. 1360–1372, 2011.
 11. Baky, M.A.H.; Hassan Khan, M.N.; Kader, M.F.; Chowdhury, H.A. Production of Biogas by Anaerobic Digestion of Food Waste and Process Simulation. In Proceedings of the ASME 2014 8th International Conference on Energy Sustainability collocated with the ASME 2014 12th International Conference on Fuel Cell Science, Engineering and Technology, Boston, MA, USA, 30 June 2014.
 12. Balasubramaniam, M. K., & Rajarathinam, R. (2013). Implementation of white rot fungal pretreated rice straw for sustainable bioethanol production by *Saccharomyces cerevisiae*. *International Journal of Engineering Research & Technology*, 2(11), 4047–4053
 13. Balat, M., Balat, H., & Cahide, O. Z. (2008). Progress in bioethanol processing. *Progress in Energy & Combustion*, 34(5), 551–573. doi:10.1016/j.pecs.2007.11.001

14. Banerjee, S., Sen, R., Morone, A., Chakrabarti, T., Pandey, R. & Mudliar, S. (2012). Improved wet air oxidation pretreatment for enhanced enzymatic hydrolysis of rice husk for bioethanol production. *Dynamic biochemistry, process biotechnology and molecular biology*, 6, 43-45.
15. BeMiller, J.N., Whistler, R.L., 1996. *Carbohydrates. Food Chemistry*, third ed. Marcel Dekker,
16. Bensah, E. C., & Mensah, M. (2013). Chemical Pretreatment methods for the production of cellulosic ethanol: Technologies and innovations. *International Journal of Chemical Engineering*, 2013, 1–21. doi:10.1155/2013/719607
17. Biswas, R., Uellendahl, H., & Ahring, B. K. (2015). Wet Explosion: A universal and efficient pretreatment process for lignocellulosic biorefineries. *BioEnergy Research*, 8(3), 1101–1116. doi:10.1007/12155-015-9590-5
18. Braide, W. and Nwaoguikpe, R.N. Ethanol Production from cocoyam *International journal of plant Physiology and biochemistry.*, 2011, 3(3), 64-66.
19. Bychkov, A. L., Buchtovarov, V. A., & Lomovsky, O. L. (2014). Mechanical pretreatment of corn straw in a centrifugal roller mill. *Cellulose Chemistry and Technology*, 48(5-6), 545–551.
20. C. B. L. Jumbe, F. B. M. Msiska, and M. Madjera, “Biofuels development in Sub-Saharan Africa: are the policies conducive?”
21. Chandel, A.K., Chan, E.S., Rudravaram, R., Narasu, M.L., Rao, L.V., Pogaku, R., 2007. Economics and environmental impact of bioethanol production technologies: an appraisal. *Biotechnology and Molecular Biology Reviews* 2 (1), 014–032.
22. Christakopoulos, P., Koullas, D.P., Kekos, D., Koukios, E.G., Macris, B.J., 1991. Direct ethanol conversion of pretreated straw by *Fusarium-Oxysporum*. *Bioresource Technology* 35, 297–300
23. Chundawat, S. P., Venkatesh, B., & Dale, B. E. (2006). Effect of particle size based separation of milled corn stover on AFEX pretreatment and enzymatic digestibility. *Biotechnology and Bioengineering*, 96(2), 219–231. doi:10.1002/bit.21132 PMID:16903002
24. Claassen, P.A.M., Lopez Contreras, A.M., Sijtsma, L., Weusthuis, R.A., Van Lier, J.B., Van Niel, E.W.J., Stams, A.J.M., De Vries, S.S., 1999. Utilisation of biomass for the supply of energy carriers. *Applied Microbiology and Biotechnology* 52, 741–755

25. Da Silva, A. S., Inoue, H., Endo, T., Yano, S., & Bon, E. P. (2010). Milling pretreatment of sugarcane bagasse and straw for enzymatic hydrolysis and ethanol fermentation. *Bioresource Technology*, 101(19), 7402–7409. doi:10.1016/j.biortech.2010.05.008
26. Das, H. and Singh, S. Useful byproducts from cellulosic wastes of agriculture and food industry_a critical appraisal, *Critical Reviews in Food Science and Nutrition.*, 2004, 44(2), 77–89
27. Dashtban, M., Schraft, H., & Qin, W. (2009). Fungal bioconversion of lignocellulosic residues; opportunities & perspectives. *International Journal of Biological Sciences*, 5(6), 578–595. doi:10.7150/ijbs.5.578 PMID:19774110
28. Demirbas, A. (2005). Bioethanol from Cellulosic Materials: A Renewable Motor Fuel from Biomass. *Energy Sources*, 27(4), 327–337. doi:10.1080/00908310390266643
29. Demirbas, A. (2008). The importance of bioethanol and biodiesel from biomass. *Energy Source. Part B*, 3, 177–185
30. Demirbas, A., 2005. Bioethanol from cellulosic materials: a renewable motor fuel from biomass. *Energy sources* 27, 327–337.
31. Du, H., Kommalapati, R. R., & Huque, Z. (2016). Assessment of Bioethanol applications on transportation vehicles in houston. *JFundam Renewable Energy App*, 6, 207.
32. Duff, S. J. B., & Murray, W. D. (1996). Bioconversion of forest products industry waste cellulose to fuel ethanol: A review. *Bioresource Technology*, 55(1). doi:10.1016/0960-8524(95)00122-0
33. E. Chakauya, G. Beyene, and R. K. Chikwamba, “Food production needs fuel too: perspectives on the impact of biofuels in southern Africa,” *South African Journal of Science*, vol. 105, no. 5-6, pp. 174–181, 2009.
34. E. Chakauya, G. Beyene, and R. K. Chikwamba, “Food production needs fuel too: perspectives on the impact of biofuels in southern Africa,” *South African Journal of Science*, vol. 105, no. 5-6, pp. 174–181, 2009.
35. *Energy Policy*, vol. 37, no. 11, pp. 4980–4986, 2009.
36. Fong, J.C.N., Svenson, C.J., Nakasugi, K., Leong, C.T.C., Bowman, J.P., Chen, B., Glenn, D.R., Neilan, B.A., Rogers, P.L., 2006. Isolation and characterization of two novel ethanol-tolerant facultative-anaerobic thermophilic bacteria strains from waste compost. *Extremophiles* 10, 363–372.

37. Food and Agriculture Organization of United Nations. Available online: <http://faostat.fao.org/site/339/default> (accessed on 5 March 2020).
38. Foyle, T., Jennings, L. and Mulcahy, P. (2007) Compositional analysis of lignocellulosic materials: evaluation of methods used for sugar analysis of waste paper and straw, *Bioresource Technology*, 2007, 98 (16), 3026–3036.
39. Galbe, M., Zacchi, G., 2002. A review of the production of ethanol from softwood. *Applied Microbiology and Biotechnology* 59, 618–628
40. García-Cubero, M. T., González-Benito, G., Indacochea, I., Coca, M., & Bolado, S. (2009). Effect of ozonolysis pretreatment on enzymatic digestibility of wheat and rye straw. *Bioresource Technology*, 100(4), 1608–1613. doi:10.1016/j.biortech.2008.09.012 PMID:18951781
41. Georgieva, T., Mikkelsen, M., Ahring, B., 2008. Ethanol production from wetexploded wheat straw hydrolysate by thermophilic anaerobic bacterium *Thermoanaerobacter* BG1L1 in a continuous immobilized reactor. *Applied Biochemistry and Biotechnology* 145, 99–110.
42. Goh, C. S., Tan, K. T., Lee, K. T., & Bhatia, S. (2010). Bioethanol from lignocellulose: Status, perspectives and challenges in Malaysia. *Bioresource Technology*, 101(13), 4834–4841. doi:10.1016/j.biortech.2009.08.080 PMID:19762229
43. Hahn-Hagerdal, B., Karhumaa, K., Fonseca, C., Spencer-Martins, I., Gorwa-Grauslund, M., 2007. Towards industrial pentose-fermenting yeast strains. *Applied Microbiology and Biotechnology* 74, 937–953.
44. Harmsen, P., Huijgen, W., Bermudez, L., & Bakker, R. (2010). Literature review of physical and chemical pretreatment processes for lignocellulosic biomass (Tech. Rep. 1184). Biosynergy, Wageningen UR Food & Biobased Research.
45. Hendriks, A., & Zeeman, G. (2009). Pretreatments to enhance the digestibility of lignocellulosic biomass. *Bioresource Technology*, 100(1), 10–18. doi:10.1016/j.biortech.2008.05.027 PMID:18599291
46. Henniges, U., Hasani, M., Potthast, A., Westman, G., & Rosenau, T. (2013). Electron beam irradiation of cellulosic materials-opportunities and limitations. *Materials (Basel)*, 6(5), 1584–1598. doi:10.3390/ma6051584 PMID:28809230
47. Imman, S., Arnthong, J., Laosiripojana, N., & Champreda, V. (2012). Liquid hot water pretreatment of rice straw for enzymatic hydrolysis. In *Proceedings of the 4th*

International Conference on Sustainable Energy and Environment: A Paradigm Shift to Low Carbon Society, Bangkok.

48. Ingram, T., Rogalinski, T., Bockemühl, V., Antranikian, G., & Brunner, G. (2009). Semi-continuous liquid hot water pretreatment of rye straw. *The Journal of Supercritical Fluids*, 48(3), 238–246. doi:10.1016/j.supflu.2008.10.023
- Jaisamut, K., Paulova, L., Patakova, P., Rychtera, M., & Melzoch, K. (2013). Optimization of alkali pretreatment of wheat straw to be used as substrate for biofuels production. *Plant, Soil and Environment*, 59(12), 537–542. doi:10.17221/7129-PSE
49. Islam, A.; Chan, E.S.; Taufiq-Yap, Y.H.; Mondal, M.A.H.; Moniruzzaman, M.; Mridha, M. Energy security in Bangladesh perspective—An assessment and implication. *Renew. Sustain. Energy Rev.* 2014, 32, 154–171. [CrossRef]
- Halder, P.K.; Paul, N.; Beg, M.R.A. Prospect of *Pongamia pinnata* (Karanja) in Bangladesh: A Sustainable Source of Liquid Fuel. *J. Renew. Energy* 2014, 2014, 1–12. [CrossRef]
50. Jorgensen, H., 2009. Effect of nutrients on fermentation of pretreated wheat straw at very high dry matter content by *Saccharomyces cerevisiae*. *Applied Biochemistry and Biotechnology* 153, 44–57
51. Kadar, Z., Szengyel, Z., Reczey, K., 2004. Simultaneous saccharification and fermentation (SSF) of industrial wastes for the production of ethanol. *Industrial Crops and Products* 20, 103–110.
52. Karakashev, D., Thomsen, A.B., Angelidaki, I., 2007. Anaerobic biotechnological approaches for production of liquid energy carriers from biomass. *Biotechnology Letters* 29, 1005–1012.
53. Karhumaa, K., Sanchez, R.G., Hahn-Hagerdal, B., Gorwa-Grauslund, M.F., 2007. Comparison of the xylose reductase-xylitol dehydrogenase and the xylose isomerase pathways for xylose fermentation by recombinant *Saccharomyces cerevisiae*. *Microbial Cell Factories* 6.
54. Krishnan, C., Sousa, L. D., Jin, M., Chang, L., Dale, B. E., & Balan, V. (2010). Alkali-based AFEX pretreatment for the conversion of sugarcane bagasse and cane leaf residues to ethanol. *Biotechnology and Bioengineering*, 107(3), 441–450. doi:10.1002/bit.22824 PMID:20521302
55. Krishnan, M., Blanco, M., Shattuck, C., Nghiem, N., Davison, B., 2000. Ethanol production from glucose and xylose by immobilized *Zymomonas mobilis* CP4 (pZB5). *Applied Biochemistry and Biotechnology*, 525–541

- 56.Kumar, A. K., & Sharma, S. (2017). Recent updates on different methods of pretreatment of lignocellulosic feedstocks: A review. *Bioresources and Bioprocessing*, 4(1), 7. doi:10.118640643-017-0137-9 PMID:28163994
- 57.Kumar, A. K., & Sharma, S. (2017). Recent updates on different methods of pretreatment of lignocellulosic feedstocks: A review. *Bioresources and Bioprocessing*, 4(1), 7. doi:10.118640643-017-0137-9 PMID:28163994
- Kumar, P. D., Barrett, D. M., Delwiche, M. J., & Stroeve, P. (2009). Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. *Industrial & Engineering Chemistry Research*, 48(8), 3713–3729. doi:10.1021/ie801542g
- 58.Kumar, R., Singh, S., & Singh, O. (2008). Bioconversion of lignocellulosic biomass: Biochemical and molecular perspectives. *Journal of Industrial Microbiology & Biotechnology*, 35(5), 377–391. doi:10.100710295-008-0327-8 PMID:18338189
59. Lee, J. (1997). Biological conversion of lignocellulosic biomass to ethanol. *Journal of Biotechnology*, 56(1), 1–24. doi:10.1016/S0168-1656(97)00073-4 PMID:9246788
- 60.Linde, M., Galbe, M., & Zacchi, G. (2008). Bioethanol production from non-starch carbohydrate residues in process streams from a dry-mill ethanol plant. *Bioresource Technology*, 99(14), 6505–6511. doi:10.1016/j.biortech.2007.11.032 PMID:18281213
- 61.Martín, C., Galbe, M., Wahlbom, C.F., Hahn-Hägerdal, B., Jönsson, L.J. Ethanol production from enzymatic hydrolysates of sugarcane bagasse using recombinant xylose-utilizing *Saccharomyces cerevisiae*. *Enzyme and Microbial Technology*, 2002, 31, 274–282.
- 62.Menezes, E. G., Do Carmo, J. R., Alves, J. G., Menezes, A. G., Guimaraes, I. C., Queiroz, F., & Pimenta, C. J. (2014). Optimization of alkaline pretreatment of coffee pulp for production of bioethanol. *Biotechnology Progress*, 30(2), 451–462. doi:10.1002/btpr.1856 PMID:24376222
- 63.Moiser, N. (2005b). Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresource Technology*, 96(6), 673–686. doi:10.1016/j.biortech.2004.06.025 PMID:15588770
- 64.Mtui, G.Y.S. (2009). Recent advances in pretreatment of lignocellulosic wastes and production of value added products. *African Journal of Biotechnology*, 8(8).
- 65.Nabi, M.N.; Hoque, S.M.N.; Akhter, M.S. Karanja (*Pongamia Pinnata*) biodiesel production in Bangladesh, characterization of karanja biodiesel and its effect on diesel emissions. *Fuel Process. Technol.* 2009, 90, 1080–1086. [CrossRef]

66. Nester, E.W., Anderson, D.G., Roberts, C.E., Pearsall, N.N and Nester, M.T. Dynamics of prokaryotic growth in Microbiology. A human perspective. 3rd edition. McGraw-Hill, New York. 2001, Pp.87-108.
67. New York; New York, pp. 157e223
http://www.bioenergybaltic.ee/bw_client_files/bioenergybaltic/public/img/File/Lep7028WFinalB.pdf
68. Nigam, J.N., 2001. Ethanol production from wheat straw hemicellulose hydrolysate by *Pichia stipitis*. *Journal of Biotechnology* 87, 17–27.
69. Nwosu-Obieogu, K., Chiemenem, L. I., & Adekunle, K. F. (2016). Utilization of agricultural waste for bioethanol production- a review. *International Journal of Current Research & Review*, 8(19)
70. Okeke, B.C. and Obi, S.K.C. Saccharification of agrowaste materials by fungal cellulases and hemicellulases, *Bioresource Technology*, 1994, 51(1), 23–27.
71. Olofsson, K.; Winman, M.; Lidén, G. Controlled feeding of cellulases improves conversion of xylose in simultaneous saccharification and co-fermentation for bioethanol production. *J. Biotechnol.* **2010**, 145, 168–175. [[CrossRef](#)] [[PubMed](#)]
72. Panagiotou, G., Olsson, L., 2007. Effect of compounds released during pretreatment of wheat straw on microbial growth and enzymatic hydrolysis rates. *Biotechnology and Bioengineering* 96 (2), 250–258.
73. Pimentel, D., & Patzek, T. W. (2005). Ethanol Production Using Corn, Switch grass and wood; biodiesel production using soybean and sunflower. *Natural Resources Research*, 14(1), 65–76. doi:10.1007/11053-005-4679-8
74. Prasad, S., Singh, A., & Joshi, H. C. (2007b). Ethanol as an alternative fuel from agricultural, industrial and urban residues. *Conservation & Recycling*, 50(1). doi:10.1016/j.resconrec.2006.05.007
75. Prasad, S., Singh, A., Jain, N., & Joshi, H. C. (2007a). Ethanol production from sweet sorghum syrup for utilization as automotive fuel in India. *Energy & Fuels*, 21(4), 2415–2420. doi:10.1021/ef060328z
76. R. B. Mangoyana, “Bioenergy for sustainable development: an African context,” *Physics and Chemistry of the Earth*, vol. 34, no. 1-2, pp. 59–64, 2009.
77. Rehman, M. S. U., Kim, I., Chisti, Y., & Han, J. I. (2013). Use of ultrasound in the production of bioethanol from lignocellulosic biomass. *Energy Education Science and Technology, Part A. Energy Science and Research*, 30(2), 1391–1410.

78. Renewables 2015 Global Status Report. Available online: http://www.ren21.net/wp-content/uploads/2015/07/REN12-GSR2015_Onlinebook_low1.pdf (accessed on 5 May 2020).
79. Rofiqul Islam, M.; Rabiul Islam, M.; Rafiqul Alam Beg, M. Renewable energy resources and technologies practice in Bangladesh. *Renew. Sustain. Energy Rev.* 2008, 12, 299–343. [CrossRef]
80. Rogers, P.L., Jeon, Y.J., Lee, K.J., Lawford, H.G., 2007. *Zymomonas mobilis* for fuel ethanol and higher value products. *Advances in Biochemical Engineering/Biotechnology*, 263–288.
81. S. Karekezi, “Renewables in Africa-Meeting the energy needs of the poor,” *Energy Policy*, vol. 30, no. 11-12, pp. 1059–1069,
82. Saha, B.C., Cotta, M.A., 2006. Ethanol production from alkaline peroxide pretreated enzymatically saccharified wheat straw. *Biotechnology Progress* 22, 449–453.
83. Saha, B.C., Cotta, M.A., 2007. Enzymatic hydrolysis and fermentation of lime pretreated wheat straw to ethanol. *Journal of Chemical Technology & Biotechnology* 82, 913–919.
84. Sanchez, C. (2009). Lignocellulosic residues: Biodegradation and bioconversion by fungi. *Biotechnology Advances*, 27(2), 185–194. doi:10.1016/j.biotechadv.2008.11.001 PMID:19100826
85. Saravanakumar, K., & Kathiresan, K. (2013). Bioconversion of lignocellulosic waste to bioethanol by *Trichoderma* and yeast fermentation. *Biotech*, 4(5), 493–499. PMID:28324381
86. Saritha, M., Arora, A., & Lata. (2011). Biological pretreatment of lignocellulosic substrates for enhanced delignification and enzymatic digestibility. *Indian Journal of Microbiology*, 52(2), 122–130. doi:10.1007/12088-011-0199-x PMID:23729871
87. Sarkar, N., Ghosh, S. K., Bannerjee, S., & Aikat, K. (2011). Bioethanol production from agricultural wastes: An overview. *Renewable Energy*, 37(1), 19–27. doi:10.1016/j.renene.2011.06.045
88. Schugerl, K. Agricultural waste: A source of bulk products. *Journal of chemical engineering and technology*, 1994, 17, 291.
89. Sheoran, A., Yadav, B.S., Nigam, P. and Singh, D. Continuous ethanol production from sugarcane molasses using a column reactor of immobilized *Saccharomyces cerevisiae*. *Journal of Basic Microbiology*, 1998, 38, 123–128. Verbelen, P.J., Saerens, S.M.G.,

- Van Mulders, S.E., and Delvaux, F.R. The role of oxygen in yeast metabolism during high cell density brewery fermentations *Applied Microbiology and Biotechnology*, 2009, 82, 6-10
90. Singh, D. P., & Trivedi, R. K. (2013). Acid and Alkaline pretreatment of lignocellulosic biomass to produce ethanol as biofuel. *International Journal of Chemtech Research*, 5(2), 727–734.
91. Sun, Y., & Cheng, J. (2002). Hydrolysis of lignocellulosic materials for ethanol production: A review. *Bioresource Technology*, 83(1). doi:10.1016/S0960-8524(01)00212-7 PMID:12058826
92. Taherzadeh, M. J., & Karimi, K. (2008). Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: A review. *International Journal of Molecular Sciences*, 9(9), 1621–1651. doi:10.3390/ijms9091621 PMID:19325822
93. Tomas-Pejo, E., Oliva, J.M., Gonzalez, A., Ballesteros, I., Ballesteros, M., 2009. Bioethanol production from wheat straw by the thermotolerant yeast *Kluyveromyces marxianus* CECT 10875 in a simultaneous saccharification and fermentation fed-batch process. *Fuel* 88, 2142–2147
94. Ullmann, 1990a. Ullmann's Encyclopedia of Industrial Chemistry, A9, pp. 588e589. Ethanol.
95. Ullmann, 1990b. Ullmann's Encyclopedia of Industrial Chemistry, A1, pp. 281e282. Ethanol.
96. Ullmann, 1990c. Ullmann's Encyclopedia of Industrial Chemistry, A9, pp. 590e596. Ethanol.
97. Ullmann, 1990d. Ullmann's Encyclopedia of Industrial Chemistry, A9, p. 596. Ethanol.
98. Ullmann, 1990e. Ullmann's Encyclopedia of Industrial Chemistry, A9, pp. 637e639. Ethanol.
99. Ullmann, 1990f. Ullmann's Encyclopedia of Industrial Chemistry, A9, p. 633. Ethanol.
100. Um, B.H., Karim, M.N., Henk, L.L., 2003. Effect of sulfuric and phosphoric acid pretreatment on enzymatic hydrolysis of corn stover. *Applied Biochemistry and Biotechnology* 105, 115e125.
101. Varga, E., Schmidt, A. S., Reczey, K., & Thomsen, A. B. (2003). Pretreatment of corn stover using wet oxidation to enhance enzymatic digestibility. *Applied Biochemistry and Biotechnology*, 104(1), 37–50. doi:10.1385/ABAB:104:1:37 PMID:12495204

102. Vidal, P. F., & Molinier, J. (1988). Ozonolysis of lignin improvement of in vitro digestibility of poplar sawdust. *Biomass*, 16(1), 1–17. doi:10.1016/0144-4565(88)90012-1
103. Wang, G. S., Lee, J.W., Zhu, J. Y. and Jeffries, T. W. Dilute acid pretreatment of corncob for efficient sugar production. *Applied Biochemistry and Biotechnology*, 2011, 163, 658–668.
104. Wang, Q., & Zhu, S. (2010). Genetically modified lignocellulosic biomass for improvement of ethanol production. *BioResources*, 5(1), 3–4.
105. Wang, Q., & Zhu, S. (2010). Genetically modified lignocellulosic biomass for improvement of ethanol production. *BioResources*, 5(1), 3–4.
106. Watanabe, S., Abu Saleh, A., Pack, S.P., Annaluru, N., Kodaki, T., Makino, K., 2007. Ethanol production from xylose by recombinant *Saccharomyces cerevisiae* expressing protein engineered NADP(+)-dependent xylitol dehydrogenase. *Journal of Biotechnology* 130, 316–319.
107. Wilda, P. J. D., Huijgen, W. J. J., & Heeres, H. J. (2012). Pyrolysis of wheat straw-derived organosolv lignin. *Journal of Analytical and Applied Pyrolysis*, 93, 95–103. doi:10.1016/j.jaap.2011.10.002
108. Wyman, C.E. Ethanol production from lignocellulosic biomass: overview. In: Wyman, C.E. (Ed.), *Handbook on bioethanol: production and utilization*. Taylor & Francis, Washington, DC, 1996, Pp. 11–12
109. Zayed, G., Meyer, O., 1996. The single-batch bioconversion of wheat straw to ethanol employing the fungus *Trichoderma viride* and the yeast *Pachysolen tannophilus*. *Applied Microbiology and Biotechnology* 45, 551–555
110. Zheng, Y. I., Pan, Z., & Zhang, R. (2009). Overview of biomass pretreatment for cellulosic ethanol production. *International Journal of Agricultural and Biological Engineering*, 2(3), 51–68.