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Bioethanol Production from Lignocellulosic Waste-A Review

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Petroleum and other fossil fuel has been the main energy source for a long period of time in human life. Through these energy sources, the world has been a developing and industrializing entity. However, it is agreed that these traditional sources of energy cannot remain forever as they are non-renewable. Many experts predicted that oil production will keep on decreasing, as the present oil wells keep on decreasing and fewer oil reserves are discovered. This led to increasing price of the minerals and eventually makes them economically unsustainable. As such, renewable source of energy has to be sourced. Bioethanol; a renewable energy source is being produced from food materials such as sugar cane, maize etc. However, if these are to be used for energy production, the world will be entering into another crisis as they will be competed for food and energy. Lignocellulosic wastes such as Rice straw, Wheat straw, Corn straw and Bagasse contain same sugar molecules for bioethanol production as such can be used to generate renewable energy using appropriate physical, chemical and biological techniques. This paper aims at exploring the process of bioethanol production from lignocellulosic wastes.

Key words: Lignocellulosic waste, Bioethanol, Lignin.

The world's non-renewable energy (crude oil) reserve is entering into a declining phase while energy demand is increasing. Oil production is expected to decline in the coming one to ten decades [1]. As a result of this awaiting energy crisis, both governments and private industry are exploring alternative sources of energy. Other non-renewable sources of energy exist, such as coal and uranium; however, these sources are limited and will also inevitably decline in availability [2].

In order to get a stable energy alternative that will meet world demand and at the same time while moderating climate change, it is necessary to develop renewable clean fuels. Unluckily, most renewable energy initiatives are focused on electricity generation, while, about two thirds of the majority of world energy consumption is derived from liquid fuels [3]. However, the need for renewable sources of portable, liquid fuel is starting to receive greater attention, which mainly focuses on biomass-derived liquid fuels, or biofuels [4, 5]. Biofuels such as ethanol is produced from agricultural products including starchy and cereal crops such as sugarcane, corn, beets, wheat, millet and sorghum. However, this renders food security at stake taking into account the world growing population and dwindling arable land due to rapid urbanization, it is apparent that biofuel production from food stuffs is not a sustainable idea. Cost is an important factor for large scale expansion of bioethanol production. On the other hand millions of tons of agricultural residues are abundantly available [6] and since no economically viable technologies are available for their conversion, most farmers burn them in the field. This not only pollute the environment, but also causes other problems such as the disruption of air transportation by smoke clouds in the sky [7]. The

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fuel from lignocellulosic wastes scavenges the competition of food versus fuel caused by grain based bioethanol production [8]. Lignocellulose is a complex carbohydrate polymer of cellulose, hemicellulose and lignin. Cellulose and hemicellulose are sugar polymers. Cellulose is composed of repeating sugar units of glucose linked by b-1,4glycosidic bonds, while Hemicellulose is a heteropolymer of D-xylose, D-arabinose, D-glucose, D-galactose, and D-mannose (Table 4). Lignin is hydrophobic in nature and is tightly bound to these two carbohydrate polymers. It thus protects these polymers from microbial attack [9]. A lignocellulosic material includes crop residues, grasses, sawdust, wood chips, etc. These are renewable, low cost and are abundantly available. Kim 2004 [10], reported that about 442 billion liters of bioethanol can be produced from lignocellulosic biomass per year. This is about 16 times higher than the actual world bioethanol production. Hence bioethanol production could be the route to the effective utilization of agricultural wastes. Rice straw, wheat straw, corn straw, and sugarcane bagasse are the major agricultural wastes in terms of quantity of biomass available [10]. This review aims to present a brief overview of the available and accessible technologies for bioethanol production using these food wastes.

MATERIALS

Rice straw, wheat straw, corn straw and baggase are the major agrowastes which are most favorable feedstocks for bioethanol production due to their availability throughout the year. Table 1. gives the estimate worldwide production of these agrowastes. They also vary in chemical composition as given in Table 2. The utilization fraction of wheat straw, rice straw and corn straw is too low and varies with geographic region [10]. Each year a large portion of agricultural residues is disposed of as waste. For instance, approximately 600 to 900 million tons per year rice straw is produced globally [11]. The options for the disposal of rice straw are limited by the great bulk of material, slow degradation in the soil, harboring of rice stem diseases, and high mineral content. Only a small portion of globally produced rice straw is used as animal feed, the rest is removed from the field by burning, a common practice all over the world, increasing air pollution and affecting human health [12].

Globally, bioethanol production from the above mentioned agrowaste is now a matter of interest (Table 3). Rice straw is the most abundant waste compared to the other major wastes (Table 1) and rice straw can potentially produce 205 billion liters bioethanol per year, which is the highest among these four mentioned agricultural wastes. Lignocellulosics are processed for bioethanol production through three major operations: pretreatment for delignification is necessary to liberate cellulose and hemicellulose before hydrolysis; hydrolysis of cellulose and hemicellulose to produce fermentable sugars including glucose, xylose, arabinose, galactose, mannose and fermentation of reducing sugars. The non-carbohydrate components of lignin also have value added applications [13].

Pretreatment

Pretreatment methods refer to the solubilization and separation of one or more of these components of lignocellulosic biomass. The lignocellulosic complex is made up of a matrix of cellulose and lignin. This step is taken to make the solid biomass more accessible to chemical or biological treatment [14]. By pretreating the complex, the matrix is broken to decrease the degree of crystallinity of the cellulose, and also, increase the fraction of amorphous cellulose, which makes it more susceptible for enzymatic attack [15]. The lignocellulosic biomass is made susceptible by quick hydrolysis to increase yields of monomeric sugars [16]. Pretreatment aimed at: (i) formation of sugars directly or subsequently by hydrolysis (ii) to avoid loss and/ or degradation of sugars formed (iii) to limit formation of inhibitory products (iv) to reduce energy demands and (v) to minimize costs. The three fundamental types of pretreatment are Physical, chemical and biological treatments. For an effective yield, all the three method are followed.

Physical pretreatment

Physical pretreatment can be of following types:

Mechanical size reduction

The first step involve milling, grinding or chipping. It is carried out to reduce cellulose crystallinity [17] and improves the efficiency of the subsequent steps. The power input for this

step depends on the initial and final particle sizes, moisture content and the nature of the raw material (hardwood, softwood, fibrous, etc.) being handled [18, 19]. Reducing the size may give better results but very fine particle size may lead to negative effects on the subsequent processes including such as enzymatic hydrolysis [20].

Pyrolysis

In this process, the materials are treated at a temperature greater than 300^0C. It leads to rapid decomposition of cellulose to produce gaseous products such as H_2 and CO and residual char. If lower temperature is used, the decomposition is slower and less volatile products are formed [15]. The residual char is now further treated by leaching with water or with mild acid. The water leachate collected contains enough carbon source to support microbial growth for bioethanol production. The water leachate consist of mainly glucose. About 55% of total weight of the biomass is lost during water leaching [21]. Mild acid leaching gives 80 to 85% conversion of cellulose to reducing sugars with more than 50% glucose.

Microwave oven and electron beam irradiation pretreatment

This is another way of pretreatment method of lignocellulosic biomass. It involves utilization of thermal and non-thermal effects generated by microwaves in aqueous environments. In the thermal method, heat is generated within the biomass by microwave radiation through vibrations of the polar bonds in the biomass and the surrounding aqueous medium. As such, a hot spot is created within the inhomogeneous material. The result is in an explosion effect among the particles and improved disruption of recalcitrant structures of lignocellulose [22]. Thermal pretreatment course the release of acetic acid from the lignocellulosic material thereby creating an acidic environment for autohydrolysis. In the non-thermal method, i.e., the electron beam irradiation method, polar bonds are allowed to vibrate by aligning with a continuously changing magnetic field. This results in disruption and shock to the polar bonds which in turn accelerates chemical, biological and physical processes [20]. High energy radiation gives more changes in cellulosic biomass including increase of specific surface area, decrease of degree of polymerization and crystallinity of cellulose, hydrolysis of hemicellulose and partial depolymerization of lignin. It was reported by Kitchaiya et al.,[22], that microwave pretreatment of rice straw and bagasse followed by lignin extraction give a yield of 43 to 55% of total available reducing sugars[22].

Chemical pretreatment

Chemical pretreatment methods involve the usage of dilute acid, alkali, ammonia, organic solvent, SO_2, CO_2 and other chemicals. These

Table 1. Quantities of agricultural waste (million tons) reportedly available for bioethanol production.

Agrowaste	Africa	Asia	Europe	America	Oceania
Rice straw	20.9	667.6	3.9	37.2	1.7
Wheat straw	5.34	145.20	132.59	62.64	8.57
Corn straw	0.00	33.90	28.61	140.86	0.24
Bagasse	11.73	74.88	0.01	87.62	6.49

Source: N. Sarkar et al. [30].

Table 2. Chemical composition of agricultural wastes

Agrowaste (%)	Cellulose (%)	Hemi-cellulose (%)	Lignin (%)	Protein (%)	Ash (%)
Rice straw	32-47	19-27	5-24	-	12.4
Wheat straw	35-45	20-30	8-15	3.1	10
Corn straw	42.6	21.3	8.2	5.1	4.3
Bagasse	65(total carbohydrate)	18.4	18.4	3	2.4

Source: N. Sarkar et al. [30].

methods are easy in operation and have good conversion yields in short time.

Acid pretreatment

Acid pretreatment method aims for high yields of sugars from lignocellulosics. It is usually carried out by concentrated or diluted acids (usually between 0.2% and 2.5% w/w) at temperatures between 130^{0}C and 210^{0}C to improve cellulose hydrolysis. Sulfuric acid is the most widely used acid [23]. According to Moiser et al., [16], lignocellulose pretreated with dilute H_2SO_4 give higher yield compared to other acids. A saccharification yield of 74% was obtained from wheat straw when subjected to 0.75% v/v of H_2SO_4 at 121^{0}C for 1 hour [24]. The acid medium cleave the polysaccharides, especially hemicelluloses which are easier to hydrolyze than cellulose [23]. However, during acid treatment, various microbial growth inhibitors like acetic acid, furfural and 5-hydroxymethylfurfural are produced. As such hydrolysis products to be used for fermentation have to be detoxified.

Alkaline pretreatment

Alkali treatment of lignocellulose disrupts the cell wall by dissolving hemicelluloses, lignin, and silica, by hydrolyzing uronic and acetic esters, and by swelling cellulose thereby digesting the lignin matrix. By this process, the substrates can be fractionated into alkali-soluble lignin, hemicelluloses and residue, which makes it easy to utilize them for more valuable products [16]. Hydroxides of sodium, potassium, calcium and ammonium are used in this process. Low temperature and pressure are used in this method [16]. Maximum release of 60% and 80% for lignin and hemicellulose respectively was found to be obtained by treating wheat straw with 1.5% NaOH for 144 h at 20^{0}C (Sun *et al.*) [17]. NaOH has been reported to increase hardwood digestibility from 14% to 55% by reducing lignin content from 24-55% to 20%.

Biological pretreatment

The degradation of the lignocellulosic complex can be achieved by microbial fermentation to liberate cellulose and hemicellulose in a mild condition (Table 6). Both bacteria and fungi have been explored, but rot fungi associated with wood decay are the predominant species in lignocellulose degradation for the purpose of biofuel production [18]. Brown rot attacks cellulose while white and soft rots attack both cellulose and lignin due to their abundant ligninolytic enzymes, including lignin peroxidase and manganese peroxidase, laccases and other enzymes, and better selectivity in lignin degradation [25]. Cellulase-less mutant was developed for the selective degradation of lignin and to prevent the loss of cellulose but in most cases of biological pretreatment the rate of hydrolysis is very low. Biological pretreatment of bamboo culms with white rot fungi has been performed at low temperature (25 ^{0}C) [26]. A study conducted by Singh et al, [27] indicates that *Aspergillus terreus* reduces the cellulose content of lignocellulosic material by about 55.2% while delignification was found to be about 92%.

Enzymatic hydrolysis

Table 3. Worldwide potential bioethanol production from agricultural wastes

Agrowaste	Potential annual bioethanol production (globally) (giga liter)
Rice straw	*205*
Wheat straw	204
Corn straw	58.6
Bagasse	51.3

Source: N. Sarkar et al. [30].

Table 4. Carbohydrate content of agricultural waste (%).

Agrowaste (%)	Glucose	Xylose	mannose	Galactose
Rice straw	41-43.4	14.8-20.2	1.8	0.4
Wheat straw	38.8	22.2	1.7	2.7
orn straw	39	14.8	0.3	0.8
Bagasse	38.1	23.3	-	1.1

Source: N. Sarkar et al. [30].

Table 5. Different pretreatments and respective yields for sugarcane bagasse, wheat straw, rice straw, and corn straw.

Substrate	pretreatment	hydrolysis	Yield of sugar
Rice straw	Chopped to 5 -6mm size range	4.4% H^2SO_4 at 1:10 solid to liquid ratio in boiling water bath, 1 h, filtered and pH adjusted to 5.5 Soaked in water at 170^{0}C and 7.6 kg/cm2, 30 min, cooled and pH 5.5.	Total sugar (20 g/L)
Wheat straw	Knife milling with 0.7-1.0 mm rejection screen, washed with water and dried	At 90 ^{0}C with 1.85% (w:v) $H^2SO_4$for 18 h; liquid to solid ratio of 20:1. Suspension centrifuged and the residue is washed with hot water.	D-xylose: 12.80±0.25 g/L, D-glucose: 1.70 ±0.30 g/L
Corn straw	Chopped, steam exploded (3.5 MPa, 275 ^{0}C, 2 min) 2% NaOH, 80^{0}C, 1 h.	Enzymatic saccharification (cytolase, novozyme) (50^{0}C, 120 h) Enzymatic hydrolysis by cellulose of TrichodermareeseiZU-02 and cellobiose of Aspergillus nigerZU-07.	Xylose 23.6 g/L, glucose 56.7 g/L, arabinose 5.7 g/Lv
Sugarcane Bagasse	Ball milling (4 h) 1% sulfuric acid (v/v) at 60^{0}C,24 h (SLR 1:6)	Enzymatic acremonium cellulase at 5 FPU/g substrate of cellulase and 20 U/g substrate of xylanase from Optimash BG at 45 0C, pH 5.0 for 72 h In an autoclave at 121 _C for 40 min after removing the excess acid (1% (v/v) sulfuric acid)	89.2 ±0.7% (glucose), 77.2 ±0.9% (xylose)

Source: N. Sarkar et al. [30].

Table 6. Summary of some bio-delignification processes.

Agrowaste	Microorganism for lignin degradation	Time (pretreatment)	% of substrate converted to reducing sugars
Rice straw	*Pleurotusostreatus*	60 days	41% lignin degraded
Wheat straw	*Pleurotusostreatus*	5 weeks	35%
	Phanerochaetesordida; Pycnoporuscinnabarinus115	4 weeks	35%
Corn straw	*Phlebia sp*. MG-60 (A marine fungus)		>50%
Sugarcane trash	*S Aspergillus terreus*	5 days	92% delignification; total reducing sugar yield 11.26 ± 0.73 mg/g
	Bacillus macerans		71% delignification; total reducing sugar yield 11.56 ± 0.51 mg/g.
	Trichodermareesei		73.6% delignification; Total Reducing sugaryield 11.16 ± 0.64

Source: N. Sarkar et al. [30].

Saccharification is the critical step for bioethanol production where complex carbohydrates are converted to simple monomers (Table 5). Compared to acid hydrolysis, enzymatic hydrolysis requires less energy and mild environment conditions [28]. The optimum conditions for cellulase have been reported as temperature of 40 to 50^{0}C and pH 4 to 5 [29]. Assay conditions for xylanase have also been reported to be 50 ^{0}C temperature and pH 4 to 5. Therefore, enzymatic hydrolysis is advantageous because of its low toxicity, low utility cost and low corrosion compared to acid or alkaline hydrolysis [19]. Moreover, no inhibitory by-product is formed in enzymatic hydrolysis [30]. However, enzymatic hydrolysis is carried out by cellulase enzymes that are highly substrate specific [31]. Here cellulase and hemicellulase enzymes cleave the bonds of cellulose and hemicellulose respectively (Cellulose contains glucan and hemicellulose contains different sugar units such as mannan, xylan, glucan, galactan and arabinan). Cellulose is hydrolysed to glucose whereas hemicellulose gives rise to several pentoses and hexoses. Several species of *Clostridium*, *Cellulomonas, Thermonospora*, *Bacillus, Bacteriodes*, *Ruminococcus, Erwinia, Acetovibrio, Microbispora, Streptomyces* are able to produce cellulase enzyme. Many fungi such as *Trichoderma, Penicillium, Fusarium, Phanerochaete, Humicola, Schizophillum*sp, also have been reported for cellulase production [32]. Among the various cellulolytic microbial strains *Trichoderma* is one of the most well studied cellulase and hemicellulose producing fungal strains [33]. On the other hand *Aspergillus* is a very efficient â-glucosidase producer [19]. *Trichoderma*cellulase supplemented with extra b-glucosidase has been studied several times [34]. Combination of *Trichodermareesei* ZU-02 cellulase and cellobiase from *Aspergillus niger* ZU-07 improved the hydrolysis yield to 81.2% [35]. Various factors influence yields of monomer sugars from lignocellulose. Temperature, pH and mixing rate are the main factors of enzymatic hydrolysis of lignocellulosic material [36]. Other factors that affect yield are substrate concentration, cellulose enzyme loading, and surfactant addition [37]. High substrate concentration may lead to substrate inhibition. Cellulase contributes to the major cost of the lignocellulosic ethanol technology [33]. Therefore, an efficient pretreatment is to be selected to decrease cellulose crystallinity and to remove lignin to the maximum extent, so that hydrolysis time as well as cellulase loading will be minimized [38]. Surfactants modify the cellulose surface by adsorbing lignin onto surfactant and thus the surfactant prevents the enzyme from unproductive binding with lignin and lowers enzyme loading [39]. Belkacemi and Hamoudi[40] studied enzymatic hydrolysis of corn stalk hemicellulose at 30 ^{0}C and pH 5. Saccharification was 90% and sugar was released after 10 hours. Chen et al. [35] studied enzymatic hydrolysis of maize straw using cellulase from *T. reesei*ZU-02 and cellobiase from *A. niger*ZU-07. Addition of 5 g/L Tween 80 improved hydrolysis yield by 7.5%. Borjesson et al. [37] reported that PEG addition increased the enzymatic conversion of soft

Table 7. Ethanol yields from various substrates by various microorganisms.

Agrowaste	Fermenting microbe	Yield of ethanol
Rice straw	*Candida shehatae*NCL-3501	0.45 g/g and 0.5 g/g of sugar utilized produced from autohydrolysate by freeandimmobilized cells in 48 h 0.37 g/g and 0.47 g/g of sugar utilized produced from acid hydrolysate by free and immobilized cells in 48 h
	Saccharomyces cerevisiae ATCC 26603	Maximum ethanol production achieved 4 g/L
	*Pichia stipitis*NRRL Y-7124	Maximum ethanol production achieved 6 g/L (78% of theoretical maximum)
Wheat straw	*Pichia stipitis NRRL Y-7124*	0.35 g/g yield
	Pichia stipitis	0.41 g/g yield
Bagasse	*Genetically modified E. coli KO11*	91.50 % yield and 3.15 % (w/v) ethanol titre

Source: N. Sarkar et al. [30].

lignocellulose from 42% to 78% at 16 hours where optimum hydrolysis temperature was 50 ^{0}C. Xu et al. [33] reported that *T. reesei* decomposed 68.21% of alkali pretreated rice straw whereas 73.96% conversion was obtained from alkali assisted photocatalysis of rice straw after enzymatic hydrolysis. Alkaline peroxide pretreated wheat straw showed 96.75% yield after enzymatic hydrolysis whereas atmospheric autocatalytic organosolvent pretreated wet wheat straw gave above 75% yield [40].

Fermentation

Selection of appropriate microorganism that can efficiently convert hexoses and pentoses to ethanol is one of the problems concerned with fermentation of lignocellulosic materials[41]. To improve the productivity, genetically modified organisms are used to get complete fermentation of the sugars. By simultaneous saccharification and co-fermentation (SSCF) and separate hydrolysis and fermentation (SHF) maximum yield could be achieved [29]. Another fermentation method is consolidated bioprocessing (CBP) where cellulase production, saccharification and ethanol production take place in same reactor [8]. CBP requires no capital investment for obtaining enzyme or its production [42], but ethanol production is poor[43].

In many cases, single or co-culture of microbes is used. A combination of *Candida shehatae*and *Saccharomyces cerevisiae* was reported to be an effective combination [29]. Sequential fermentation with two different microorganisms in a given time interval results in better utilization of sugar by using *S. cerevisiae* in the first phase for hexose utilization and *C. shehatae* in the second phase for pentose utilization, but it gives poor ethanol yield. Some wild type microorganisms used in the fermentation are *S. cerevisiae, Escherichia coli, Zymomonasmobilis, Pachysolentannophilus, C. shehatae, Pichia stipitis, Candida brassicae*, *Mucorindicus* etc. [15]. *S. cerevisiae* and *Z.mobilis* are the most commonly used microbes used in bioethanol production from hexoses and pentoses arerespectively; but S. cerevisiae cannot utilize the main C-5 sugar xylose of the hydrolysate [19]. Native organisms such as *Pichia* and *Candida* species can utilize xylose though they have lower bioethanol production rate[12]. Table 7 show some microbes with different bioethanol yield. Many genetically modified microorganisms such as *P. stipitis* BCC15191 [44], *P. stipitis*NRRLY-7124 [45], recombinant *E. coli* KO11, *C. shehatae* NCL-3501 [46], *S. cerevisiae* ATCC 26603 [47] have been developed to improve bioethanol production. Obligate anaerobic hemophilic bacteria such as *Clostridium* sp. and *Thermoanaerobacter* sp. were thoughtto explore the benefits of fermentation at higher temperatures[19]. Also, some other thermo-tolerant microorganisms developed are *K. marxianus, Candida lusitanieae* and *Z. mobilis*[8].

CONCLUSION

To cater for the increasing demand for energy source that will replace the petroleum and gas, bioethanol production came into play. Though sugar and starch (g ethanol/g substrate) are the best sources for bioethanol production, they cannot be used for worldwide production of bioethanol due to high demand and less in abundance. Agricultural wastes called lignocellulosic biomass are potential raw materials for bioethanol production. They grow abundantly and do not demand separate land, water, and energy. However feedstock, conversion technology, hydrolysis process, and fermentation configuration serve as drawback to the production of bioethanol from lignocellulosic materials. In conclusion it may be said that to solve the technology problems of the conversion process, science and efficient technology are to be applied, so that bioethanol can be sufficiently produced in the near for future to defeat the current energy demand from the depleting oil and gas.

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