Bioethanol from Lignocellulosic Feedstock: A Review

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ABSTRACT

Ethanol is the most commonly used biofuel in the world, its demand increasing with time. Due to the steady rate of depletion of fossil fuels, bioethanol is emerging as an alternative fuel source. Unlike fossil fuels, bioethanol is a clean fuel that is environment friendly and causes little pollution. It is an alcohol, resulting from the fermentation of sugars, starches or cellulose, derived from crops such as wheat, corn, sugar beets, sugarcane, agricultural residues or any other lignocellulosic biomass. The production is a multi-step process that includes a range of pre-treatments, enzymatic hydrolysis and fermentation. The final product, ethanol, is obtained from the final step of fermentation that is carried out with the help of microorganisms like yeast or bacteria, or even a combination of microorganisms. Researches are being carried out to genetically engineer or find natural indigenous microorganisms that are capable of producing specific enzymes required for the production of bioethanol in large amounts that is suitable for industrial scale. Bioethanol is currently being blended with gasoline and used as automobile fuel for vehicles with gasoline engines. In this article, the various sources of raw materials that can be used for bioethanol production have been discussed, along with descriptions of the different production steps involved in the generation of bioethanol on an industrial scale.

Keywords: Bioethanol; Lignocellulosic feedstock; enzymes; microorganisms; Ozonolysis; fuel
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INTRODUCTION

The world, as we know it, is faced with the grave problem of depleting levels of non-renewable energy resources. At the same time, the demand and consumption of energy is on the rise. According to the IEA (International Energy Agency), the transportation sector dominates the world oil consumption. Today, the transportation sector is almost entirely dependent on petroleum-based fuels. It accounts for more than 70% of the global carbon monoxide (CO) emissions and 19% of the global carbon dioxide (CO_2) emissions [1]. Intensive utilization of fossil fuels such as petroleum and gasoline, has led to the generation and release of green house gases into the atmosphere, which is the primary cause of global climate change and global warming. With the world's oil reserves running low, there has been a dramatic increase in the price of petroleum worldwide. Due to concerns about global warming, low reserves of fossil fuels and health issues, the search for renewable sources of energy, which would help reduce green house gas emissions, has become a matter of utmost importance.

Biofuels or biomass-based fuels, offer many advantages over petroleum-based fuels [2]. Biofuels are easily available from common biomass sources. They are considerably environment friendly, they are biodegradable, nontoxic, and has low emission profiles, and so it is environmentally beneficial and contribute to sustainability [3]. Bioethanol, which is a biofuel, has been recognized as a potential alternative to petroleum-derived transportation fuels [4]. It began with the use of ethanol in the internal combustion engine (ICE) invented by Nikolas Otto in 1897 [5]. Bioethanol has also been used in as fuel Brazil since 1925. Fuel ethanol blends can be successfully used in all types of vehicles and engines that require gasoline [6]. Ethanol is also a safer alternative to methyl tertiary butyl ether (MTBE), a common additive to gasoline used to provide cleaner combustion [7]. MTBE is a toxic chemical compound that contaminates ground water. Bioethanol was widely used in Europe and in the United States until early 1900s, when ethanol production became more expensive than petroleum based fuels after World War II. Its potential was mostly ignored until the oil crisis of the 1970s [8]. Bioethanol is a renewable source of energy that can be derived from a range of feedstock like wheat, straw, sugar beet, rice, corn and wood. A potential starting material for ethanol is lignocellulosic biomass, which includes materials such as agricultural residues, herbaceous crops, forestry wastes, waste paper and other cellulosic wastes [9]. However, there are a few major bottlenecks in the current production of biofuels. There is a lack of biocatalysts that can work efficiently and inexpensively at high temperatures and/or low pH conditions for use in the bioconversion of

lignocellulosic biomass to bioethanol. Also, there is a need for cost effective fermentation of derived sugars from cellulose and hemicellulose [10]. The process requires enzymes to be stable and active at low or high pH.

Bioethanol, also known as ethyl alcohol, grain alcohol or ETOH, has the chemical formula CH_3-CH_2-OH. It is a liquid biofuel which can be produced from different biomass feedstocks. Ethanol is an oxygenated fuel that contains 35% oxygen which reduces particulate and NO_x emissions from combustion. The presence of oxygen improves combustion and therefore reduces hydrocarbon, carbon monoxide, and particulate emissions. However, oxygenated fuels also tend to increase nitrogen oxide emissions. Bioethanol has a higher octane number (108), broader flammability limits, higher flame speeds, and higher heat of vaporization than gasoline. These properties allow a higher compression ratio, shorter burn time and leaner burn engine that give efficiency advantages over gasoline in an ICE [11]. Ethanol has 66% of the energy possessed by gasoline. It is corrosive, has low flame luminosity, and has lower vapour pressure making cold starts difficult. Its miscibility with water is high, and it is toxic to ecosystems [12]. Bioethanol can be used as an appropriate mixed fuel in gasoline engines because of its high octane number, and the high heat of vaporization obstructs self ignition in diesel engines. Thus, ignition improver, glow-plug, surface ignition and pilot injection are applied to promote self-ignition by using diesel-bioethanol blended fuels [13]. The most popular blend for light-duty vehicles is E85 that contains 85% bioethanol and 15% gasoline.

This review aims at describing the various raw materials, particularly the lignocellulosic materials used in the large scale production of bioethanol commercially.

FEEDSTOCKS FOR BIOETHANOL PRODUCTION

Bioethanol can be produced from different kinds of raw materials, mainly from three kinds of agricultural raw materials: sucrose containing feedstocks (eg. sugarcane, sugar beets, fruits, etc.), starch materials (corn, wheat, rice, barley, etc.) and lignocellulosic materials (wood, straw, grasses). Globally, bioethanol production from rice straw, wheat straw, corn straw and sugarcane bagasse is now gaining importance [14]. Currently, the major emphasis is on generating bioethanol from agricultural wastes. Though there are a few concerns related to the raw materials to be used for bioethanol production like chemical composition of the biomass, cultivation practices, availability of land, use of resources, emission of greenhouse gases, acidifying gases and ozone depletion gases, injection of pesticides, soil erosion, biodiversity and landscape value losses, farm-gate price of biomass, transportation and storage cost of biomass, water requirements and water availability, considerable variability of feedstock availability from season to season, dependence on the geographic location, and creation and maintenance of employment [3]. Globally, Brazil utilizes sugarcane, while the United States and Europe utilizes starch from corn, wheat and barley for the production of bioethanol.

Sucrose-containing feedstock
This group mostly contains biomass from sugarcane and sugar beet [15]. These two plants are geographically discrete. While sugarcane is found in tropical and subtropical counties, sugar beet is found in temperate climate counties only. Brazil is the single largest producer of sugarcane in the world. In Asian countries like India, Thailand and Philippines, sugarcane is produced on small fields owned by small farmers [16]. In European countries, beet molasses are the most utilized sucrose containing feedstock [17]. The advantages of sugarcane and sugar beet include a lower cycle of crop production, higher yield, higher tolerance for a wide range of climatic variations, low water requirements and low fertilizer requirements. Compared to sugar cane, sugar beet requires 35-40% less water and fertilizers [18].

Starchy feedstock
Starch is a homopolymer, made up of D-glucose monomers [19]. In order to produce bioethanol from starch sources, it is necessary to break the chains of this carbohydrate to obtain glucose units that can be converted to ethanol by yeasts. Starch can be converted to fermentable sugar by the method of hydrolysis. Hydrolysis is the reaction of starch using water, which is normally used to break down starch into fermentable sugar [20]. It could be either enzymatic or acid hydrolysis. When starch is hydrolysed by the enzyme amylase, the process is industrially known as liquefaction. Factors like choice of substrate, enzyme activity and reaction conditions like temperature and pH affect the enzymatic hydrolysis of starch [21].

Corn and wheat fall under the starchy feedstock category and are majorly utilized for bioethanol production in North America and Europe [17]. The United States has a large corn-based bioethanol industry. About 5% of corn in the world is wasted. If wasted corn could be fully utilized as feedstock for bioethanol production, then 9.3GL of bioethanol could be produced, thereby replacing 6.7GL of gasoline if bioethanol is used alternative vehicle fuel [22]. However, one small disadvantage of using corn for the production of bioethanol is the cost of corn. Corn prices vary from year to year, and it also varies in different locations because of shipping distance from the field to the plant [23]. Like corn, barley can also be used as feedstock for bioethanol production. Wasted barley, amounting to 3.4% worldwide, has the potential of producing 1.5GL of bioethanol, replacing 1.1GL of gasoline if ethanol is used as vehicle fuel [22].

China is the largest producer of wheat, closely followed by India. Wheat straw is an abundant agricultural residue with low commercial value making it an attractive feedstock for bioethanol production [24]. Utilization of wasted wheat could produce 7.0GL of bioethanol replacing 5.0GL of gasoline.

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Lignocellulosic feedstock
Lignocellulosic biomass includes agricultural residues such as corn stover, rice straw and wheat straw, wood and energy crops. Lignocellulosic materials are attractive raw materials for bioethanol production because it is the most abundant and reproducible resource on earth. The total potential for bioethanol production from crop residues is about 16 times higher than the current world bioethanol production from all other sources combined [25]. Lignocellulosic perennial crops are promising feedstocks because of their high yields, low costs, suitability to less fertile lands and low environmental impacts. Lignin content of most feedstocks is about 27%, although bit grasses contain significantly less [26]. One of the main challenges of producing bioethanol from lignocellulosic biomass is the high cost of hydrolysis process [27].
Rice straw is one of the most abundant lignocellulosic waste materials worldwide, with annual production of 731 million tons distributed over Africa, Asia, Europe, America and Oceania [28]. Such amounts of rice straw can produce around 205 billion litres of bioethanol per year, the largest amount from a single biomass feedstock [29].

Sources of Lignocellulosic feedstock
Forest woody feedstocks: Woody materials are classified into two broad categories: hardwoods and softwoods. Softwoods originate from coniferous trees and gymnosperms and unlike hardwoods, softwoods possess lower densities and grow faster [30]. Gymnosperms mostly consist of evergreen plants like cedar, pine, spruce, cypress, redwood, fir and hemlock [31]. Hardwoods on the other hand are angiosperms, and are mostly deciduous trees found mainly in the northern hemisphere [32]. It includes trees such as willow, oak, cottonwood and aspen. Unlike agricultural biomass, woody raw materials offer flexible harvesting times and avoid long latency periods of storage [33]. Woody feedstock also comprises of more lignin than agricultural residues and less ash content. Forestry wastes like sawdust from saw mills, slashes, wood chips and branches from dead trees can also be used as bioethanol feedstocks [34].

Agricultural residues, herbaceous and municipal solid wastes (MSW): Crop residues mostly consist of agricultural wastes like corn stover, corn stalks, rice and wheat straws as well as sugarcane bagasse [14]. Rice and wheat straws and corn stalks have the maximum potential to be used as feed for bioethanol production. Crop residues contain more hemicellulosic materials than woody biomass (25-35% approx.) [35]. Agricultural residues are environmentally friendly and help to avoid dependency on forest–woody biomass and hence reduce deforestation (non-sustainable cutting of trees).
Switch grass is a kind of herbaceous prairie grass grown as an energy crop in the northern parts of North America and Canada. Due to their low investment, abundance, disease resistance, low maintenance and high yield of sugar substrates per acre, these perennial grasses are potential candidates for the production of bioethanol. *Miscanthus giganteus* is yet another fast growing grass, native to Asia and grown in Europe. It is also a potential candidate for bioethanol production [36]. Apart from cellulosic feedstock, municipal and industrial solid wastes can also be used as raw materials for bioethanol production. Their utilization restricts environmental problems associated with the disposal of household garbage, processing papers, food processing by-products, black liquors and pulps [37]. Although enough biomass can potentially be available to replace 30% of the petroleum-derived gasoline by the year 2030, the high cost of biomass could be a serious hindrance if potential lands and feedstocks are not managed and utilized efficiently [34,37].

Marine algae: Interest in algae as a potential biofuel feedstock has existed since 1978 in the U.S. and has recently gained support from the DOE Aquatic Program. Assessment of several aspects of algal biomass like water uptake, productivity per acre, by-product and co-products recovered during bioethanol production were carried out, but improving the efficiency of algal feedstock and its development as a viable source of raw material to be used commercially remained limited during the 20th century. But recently, algal biomass is recapturing interest as a third generation biofuel feedstock due to a shortage on current energy crops designated for bioethanol and biodiesel industries [38]. Besides being a prospective raw material for bioethanol production, algae can also be used as feedstock for biodiesel and aviation fuel production, as well as possible applications in bio-crude oils and bio-plastics [39]. Algae are capable of generating 10 times more ethanol than corn per growing area [40]. Unlike corn and sugarcane, algal biomass does not compete directly with food and doesn't need fresh water to be cultivated in. It consumes a large amount of CO_2 during its growth phase, which makes it environmentally attractive as a carbon sink [41].

Composition of Lignocellulosic material:L Lignocellulosic materials have three main components: cellulose (30-50%), hemicellulose (15-35%) and lignin (10-20%) [42]. Almost 70% of the entire biomass is made up of hemicelluloses and cellulose. They are tightly linked to the lignin component by covalent and hydrogen bonding, making the structure highly tough and resistant to treatment.

Hemicellulose: Hemicellulose, also known as polyose, is an amorphous and variable structure made up of heteropolymers of hexoses (D-glucose, D-galactose, D-mannose) and pentoses (D-xylose, L-arabinose). It may also contain sugar acids like D-glucuronic, D-galacturonic and methylgalacturonic acids [43]. Its backbone is made up of xylan β(1→4) linkages [42]. Xylan composition varies in each feedstock [44]. Due to the presence of different sugars, hemicellulose requires a range of enzymes to be completely hydrolysed to free monomers. However, hemicellulose is more readily hydrolysed compared to cellulose because of its branched, amorphous nature [45].

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Cellulose: Cellulose fibres provide strength to wood and comprise 40-50% weight of dry wood [46]. Cellulose, found in the plant cell wall, consists of long chains of glucose monomers linked by β(1→4) glycosidic bonds, with extensive hydrogen linkages among molecules leading to a crystalline and strong matrix structure [47]. Each cellulose molecule can have up to several thousand glucose units. The molecules are strong and compact due to the cross linkages of numerous hydroxyl groups. Cellulose requires a temperature of 320°C and a pressure of 25 MPa to shift from a rigid crystalline structure to an amorphous structure in water [48].

Lignin: Lignin is an aromatic and rigid biopolymer bonded by covalent bonds to xylan, providing rigidity and a high level of compactness to the plant cell wall [49]. Lignin is comprised of three phenolic monomers of phenyl propionic alcohol namely, coumaryl, coniferyl and sinapyl alcohol [38]. Among woody biomass, softwood barks have the highest level of lignin (30-60%) followed by hardwood barks (30-55%) while agricultural residues contain the lowest level of lignin (3-15%) [50, 51]. Lignin components have been received with little interest previously, but are now gaining importance because of their dilution effect on the process of fermentation in bioreactors [52]. Phenolic groups are formed from the degradation of lignin which significantly deactivates cellulolytic enzymes thus influencing enzymatic hydrolysis. This negative impact of lignin has led to interests in lowering the lignin negative effect [53]. Although lignin modification via genetic engineering practises that target biosynthetic pathways could be performed to reduce lignin formation and improve ethanol yield, this process may prove to be problematic as lignin components serve as the major plant defence system to pathogens and insects. Thus its modification could disrupt the plants' natural protection [54,55].

PROCESSING OF LIGNOCELLULOSICS FEEDSTOCK TO BIOETHANOL

The bioconversion of cellulose and hemicellulose to monomeric sugars is hard to accomplish. Efficient de-polymerization of cellulose and hemicelluloses to soluble sugars is required.

Pre-treatment
The first step in bioconversion of lignocellulosics to bioethanol is size reduction and pre-treatment [56]. The objective of pre-treatment technology is to alter or remove structural and compositional impairments in order to improve the rate of enzyme hydrolysis and increase yields of fermentable sugars from cellulose and hemicelluloses [57]. Pre-treatment has been viewed as one of the most expensive processing steps within the conversion of biomass to fermentable sugars. During pre-treatment, the matrix of cellulose and lignin bound by hemicelluloses chains needs to be broken in order to reduce the degree of crystallinity of cellulose and increase the fraction of amorphous cellulose. Amorphous cellulose is the most suitable form for enzymatic attack. Additionally, the main part of hemicellulose should be hydrolysed and lignin should be released or degraded [58]. The purpose of pre-treatment is to remove lignin and the hemicellulose, reduce cellulose crystallinity and increase the porosity of the materials. Pre-treatment must also meet the following requirements: (1) improve the formation of sugars or the ability to subsequently form sugars by enzymatic hydrolysis, (2) avoid the degradation or loss of carbohydrate, (3) avoid the formation of by-products inhibitory to the subsequent hydrolysis and fermentation processes, (4) to reduce energy demands, and (5) be cost-effective [14,27]. For pre-treatment of lignocelluloses, several physical, physic-chemical, chemical and biological processes have been proposed and developed.

Physical pre-treatment
Mechanical comminution: Waste materials can be comminuted by a combination of chipping, grinding, and milling to reduce cellulose crystallinity [27] and improve the efficiency of downstream processing. The size of the materials is usually 10-30mm after chipping, and 0.2-2mm after grinding or milling [59]. Wet milling, dry milling, vibratory ball milling, and compression milling are usually done [14]. Vibratory milling has been found to be more effective than ordinary ball milling in reducing cellulose crystallinity and in improving digestibility [60]. Power requirements of mechanical comminution depend on the initial and final particle size, moisture content, and waste biomass characteristics [61]. Size reduction may provide better results but very fine particles may have negative effects on subsequent processing steps like by generating clumps in steps involving liquid, and may thereafter lead to channelling [14]. Mechanical pre-treatment techniques are time consuming, energy demanding, and expensive to process. Compression milling is apparently the poly comminution process that has been tested using a production scale apparatus [9].

Pyrolysis: Pyrolysis is an endothermic process where less input of energy is required. Here, materials are treated at temperatures greater than 300°C where cellulose rapidly decomposes to produce gaseous products like H_2 and CO, and residual char [62]. The decomposition is much slower and less volatile products are formed at lower temperatures [58, 63]. Residual char can be further treated by leaching with water or mild acid [14]. Studies have shown 80-85% conversion to cellulose to reducing sugars with more than 50% glucose through mild acid leaching (1N H_2SO_4) at 95°C for one hour [64]. The water leachete contains enough carbon source to support microbial growth for bioethanol

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production, where glucose is the main component. An average of 55% of the total weight of biomass is lost during water leaching [65].

Microwave oven and electron beam irradiation pre-treatment: Pre-treatment of lignocellulosic biomass in a microwave oven is also a feasible method which uses the high heating efficiency of a microwave oven and is also easy to operate [66]. Microwave treatment utilizes thermal and non-thermal effects generated by microwaves in aqueous environments. In thermal method, vibration of polar bonds in the biomass and surrounding aqueous medium results in the generation of internal heat in the biomass. This unique heating feature results in an explosion effect among the particles and improves the disruption of recalcitrant structures of lignocellulose [67]. Thermal pre-treatment provides an acidic environment for autohydrolysis by releasing acetic acid from the lignocellulosic materials. In non-thermal method, that is electron beam irradiation method, polar bonds vibrate, as they are aligned with a continuously changing magnetic field. The disruption and shock to the polar bonds accelerates chemical, biological and physical processes [68]. High energy radiation results in more changes in cellulosic biomass including increase of specific surface area decrease of polymerization and chrystallinity of cellulose, hydrolysis of hemicelluloses, and partial de-polymerization of lignin.

Physico-chemical pre-treatment
Steam explosion (autohydrolysis): Steam explosion is one of the biomass fractionation processes. Here, chipped biomass is treated with high-pressure saturated steam and then the pressure is swiftly reduced which makes the materials undergo an explosive decompression [27]. It is typically initiated at a temperature of 160-260°C with corresponding pressure of 0.69-4.83MPa for several seconds to a few minutes before the material is exposed to atmospheric pressure. Steam explosion treatment on lignocellulosics have the following effects [69]: (1) increases crystallinity of cellulose by promoting crystallization of the amorphous portions, (2) hemicelluloses is easily hydrolysed, (3) it promotes delignification, and (4) it increases the potential of cellulose hydrolysis. The factors that affect steam explosion pre-treatment are residence time, temperature, chip size, and moisture content [70]. Recent studies indicate that lower temperature and longer residence time is more favourable [71]. Addition of H_2SO_4 or SO_2 or CO_2 in steam explosion can effectively improve enzymatic hydrolysis, decrease the production of inhibitory compounds and lead to more complete removal of hemicelluloses [72]. The advantages of steam explosion pre-treatment include low energy requirements compared to mechanical comminution and no recycling or environmental costs. Conventional mechanical methods require 70% more energy than steam explosion to achieve the same size reduction [73]. Limitations of steam explosion include destruction of a portion of the xylan fraction, incomplete disruption of the lignin-carbohydrate matrix, and generation of compounds that may be inhibitory to microorganisms used in downstream processes [74]. Because of the formation of inhibitory products that inhibit microbial growth, enzymatic hydrolysis and fermentation, pre-treated biomass needs to be washed by water to remove the inhibitory materials along with water soluble hemicellulose [75].

Ammonia fibre explosion (AFEX): AFEX is one of the alkaline physico-chemical pre-treatment processes. Here, the material is subjected to liquid ammonia at high temperature and pressure and the subsequent fast decompression similar to the steam explosion which causes a fast saccharification of the lignocellulosic material [76]. In a typical AFEX process, the amount of liquid ammonia used is 1-2 kg ammonia per kg of dry biomass, the temperature is 363K and the residence time is 30 minutes [27]. The effective parameters involved are ammonia loading, temperature, water loading, blowdown pressure, time, and number of treatments [77]. This system does not directly liberate any sugars but allows the polymers to be attacked enzymatically and reduced to sugars [78]. AFEX markedly improves the saccharification rates of numerous herbaceous crops and grasses [75]. It has been applied to various lignocellulosic raw materials like rice straw, municipal solid wastes, newspaper, sugar beet pulp, sugar cane bagasse, corn stover, switchgrass, miscanthus, apsen chips, etc. [79]. AFEX works only moderately and is not attractive for biomass that contains high amounts of lignin. Since grasses contain relatively lower amounts of lignin (15-20%) than hardwood and softwood (20-35%), grasses can be more easily digested by AFEX treatment [80]. Ammonia must be recycled after the pre-treatment to reduce the cost and protect the environment [77].

Liquid hot-water pre-treatment (LHW): Cooking of lignocellulosic materials in LHW is one of the hydrothermal pre-treatment methods applied for pre-treatment of lignocellulosic materials. This method has been used for several decades, for example in pulp industries [77]. LHW subjects biomass to hot water in liquid state at high pressure for a fixed period. It presents elevated recovery rates for pentoses and generates low amount of inhibitors [81]. This process usually involves temperatures of 473-503K for durations up to 15 minutes. Around 40-60% of the total mass is dissolved in this process, with 4-22% of the cellulose, 35-60% of the lignin, and all of the hemicelluloses being removed [82]. If the pH can be maintained between 4 and 7, degradation of monosaccharide sugars can be minimised [83].

CO_2 explosion: Similar to steam and ammonia explosion pre-treatment, CO_2 explosion is also used for pre-treatment of lignocellulosic materials. It was hypothesized that CO_2 would form carbonic acid and increase the hydrolysis rate [27]. The yields are relatively low when compare to steam or ammonia explosion pre-treatment, but high when compared to

enzymatic hydrolysis without pre-treatment. CO_2 explosion when compare to steam and ammonia explosion was found to be more cost effective and did not cause the formation of inhibitory compounds [84].

Chemical pre-treatment
Ozonolysis: Ozonolysis involves using ozone gas to break down the lignin and hemicellulose and increase the biodegradability of the cellulose. It is usually carried out at room temperature and is effective at lignin removal without the formation of toxic by-products [85]. Ozonation has been widely used to reduce the lignin content of both agricultural and forestry wastes [86]. Ozonolysis pre-treatment has the following advantages: (1) it effectively removes lignin, (2) does not produce toxic residues from downstream processes, and (3) reactions are carried out at room temperature and pressure. However, a large amount of ozone is required, making the process expensive.

Alkaline hydrolysis: Alkali pre-treatment refers to the application of alkaline solutions to remove lignin and various uronic acid substitutions on hemicelluloses that lower the accessibility of enzyme to the hemicelluloses and cellulose [87]. The mechanism of alkaline hydrolysis is believed to be saponification of inter-molecular ester bonds cross-linking xylan hemicelluloses and other components like lignin. The porosity of lignocellulosic materials increases with the removal of cross-links [88]. Dilute NaOH treatment of lignocellulosic materials causes swelling and leads to an increase in internal surface area, a decrease in the degree of polymerization, decrease in crystallinity, separation of structural linkages between lignin and carbohydrates, and disruption of the lignin structure [64]. The process also utilises lower temperatures and pressures compared to other pre-treatment technologies. Lime ($Ca(OH)_2$) as compared to NaOH and KOH has lower cost and less significant safety requirements. It can be recovered from the hydrolysate by reaction with CO_2, so that formed carbonate can be reconverted to lime [89]. To make lime as efficient as other alkalis in enhancing the digestibility of lignocellulose, appropriate pre-treatment conditions need to be employed.
Alkaline peroxide is one of the effective pre-treatment methods that can improve the enzymatic hydrolysis by delignification of lignocellulosic materials. Here, the lignocelluloses are soaked in pH adjusted water containing H_2O_2 at room temperatures for a period of time (6-24 hours) [77].

Acid hydrolysis: Acid hydrolysis is considered as one of the most important techniques that aims for high yields of sugars from lignocellulosics. It is usually carried out by concentrated or diluted acids such as H_2SO_4, HNO_3 or HCl, at temperatures between 130°C and 210°C in order to remove hemicelluloses components and expose cellulose for enzymatic digestion [87]. Although they are very powerful agents for cellulose hydrolysis, concentrated acids are toxic, corrosive and hazardous, and thus require reactors that are resistant to corrosion. The concentrated acid must be recovered after hydrolysis to make the process economically feasible [90]. Acid pre-treatment can utilise either dilute or concentrated acids to improve cellulose hydrolysis. The acid medium attacks the polysaccharides, especially hemicelluloses which are easier to hydrolyse than cellulose [91]. Dilute acid hydrolysis using dilute sulphuric acid at high temperature can achieve high reaction rates and significantly improve cellulose hydrolysis [92]. Recently developed dilute acid hydrolysis processes use less severe conditions and achieve high xylan to xylanose conversion yields, which is necessary to achieve favourable overall process economics because xylan accounts for up to a third of the total carbohydrate in many lignocellulosic materials [93]. There are two types of dilute acid pre-treatment processes: (1) high temperature (greater than 160°C), continuous flow process for low solids loading (5-10% w/w) [94], and (2) low temperature (less than 160°C), batch process for high solids loading (10-40% w/w) [95]. A neutralization of pH is necessary for the downstream enzymatic hydrolysis or fermentation processes.

Organosolv process: In the organosolv process, an organic or aqueous organic solvent mixture with inorganic acid catalysts like HCl or H_2SO_4 is used to break the internal lignin and hemicelluloses bonds. This process also known as organosolv pulping process is an alternative method the delignification of lignocellulosic materials. The organic solvents used in the process include methanol, ethanol, acetone, ethylene glycol, triethylene glycol, and tetrahydrofurfuryl alcohol [96]. Organic acids such oxalic, acetylsalicylic, and salicylic acid can also be used as catalysts in the organosolv process [97]. The utilization of organic solvent/water mixtures eliminates the need to burn the liquor and allows the isolation of lignin (by distillation of the organic solvent). At high temperatures (above 185°C) the addition of catalyst is unnecessary for satisfactory delignification [97]. A high yield of xylose can be obtained with the addition of acid. Solvents used in the process need to be drained from the reactor, evaporated, condensed, and recycled to reduce the cost. Removal of the solvents from the system is necessary because the solvents may be inhibitory to the growth of the organisms, enzymatic hydrolysis and fermentation.

Wet oxidation: In wet oxidation, the feedstock material is treated with water and either by air or oxygen at temperatures above 120°C [98]. The water is added at a ratio of 1 litre per 6gms of biomass. The transfer of hemicelluloses from sold to liquid phase is promoted by this technique. The products of hemicelluloses hydrolysis during wet oxidation are sugar oligomers [91].

Oxidative delignification: Lignin biodegradation could be catalysed by peroxidase enzyme in presence of H_2O_2 [99]. Studies have shown that this process enhances the susceptibility of lignocellulosic materials to enzymatic hydrolysis.

About 50% lignin and most hemicellulose can be solubilized by 2% H_2O_2 at 30°C within 8 hours with efficiencies as high as 95% of glucose production from cellulose when treated by cellulase at 45°C for 24 hours [99].

Biological pre-treatment: In biological pre-treatment processes, microorganisms such as brown rot, white rot, and soft rot fungi are used to degrade lignin and hemicelluloses in waste materials [100]. Brown rots attack cellulose while white and soft rots attack both cellulose and lignin. White rots have been found to be the most effective basidiomycetes for biological pre-treatment of lignocellulosic materials [64]. Cellulase-less mutant was developed for the selective degradation of lignin and to prevent the loss of cellulose. However, in most cases the rate of hydrolysis is very low. This method is a safe and energy saving process due to less mechanical support [24]. It needs no chemicals but low hydrolysis and low yields obstruct its implementation [77]. In the case of marine organisms, it was seen that when the substrate was accompanied with a nutrient medium such as Kirk's medium, better delignification was observed compared to sterilized water [91]. Bio-delignification generally requires longer periods of time. Advantages of biological pre-treatment include low energy requirement and mild environmental conditions.

There are several factors that are responsible for the low cost and an advanced pre-treatment [101], like high yield for multiple crops depending on site, ages and harvesting times; highly digestible pre-treated solids; no significant sugar degradation; formation of minimum amount of toxic compounds; size reduction not being actually necessary; process should be carried out in reasonably sized and moderately priced reactors; non-production of solid waste residues that may create disposal issues; effectiveness of the process at low moisture contents; obtaining high sugar concentration; fermentation compatibility; lignin recovery; and, minimum heat and power requirements.

Enzymatic hydrolysis

Cellulase enzyme is the primary enzyme used to carry out enzymatic hydrolysis of cellulose. The products of hydrolysis are usually reducing sugars including glucose. When compared to acid or alkaline hydrolysis, the cost of enzymatic hydrolysis is quite low. It is carried out usually at mild conditions with pH of around 4.8 and temperature ranging between 45-50°C. This technique does not have a corrosive problem [70]. Since microorganisms like bacteria and fungi are capable of producing cellulases for their own metabolic activities, these organisms can be used to produce cellulose for enzymatic hydrolysis for cellulose on an industrial scale. These microbes can be aerobic or anaerobic, mesophilic or thermophilic. Bacteria belonging to species like *Clostridium, Cellulomonas, Bacillus, Thermomonospora, Ruminococcus, Dacteriodes, Erwinia, Acetovibrio, Microbispora,* and *Streptomyces* can produce cellulases [102]. Although many cellulolitic bacteria, especially the anaerobes such as *Clostridium* produce cellulases with high specific activity, they do not produce high enzyme titres [70]. Because anaerobes have a very low growth rate and require anaerobic growth conditions, most research for commercial cellulase production has been focussed on fungi [70].

Fungal species like *Trichoderma, Aspergillus, Schizophyllum, and Penicillium* [103] have been reported to produce cellulases. Of all these fungal species, *Trichoderma* has been the most extensively studied organism for cellulose production. This species has been widely used for enzymatic hydrolysis because of the production of a substantial amount of cellulase. However, it is unable to produce sufficient amounts of β-glucosidase, and hence needs to be used in a concoction along with other fungal species that are able to produce β-glucosidase.

Cellulases are usually a mixture of several enzymes. There are three major groups of cellulases involved in enzymatic hydrolysis: (1) endoglucanase, which attacks regions of low crystallinity in the cellulose fibre, creating free chain-ends; (2) exoglucanase or cellobiohydrolase, which degrades the molecule further by removing cellobiose units from the free chain-ends; and (3) β-glucosidase, which hydrolyses cellobiose to produce glucose [104]. During enzymatic hydrolysis, cellulose is degraded by cellulases to reducing sugars that can be fermented by yeast or bacteria to ethanol. Factors that affect enzymatic hydrolysis are substrate concentration, cellulase activity, and reaction conditions. Substrate concentration affects the yield and the initial rate of enzymatic hydrolysis of cellulose. At low substrate concentration, there is an increase of yield and reaction rate of hydrolysis [105]. However, high substrate concentration may cause substrate inhibition which lowers the rate of hydrolysis [106]. Lignin interferes with hydrolysis by blocking the access of cellulases to cellulose and by irreversibly binding hydrolytic enzymes. Thus, removal of lignin can increase the hydrolysis rate by a fair amount [75]. Increasing the dosage of cellulases in the process can enhance the yield and rate of hydrolysis to a certain extent, but it would significantly increase the cost of the process. Cellulase activity is inhibited by cellobiose and glucose. Several methods have been developed to reduce the inhibition including the use of high concentrations of enzyme, supplementation of β-glucosidases during hydrolysis, and the removal of sugars by ultrasound or simultaneous saccharification and fermentation (SSF).

Substrate-related factors limiting enzymatic hydrolysis are directly connected to the pre-treatment employed. Some such factors are: (1) Cellulose crystallinity, where degree of polymerization and cellulose crystallinity determine the hydrolysis rates of relatively refined cellulosic substrates [107]; (2) Number of glycosyl residues per cellulose chain, where decreased chain length reduces the degree of polymerization thereby having an impact on cellulose hydrolysis; (3) Available surface area on substrates, where increase in substrate surface area increases the accessibility of the substrate to the cellulolytic enzymes; (4) Lignin barrier, makes accessibility of cellulase enzymes to cellulose difficult thereby limiting the rate enzymatic hydrolysis; (5) Removal of hemicellulose increases the mean pore size of the substrate and therefore increases the accessibility and probability of the cellulose to become hydrolysed [108]; (5) Feedstock particle size, where reduced particle size increases the specific surface area and subsequently the accessibility of cellulose to enzymes [27]; (6) Porosity, that helps cellulases to get trapped in the pores if the porosity is high, which

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improves hydrolysis; (7) Waxy barriers present in plant cell walls impedes penetration of enzymes; (8) Change in accessibility of glucan with conversion [109].

Fermentation

The saccharified biomass is used for fermentation by several microorganisms. The industrial utilization of lignocellulosic biomass for bioethanol production is hindered by the lack of ideal microorganism which can ferment both pentoses and hexoses efficiently [24]. For ethanol production on an industrial scale, an ideal microorganism should be selected which has broad substrate utilization, high ethanol yield and productivity, should have the ability to withstand high concentrations of ethanol and high temperature, should be tolerant to inhibitors present in the hydrolysate and have cellulolitic activity. Due to these reasons, microorganisms are modified or genetically engineered to achieve complete utilization of the sugars in the hydrolysate and for better production benefits. Generally, the process employs fermentation of lignocellulosic hydrolysate in SSF. SSF can be used for improved ethanol yields by removing end products' inhibition and eliminating the need for separate reactors. It is a cost effective process.

Fermentation involves microorganisms that use the fermentable sugars as food and produces ethyl alcohol and other by-products. These microorganisms typically utilize 6-carbon sugars like glucose. One of the most effective bioethanol producing microbes is yeast, *Saccharomyces cerevisiae.* Use of yeast has its advantage owing to its high bioethanol production from hexoses and its high tolerance to bioethanol and other inhibitory compounds. Bacteria showing the most promise for industrial exploitation are *Escherichia coli, Klebsiella oxytoca* and *Zymomonas mobilis* [110]. *Zymomonas* is known for its ability to rapidly and efficiently produce bioethanol from glucose based feedstocks, and has shown to achieve 5% higher yields and up to five-fold higher volumetric productivity when compared with traditional yeast fermentations. Productivity of microorganisms depend on the process parameters like temperature range, pH range, alcohol tolerance, growth rate, productivity, osmotic tolerance, substrate specificity, yield, genetic stability, and inhibitor tolerance [110]. The microorganisms function best between 303-311 K [111]. They generally prefer a narrow pH range of 6.5-7.5 [112]. The majority of microorganisms cannot tolerate bioethanol concentrations above 10-15% (w/v) [111].

Fermentation can be carried out in batch, fed-batch or continuous reactors, depending upon the kinetic properties of microorganisms and the type of lignocellulosic hydrolysate in addition to process economics aspects [113]. Fed-batch reactors are widely used in the industry because it combines the advantages of both batch and continuous processes [114]. The major advantage of fed-batch reactors is the ability to increase maximum viable cell concentration, prolong culture lifetime, and allow product accumulation to a higher concentration [115]. This process allows for the maintenance of critical process variables, like temperature, pH, and dissolved oxygen, at specific levels through feedback control [116].

DISCUSSION AND CONCLUSION

The demand for biofuels is on the rise due to the depletion of non-renewable energy sources and the need for clean, green fuels. Bioethanol is currently used as a biofuel worldwide, and is being developed as a transport fuel to be used as an alternative to gasoline in the near future. It is currently used as a blended automobile fuel to significantly reduce petroleum consumptions and exhaust greenhouse gas emissions. Bioethanol is derived from lignocellulosic feedstock like rice, wheat, sugarcane, sugar beets, grass straw and wood. A lot of studies are being carried out to find out good microbial sources for the enzymatic hydrolysis and fermentation of cellulose to produce bioethanol. It has been seen that biomass containing high levels of glucose or precursors of glucose are easiest to convert to bioethanol. Genetically engineered microbes are created to produce adequate amounts of cellulases and also to enable them to remain active in environments with higher product concentrations.

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