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Review

Bioethanol production from agricultural wastes: An overview

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ABSTRACT

Due to rapid growth in population and industrialization, worldwide ethanol demand is increasing continuously. Conventional crops such as corn and sugarcane are unable to meet the global demand of bioethanol production due to their primary value of food and feed. Therefore, lignocellulosic substances such as agricultural wastes are attractive feedstocks for bioethanol production. Agricultural wastes are cost effective, renewable and abundant. Bioethanol from agricultural waste could be a promising technology though the process has several challenges and limitations such as biomass transport and handling, and efficient pretreatment methods for total delignification of lignocellulosics. Proper pretreatment methods can increase concentrations of fermentable sugars after enzymatic saccharification, thereby improving the efficiency of the whole process. Conversion of glucose as well as xylose to ethanol needs some new fermentation technologies, to make the whole process cost effective. In this review, available technologies for bioethanol production from agricultural wastes are discussed.

1. Introduction

The world's present economy is highly dependent on various fossil energy sources such as oil, coal, natural gas, etc. These are being used for the production of fuel, electricity and other goods [1]. Excessive consumption of fossil fuels, particularly in large urban areas, has resulted in generation of high levels of pollution during the last few decades. The level of greenhouse gasses in the earth's atmosphere has drastically increased [2]. With the expansion of human population and increase of industrial prosperity, global energy consumption also has increased gradually. Import of transport fuel is affected by limited reserves of fossil fuel. Annual global oil production will begin to decline within the near future [3]. In this scenario, renewable sources might serve as an alternative. Wind, water, sun, biomass, geothermal heat can be the renewable sources for the energy industry whereas fuel production and the chemical industry may depend on biomass as an alternative source in the near future [4]. All petroleum-based fuels can be replaced by renewable biomass fuels such as bioethanol, bio-diesel, bio-hydrogen, etc., derived from sugarcane, corn, switchgrass, algae, etc. Requirements of electricity may be supplied by solar- and wind-farms. The energy consumption rate includes each person's share of electricity and fuel used in making foods and goods and their transport. Biogas has also been identified as a possible motor fuel on organic farms in the short and medium terms. Biogas is produced by anaerobic digestion of organic material. When used as biofuel, CO_2 is removed from the gas to increase the energy content and the gaseous fuel can be stored at high pressure. Biogas can be substituted for natural gas or propane as fuel for boilers and for electricity generation in rural areas. Approximately 1281 mega watt biogas is potentially produced from agrowastes in India [5]. Annual methane production in Sweden from organic waste is about 38 PJ, catering to 11% of the domestic energy requirement for transportation in 2007 and projected to be sufficient for fulfilling the EU target for 2020 [6].

Countries across the globe have considered and directed state policies toward the increased and economic utilization of biomass for meeting their future energy demands in order to meet carbon dioxide reduction targets as specified in the Kyoto Protocol as well as to decrease reliance and dependence on the supply of fossil fuels. Although biomass can be a huge source of transport fuels such as bioethanol, biomass is commonly used to generate both power and heat, generally through combustion. Ethanol is at present the most widely used liquid biofuel for motor vehicles [7,8]. The importance of ethanol is increasing due to a number of reasons such as global warming and climate change. Bioethanol has been receiving widespread interest at the international, national and regional levels. The global market for bioethanol has entered a phase of rapid, transitional growth. Many countries around the world are shifting their focus toward renewable sources for power production because of depleting crude oil reserves. The trend is extending to transport fuel as well. Ethanol has potential as a valuable

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replacement of gasoline in the transport fuel market. However, the cost of bioethanol production is more compared to fossil fuels. The world bioethanol production in 2001 was 31 billion liters [19]. It has grown to 39 billion liters in 2006 and is expected to reach 100 billion liters in 2015 [9]. Brazil and the USA are the two major ethanol producers accounting for 62% of the world production [18]. Large scale production of fuel ethanol is mainly based on sucrose from sugarcane in Brazil or starch, mainly from corn, in the USA. Current ethanol production based on corn, starch and sugar substances may not be desirable due to their food and feed value. Economy of the ethanol production process from grains is dependent on the market of its by-product – distillers' dried grains with solubles (DDGS) – as animal food. The market of DDGS may not expand like that of ethanol in the future [9]. Cost is an important factor for large scale expansion of bioethanol production. The green gold fuel from lignocellulosic wastes avoids the existing competition of food versus fuel caused by grain based bioethanol production [20]. It has been estimated that 442 billion liters of bioethanol can be produced from lignocellulosic biomass and that total crop residues and wasted crops can produce 491 billion liters of bioethanol per year, about 16 times higher than the actual world bioethanol production [18]. Lignocellulosic materials are renewable, low cost and are abundantly available. It includes crop residues, grasses, sawdust, wood chips, etc. Extensive research has been carried out on ethanol production from lignocellulosics in the past two decades [10–12]. 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 [18]. This review aims to present a brief overview of the available and accessible technologies for bioethanol production using these major agrowastes.

2. Raw material

The four major agrowastes mentioned in the preceding section are the most favorable feedstocks for bioethanol production due to their availability throughout the year. Worldwide production of these agrowastes is given in Table 1. Asia is the major producer of rice straw and wheat straw, whereas corn straw and bagasse are mostly produced in America (Table 1). They also vary in chemical composition (Table 2), cellulose being the major component.

These agro-residues are also utilized as animal fodder, as domestic fuel, and as fuel to run boilers. The utilization fraction of wheat straw, rice straw and corn straw is too low and varies with geographic region [18]. Each year a large portion of agricultural residues is disposed of as waste. For instance, approximately 600–900 million tons per year rice straw is produced globally [13]. 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 [14–17]. Open field burning is already banned in many countries in Western Europe and some other countries have considered it seriously. Less than 1% of corn straw is collected for industrial processing and about 5% is used as animal feed and bedding. More than 90% of corn straw in United States is left in the fields [22]. Sugarcane bagasse has its prominent use as a fuel for boilers and for cogeneration of electricity [23]. Globally, bioethanol production from rice straw, wheat straw, corn straw and sugarcane bagasse 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.

Lignocellulose is a complex carbohydrate polymer of cellulose, hemicellulose and lignin. Cellulose is linear and crystalline. It is a homopolymer of repeating sugar units of glucose linked by β-1,4 glycosidic bonds. Hemicellulose is a short and highly branched polymer. It is a heteropolymer of D-xylose, D-arabinose, D-glucose, D-galactose, and D-mannose. Lignin is hydrophobic in nature and is tightly bound to these two carbohydrate polymers. It thus protects these polymers from microbial attack [24]. It is a three-dimensional aromatic polymer of p, hydroxyphenylpropanoid units connected by C–C and C–O–C links. Sugar compositions of various agrowastes (rice straw, wheat straw, corn straw, bagasse) are given in Table 4 [25].

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 [21].

3. Pretreatment

The most important processing challenge in the production of biofuel is pretreatment of the biomass. Lignocellulosic biomass is composed of three main constituents namely hemicellulose, lignin and cellulose. Pretreatment methods refer to the solubilization and separation of one or more of these components of biomass. It makes the remaining solid biomass more accessible to further chemical or biological treatment [7]. The lignocellulosic complex is made up of a matrix of cellulose and lignin bound by hemicellulose chains. The pretreatment is done to break the matrix in order to reduce the degree of crystallinity of the cellulose and increase the fraction of amorphous cellulose, the most suitable form for enzymatic attack [26]. Pretreatment is undertaken to bring about

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

Agrowaste	Africa	Asia	Europe	America	Oceania	Reference
Rice straw	20.9	667.6	3.9	37.2	1.7	[18,21]
Wheat straw	5.34	145.20	132.59	62.64	8.57	[18]
Corn straw	0.00	33.90	28.61	140.86	0.24	[18]
Bagasse	11.73	74.88	0.01	87.62	6.49	[18]

Table 2
Chemical composition of agricultural wastes.

Substrate	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Protein (%)	Ash (%)	Reference (%)
Rice straw	32–47	19–27	5–24	–	12.4	[13,25]
Wheat straw	35–45	20–30	8–15	3.1	10.1	[24,75]
Corn straw	42.6	21.3	8.2	5.1	4.3	[75]
Baggase	65 (total carbohydrate)		18.4	3	2.4	[48,85]

Table 3
Worldwide potential bioethanol production from agricultural wastes.

Agricultural residue	Potential annual bioethanol production (globally) (giga liter)	Reference
Rice straw	205	[18]
Wheat straw	104	[85]
Corn straw	58.6	[18]
Sugarcane bagasse	51.3	[18]

Table 4
Carbohydrate content of agricultural waste (%).

	Glucose	Xylose	Mannose	Galactose	Arabinose	Reference
Rice straw	41–43.4	14.8–20.2	1.8	0.4	2.7–4.5	[13]
Wheat straw	38.8 ± 0.5	22.2 ± 0.3	1.7 ± 0.2	2.7 ± 0.1	4.7 ± 0.1	[86]
Corn straw	39	14.8	0.3	0.8	3.2	[25]
Bagasse	38.1	23.3	-	1.1	2.5	[25]

a change in the macroscopic and microscopic size and structure of biomass as well as submicroscopic structure and chemical composition. It makes the lignocellulosic biomass susceptible to quick hydrolysis with increased yields of monomeric sugars [27]. Goals of an effective pretreatment process are (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. Physical, chemical, physicochemical and biological treatments are the four fundamental types of pretreatment techniques employed. In general a combination of these processes is used in the pretreatment step.

3.1. Physical pretreatment

3.1.1. Mechanical size reduction

The first step for ethanol production from agricultural solid wastes is comminution through milling, grinding or chipping. This reduces cellulose crystallinity [28] and improves the efficiency of downstream processing. Wet milling, dry milling, vibratory ball milling and compression milling are usually done. The power input for mechanical comminution of agricultural materials depends on the initial and final particle sizes, moisture content and on the nature of waste (hardwood, softwood, fibrous, etc) being handled [28,29]. Size reduction may provide better results [20,30] but very fine particle size may impose negative effects on the subsequent processing such as pretreatment and enzymatic hydrolysis. It may generate clumps during the subsequent steps involving liquid and may lead to channeling. Specific energy consumption also increases. The specific energy consumptions for grinding wheat straw with hammer mill screen sizes of 0.8 and 3.2 mm were 51.6 and 11.4 kW h t^{-1}, respectively [29]. It is advisable to use hammer mill or ball mill for hardwood and cutter mill for softwood. Ball milling (BM) and wet disk milling (WDM) are other processes which can be used for comminution [31].

3.1.2. Pyrolysis

Pyrolysis is an endothermic process where less input of energy is required. In this process the materials are treated at a temperature greater than 300 °C, whereby cellulose rapidly decomposes to produce gaseous products such as H_2 and CO and residual char. The decomposition is much slower and less volatile products are formed at lower temperatures [26,32,33]. The residual char is further treated by leaching with water or with mild acid. The water leachate contains enough carbon source to support microbial growth for bioethanol production. Glucose is the main component of water leachate. An average of 55% of total weight of biomass is lost during water leaching [87].

Fan et al. [34] have shown 80–85% conversion of cellulose to reducing sugars with more than 50% glucose through mild acid leaching (1 N H_2SO_4, 95 °C, 1 h).

3.1.3. Microwave oven and electron beam irradiation pretreatment

Pretreatment of lignocellulosic biomass in a microwave oven is also a feasible method which uses the high heating efficiency of a microwave oven and it is also easy to operate [20]. Microwave treatment utilizes thermal and non-thermal effects generated by microwaves in aqueous environments. In the thermal method, internal heat is generated in the biomass by microwave radiation, resulting from the vibrations of the polar bonds in the biomass and the surrounding aqueous medium. Thus a hot spot is created within the inhomogeneous material. This unique heating feature results in an explosion effect among the particles and improves the disruption of recalcitrant structures of lignocellulose [35]. Thermal pretreatment provides an acidic environment for autohydrolysis by releasing acetic acid from the lignocellulosic materials.

In the non-thermal method, i.e., the electron beam irradiation method, polar bonds vibrate, as they are aligned with a continuously changing magnetic field and the disruption and shock to the polar bonds accelerates chemical, biological and physical processes [36]. High energy radiation results in 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. Ooshima et al. [37] reported an improvement in total reducing sugar production by a factor of 1.6 and 3.2 for microwave radiated rice straw and bagasse respectively. Microwave pretreatment of rice straw and bagasse followed by lignin extraction has been reported to give a yield of 43–55% of total available reducing sugars [38].

3.2. Physicochemical pretreatment

3.2.1. Steam explosion or autohydrolysis

Steam explosion is a promising method of pretreatment which makes biomass more accessible to cellulase attack [39]. This method of pretreatment without the use of any catalyst is promising and the biomass fractionates to yield levulinic acid, xylitol and alcohols [21]. In this method the biomass is heated using high-pressure steam (20–50 bar, 160–290 °C) for a few minutes; the reaction is then stopped by sudden decompression to atmospheric pressure [26,39]. When steam is allowed to expand within the lignocellulosic matrix it separates the individual fibers [21]. The high recovery of xylose (45–65%) makes steam-explosion pretreatment economically attractive [39,40].

3.2.2. Liquid hot water method

The liquid hot water method uses compressed hot liquid water (at pressure above saturation point) to hydrolyze the hemicellulose [39]. It is a hydrothermal pretreatment method which releases high fraction of hemicellulosic sugars in the form of oligomers. The treatment generally occurs at temperatures of 170–230 °C and pressures above 5 MPa for 20 min. It, however, also contributes to the production of small amounts of undesired degrading compounds such as furfural, carboxylic acid, that are very toxic to ethanol fermentation as they inhibit microbial growth [29,41]. As xylose recovery is relatively high (88–98%), and no acid or chemical is required, it is an environmentally attractive and economically interesting method [39]. Yu et al. [42] studied two step liquid hot water treatment of *Eucalyptus grandis* and obtained a xylose recovery of 86.4%. Maximal glucose yield of 70–76% corresponding to 80% of xylan removal from soybean straw was obtained through combined liquid hot water and alkaline treatments [43].

3.2.3. Ammonia fiber explosion

Ammonia fiber explosion (AFEX) pretreatment involves liquid ammonia and steam explosion [21]. AFEX is an alkaline thermal pretreatment which exposes the lignocellulosic materials by high temperature and pressure treatment followed by rapid pressure release. This method does not produce inhibitors of the downstream processes and small particle size is not required for efficacy

[27,28]. This pretreatment has the drawbacks of being less efficient for biomass containing higher lignin contents (e.g. softwood newspaper) as well as of causing solubilization of only a very small fraction of solid material particularly hemicellulose [28,29]. The advantages are that it is simple and has a short process time. It is more effective for the treatment of substrates with less content of lignin compared to sugarcane. This system does not directly liberate any sugars, but allows the polymers (hemicellulose and cellulose) to be attacked enzymatically which break down to sugars. The AFEX process requires efficient ammonia recovery to be economical due to the high cost of ammonia. A possible approach is to recover the ammonia after the pretreatment by evaporation. The major parameters influencing the AFEX process are ammonia loading, temperature, high pressure, moisture content of biomass, and residence time [29]. Moderate temperatures of 60–100 °C are used and residence time may vary from low (5–10 min) to intermediate (30 min) depending on the degree of saturation of the biomass. Ammonia loading is done at 1 kg ammonia per kg of dry substrates [90–92]. The process optimization of AFEX pretreatment of different materials has been reported [44]. At optimal conditions, 90% cellulose and hemicellulose conversions have been achieved. In AFEX treatment, low enzyme loading is required compared to other pretreatment methods [45].

3.2.4. CO_2 explosion

CO_2 explosion acts in a manner similar to that of the steam and ammonia explosion techniques. However, CO_2 explosion is more cost effective than ammonia explosion and does not cause the formation of inhibitors as in steam explosion [32,40]. Conversion yields are higher compared to the steam explosion method [40].

3.3. Chemical pretreatment

Chemical pretreatment methods involve the usage of dilute acid, alkali, ammonia, organic solvent, SO_2, CO_2 or other chemicals. These methods are easy in operation and are have good conversion yields in short span of time.

3.3.1. Acid pretreatment

Acid pretreatment is considered as one of the most important techniques and 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 °C and 210 °C. Sulfuric acid is widely used for acid pretreatment among various types of acid such as hydrochloric acid, nitric acid and phosphoric acid [46]. Acid pretreatment can utilize either dilute or concentrated acids to improve cellulose hydrolysis [21]. The acid medium attacks the polysaccharides, especially hemicelluloses which are easier to hydrolyze than cellulose [46]. However, acid pretreatment results in the production of various inhibitors like acetic acid, furfural and 5 hydroxymethylfurfural. These products are growth inhibitors of microorganisms. Hydrolysates to be used for fermentation therefore need to be detoxified. Moiser et al. [47] reported higher hydrolysis yield from lignocellulose pretreated with diluted H_2SO_4 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 °C for 1 h [51].

3.3.2. Alkaline pretreatment

Alkaline pretreatment of lignocellulosics digests the lignin matrix and makes cellulose and hemicellulose available for enzymatic degradation [48]. Alkali treatment of lignocellulose disrupts the cell wall by dissolving hemicelluloses, lignin, and silica, by hydrolyzing uronic and acetic esters, and by swelling cellulose. Crystallinity of cellulose is decreased due to swelling. 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. The end residue (mainly cellulose) can be used to produce either paper or cellulose derivatives [46]. Hydroxides of sodium, potassium, calcium and ammonium are used in this process. Alkaline pretreatment processes utilize lower temperatures and pressures than other pretreatment technologies [27]. Sun et al. [49] studied the effectiveness of different alkaline solutions by analyzing the delignification and dissolution of hemicellulose in wheat straw. They found that the optimal process condition was that using 1.5% NaOH for 144 h at 20 °C, releasing 60% and 80% lignin and hemicellulose respectively. NaOH has been reported to increase hardwood digestibility from 14% to 55% by reducing lignin content from 24–55% to 20% [50].

3.3.3. Wet oxidation

In wet oxidation, the feedstock material is treated with water and either by air or oxygen at temperatures above 120 °C [52]. The water is added to the biomass at a ratio of 1 L per 6 g of biomass. The transfer of hemicelluloses from solid phase to the liquid phase is promoted in this technique. It does not hydrolyze the liberated hemicellulose molecules. The products of hemicellulose hydrolysis during wet oxidation are sugar oligomers [46]. There have been several studies on wet oxidation as a pretreatment strategy using different substrates [52–54]. Pedarson et al. [55] obtained yields of 400 and 200 g/kg of wet oxidation treated wheat straw for glucose and xylose respectively after 24 h at 50 °C using an enzyme mixture of 36 FPU/g celluclast–1.5 L and 37 CBU/g of Novozyme-188.

3.3.4. Organosolv pretreatment

Organic solvent or organosolv pulping processes are alternative methods for the delignification of lignocellulosic materials. The utilization of organic solvent/water mixtures eliminates the need to burn the liquor and allows the isolation of the lignins (by distillation of the organic solvent). Examples of such pretreatments include the use of 90% formic acid and that of pressurized carbon dioxide in combination (50% alcohol/water mixture and 50% carbon dioxide) [46]. Other various organic solvents which can be used for delignification are methanol, ethanol, acetic acid, performic acid and peracetic acid, acetone, etc. [56]. A combination of ammonia and ionic liquid pretreatments of rice straw resulted in 97% conversion of cellulose to glucose [88]. Different types of pretreatment and respective yields for sugarcane bagasse, wheat straw, rice straw, and corn straw are given in Table 5.

3.4. Biological pretreatment

Degradation of the lignocellulosic complex to liberate cellulose can be brought about with the help of microorganisms like brown rot, white rot and soft rot fungi. Biological pretreatment renders the degradation of lignin and hemicellulose [28,29,32] and white rot fungi seem to be the most effective microorganism. Brown rot attacks cellulose while white and soft rots attack both cellulose and lignin [32]. 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. This method is safe and energy saving due to less mechanical support [28,29]. It needs no chemicals but low hydrolysis rates and low yields impede its implementation [21,40]. Biological pretreatment of bamboo culms with white rot fungi has been performed at low temperature (25 °C) [89]. In the case of a marine microorganism *Phlebia* sp. MG-60, it was seen that when the substrate was supplemented with a nutrient medium such as Kirk's Medium, better delignification was observed compared to sterilized water [46]. Bio-delignification generally needs long periods of time

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

Substrate	Pretreatment	Hydrolysis	Yield of sugars	References
Sugarcane bagasse	Ball milling (4 h)	Enzymatic (Acremonium cellulase at 5 FPU/g substrate of cellulase and 20 U/g substrate of xylanase from Optimash BG at 45 °C, pH 5.0 for 72 h	89.2 ± 0.7% (glucose), 77.2 ± 0.9% (xylose)	[78]
	1% sulfuric acid (v/v) at 60 °C, 24 h (SLR 1:6)	In an autoclave at 121 °C for 40 min after removing the excess acid (1% (v/v) sulfuric acid)	Total sugar concentration of approximately 68.0 g/L	[83]
Wheat Straw	Knife milling with 0.7–1.0 mm rejection screen, washed with water and dried	At 90 °C with 1.85% (w:v) sulfuric acid 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	[82]
Rice straw	Chopped to 5–6 mm size range	4.4% sulfuric acid at 1:10 solid to liquid ratio in boiling water bath, 1 h, filtered and pH adjusted to 5.5	Total sugar (20 g/L)	[84]
		Soaked in water at 170 °C and 7.6 kg/cm^2, 30 min, finally cooled and pH adjusted to 5.5	Total sugar (23 g/L)	
	Chopped, steam exploded (3.5 MPa, 275 °C, 2 min)	Enzymatic saccharification (cytolase, novozyme) (50 °C, 120 h)	Xylose yield (10–5 g/L)	[81]
Corn straw	2% NaOH, 80 °C, 1 h	Enzymatic hydrolysis by cellulase of *Trichoderma reesei* ZU-02 and cellobiose of *Aspergillus niger* ZU-07	Xylose 23.6 g/L, glucose 56.7 g/L, arabinose 5.7 g/L	[68]

(Table 6). In a biological pretreatment study with an aim to release the sugars from the lignocellulosic matrix of sugarcane trash using a number of microorganisms it was observed that both cellulose and lignin contents of the raw material can be drastically reduced. Reduction in the cellulose content by *Aspergillus terreus* was about 55.2% while delignification was found to be about 92% [57].

4. Enzymatic hydrolysis

Saccharification is the critical step for bioethanol production where complex carbohydrates are converted to simple monomers. Compared to acid hydrolysis, enzymatic hydrolysis requires less energy and mild environment conditions [58]. The optimum conditions for cellulase have been reported as temperature of 40–50 °C and pH 4–5 [39]. Assay conditions for xylanase have also been reported to be 50 °C temperature and pH 4–5 [93]. Therefore, enzymatic hydrolysis is advantageous because of its low toxicity, low utility cost and low corrosion compared to acid or alkaline hydrolysis [28,59]. Moreover, no inhibitory by-product is formed in enzymatic hydrolysis [58]. However, enzymatic hydrolysis is carried out by cellulase enzymes that are highly substrate specific [23,59]. 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. Cellulase enzymes involve endo and exoglucanase and β-glucosidases. Endoglucanase (endo 1,4-D glucanhydrolase or E.C. 3.2.1.4) attacks the low crystallinity regions of the cellulose fiber, exoglucanase (1,4-β-D glucan cellobiohydrolase or E.C. 3.2.1.91) removes the cellobiase units from the free chain ends and finally cellobiose units are hydrolysed to glucose by β-glucosidase (E.C. 3.2.1.21) [23,59]. Hemicellulolytic enzymes are more complex and are a mixture of at least eight enzymes such as endo-1,4-β-D-xylanases, exo-1,4-β-D xylocuronidases, α-L-arabinofuranosidases, endo-1,4-β-D mannanases, β-mannosidases, acetyl xylan esterases, α-glucoronidases and α-galactosidases [60]. 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

Table 6
Summary of some bio-delignification processes.

Substrate	Microorganism for lignin degradation	Time of pretreatment	% of substrate converted to reducing sugars	References
Wheat straw	*Pleurotus ostreatus*	5 weeks	35%	[29]
	Phanerochaete sordida; *Pycnoporus cinnabarinus* 115	4 weeks	35%	[28,32]
Sugarcane trash	*Aspergillus terreus*	45 days	92% delignification; total reducing sugar yield 11.26 ± 0.73 mg/g	[57]
	Bacillus macerans		71% delignification; total reducing sugar yield 11.56 ± 0.51 mg/g	
	Trichoderma reesei		73.6% delignification; Total Reducing sugar yield 11.16 ± 0.64	
Rice straw	*Pleurotus ostreatus*	60 days	41% lignin degraded	[20]
Sugarcane bagasse	Phlebia sp. MG-60 (A marine fungus)		>50%	[46]

[28,61]. Among the various cellulolytic microbial strains *Trichoderma* is one of the most well studied cellulase and hemicellulase producing fungal strains [62]. *Trichoderma* is able to produce at least two cellobiohydrolases and five endoglucanases and three endoxylanases [62,63]. However, *Trichoderma* lacks β-glucosidase activity that plays an efficient role in polymer conversion [59,64]. On the other hand, *Aspergillus* is a very efficient β-glucosidase producer [59]. *Trichoderma* cellulase supplemented with extra β-glucosidase has been studied several times [65–67]. Combination of *Trichoderma reesei* ZU-02 cellulase and cellobiase from *Aspergillus niger* ZU-07 improved the hydrolysis yield to 81.2% with cellobiase activity enhanced to 10 CBU/g substrate [68].

Various factors influence yields of monomer sugars from lignocellulose. Temperature, pH and mixing rate are the main factors of enzymatic hydrolysis of lignocellulosic material [59,69]. Other factors that affect yield are substrate concentration, cellulase enzyme loading, and surfactant addition [28,70,71]. High substrate concentration may lead to substrate inhibition. Cellulase contributes to the major cost of the lignocellulosic ethanol technology [23]. 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 [72]. 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 [73].

Several studies have been reported on the conversion of cellulosic biomass to sugars by enzymatic hydrolysis. Belkacemi and Hamoudi [74] studied enzymatic hydrolysis of corn stalk hemicellulose at 30 °C and pH 5. Saccharification was 90% and sugar was released after 10 h. Chen et al. [68] 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. [71] reported that PEG addition increased the enzymatic conversion of soft lignocellulose from 42% to 78% at 16 h where optimum hydrolysis temperature was 50 °C. Xu et al. [62] 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 organosolv pretreated wet wheat straw gave above 75% yield [75].

5. Fermentation

The saccharified biomass is used for fermentation by several microorganisms. But the industrial utilization of lignocelluloses for bioethanol production is hindered by the lack of ideal microorganisms which can efficiently ferment both pentose and hexose sugars [29]. For a commercially viable ethanol production method, an ideal microorganism should have 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 hydrolysate and have cellulolytic activity. Genetically modified or engineered microorganisms are thus used to achieve complete utilization of the sugars in the hydrolysate and better production benefits.

The processes usually employed in the fermentation of lignocellulosic hydrolysate are simultaneous saccharification and fermentation (SSF) and separate hydrolysis and fermentation (SHF). Conventionally or traditionally the SHF process has been employed but SSF is superior for ethanol production since it can improve ethanol yields by removing end product inhibition and eliminate the need for separate reactors. It is also cost effective but difference in optimum temperature conditions of enzyme for hydrolysis and fermentation poses some limitations [20,39,40]. The higher ethanol yield coefficient from SSF would be partially due to more conversion of xylose to xylitol under the SSF conditions [78]. A comparative study between the two processes (SHF and SSF) is presented in Table 7.

Studies have shown that SSF is a better alternative to SHF [20,21]. The slow xylose consumption during fermentation in SHF may be due to the presence of toxic compounds which inhibit the growth and fermentation activity of the microorganism [78]. The drawback of SSF can be removed by using thermo-tolerant microorganisms like *Kluyveromyces marxianus* which has been developed to withstand the higher temperatures needed for enzymatic hydrolysis [20].

Apart from SSF or SHF, the available alternatives are consolidated bioprocessing (CBP) and simultaneous saccharification and co-fermentation (SSCF) [46]. In CBP, cellulase production, biomass hydrolysis and ethanol fermentation are all together carried out in a single reactor [20]. The process is also known as direct microbial conversion (DMC). Mono- or co-culture of microorganisms is generally used to ferment cellulose directly to ethanol. Application of CBP requires no capital investment for purchasing enzyme or its production [40,79]. Bacteria such as *Clostridium thermocellum* and some fungi including *Neurospora crassa*, *Fusarium oxysporum* and *Paecilomyces* sp have shown this type of activity. However, CBP is not an efficient process because of poor ethanol yields and long fermentation periods (3–12 days) [80]. In SSCF the co-fermenting microorganisms need to be compatible in terms of operating pH and temperature [39]. A combination of *Candida shehatae* and *Saccharomyces cerevisiae* was reported as suitable for the SSCF process [39]. Sequential fermentation with two different microorganisms in different time periods of the fermentation process for better utilization of sugar has also been employed using *S. cerevisiae* in the first phase for hexose utilization and *C. shehatae* in the second phase for pentose utilization but ethanol yields achieved are not high [26].

Some native or wild type microorganisms used in the fermentation are *S. cerevisiae*, *Escherichia coli*, *Zymomonas mobilis*, *Pachysolen tannophilus*, *C. shehatae*, *Pichia stipitis*, *Candida brassicae*, *Mucor indicus* etc. [20,21,26,29,76,77,81,82]. Among all the best

Table 7
Comparison between the two main fermentation techniques.

Fermentation process	Features and advantages	Limitations	References
Simultaneous saccharification and fermentation	Low costs Higher ethanol yields due to removal of end product inhibition of saccharification step Reduces the number of reactors required	Difference in optimum temperature conditions of enzyme for hydrolysis and fermentation.	[20,21,39,40]
Separate hydrolysis and fermentation	Each step can be processed at its optimal operating conditions Separate steps minimize interaction between the steps	End product inhibition minimizes the yield of ethanol. Chance of contamination due to long period process	[21,26,39]

Table 8
Ethanol yields from various substrates by various microorganisms.

Substrate	Fermenting microbe	Yield of ethanol	Feature of the employed microorganism	Reference
Sugarcane bagasse	*Pichia stipitis* BCC15191	0.29±0.02 g ethanol/g available fermentable sugars (glucose and xylose) after 24 h	Can ferment both glucose and xylose	[78]
	Recombinant *Escherichia coli* KO11	31.50 g of ethanol/L in 48 h equivalent to a theoretical maximum yield of 91.5%	Utilizes xylose and glucose present in hydrolysates	[83]
Wheat straw	*Pichia stipitis* NRRL Y-7124 *Pichia stipitis* A	0.35 g_p/g_s 0.41 g_p/g_s Calculated as final ethanol concentration divided by total sugar in the fermentation medium	Adapted at increased concentration of hydrolysate	[82]
Rice straw	*Candida shehatae* NCL-3501	0.45 g/g and 0.5 g/g of sugar utilized produced from autohydrolysate by free and immobilized 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	Co-ferment glucose and xylose and utilizes ethanol in absence of sugar	[84]
	Saccharomyces cerevisiae ATCC 26603	Maximum ethanol production achieved 4 g/L	Ferment only glucose	[81]
	Pichia stipitis NRRL Y-7124	Maximum ethanol production achieved 6 g/L (78% of theoretical maximum)	Ferment glucose first and then xylose from the mixture	

known yeast and bacteria employed in ethanol production from hexoses are *S. cerevisiae* and *Z. mobilis* respectively [29]. But *S. cerevisiae* cannot utilize the main C-5 sugar – xylose – of the hydrolysate [29,62]. Native organisms such as *Pichia* and *Candida* species can be used in place of *S. cerevisiae* and they can utilize xylose but their ethanol production rate is at least fivefold lower than that observed with *S. cerevisiae* [62]. Different microorganisms have shown different yields of ethanol depending on their monomer utilization (Table 8).

Genetic engineering has been employed to develop the various aspects of fermentation from higher yield to better and wide substrate utilization to increased recovery rates. A number of genetically modified microorganisms such as *P. stipitis* BCC15191 [78], *P. stipitis* NRRL Y-7124 [81,82], recombinant *E. coli* KO11 [83], *C. shehatae* NCL-3501 [84], *S. cerevisiae* ATCC 26603 [81] have been developed. Strict anaerobic hemophilic bacteria such as *Clostridium* sp. and *Thermoanaerobacter* sp. have been proposed [26,29] to explore the benefits of fermentation at elevated temperatures. Some other thermo-tolerant microorganisms developed are *K. marxianus*, *Candida lusitanieae* and *Z. mobilis* [20].

6. Conclusion

Lignocellulosic biomass has been projected to be one of the main resources for economically attractive bioethanol production. Though theoretical ethanol yields from sugar and starch (g ethanol/g substrate) are higher than from lignocellulose, these conventional sources are insufficient for worldwide bioethanol production. In that aspect agricultural wastes are renewable, less costly and abundantly available in nature. Agricultural wastes do not demand separate land, water, and energy requirements. They do not have food value as well. For economically feasible bioethanol production, several hindrances are to be overcome. These refer to the four major aspects which are feedstock, conversion technology, hydrolysis process, and fermentation configuration. With regard to feedstock major obstacles are cost, supply, harvesting, and handling. As regards conversion technology the hindrances are biomass processing, proper and cost effective pretreatment technology to liberate cellulose and hemicellulose from their complex with lignin. In respect of the hydrolysis process the challenge is to achieve an efficient process for depolymerization of cellulose and hemicellulose to produce fermentable monomers with high concentration. In this aspect enzymatic hydrolysis may be the most potent alternative process for saccharification of complex polymer. Several efforts have been made to reduce the cost of cellulase enzyme to optimize the enzymatic hydrolysis process. Finally, in case of fermentation configuration, the challenges involved are xylose and glucose co-fermentation, and the use of recombinant microbial strains. In conclusion it may be said that to solve the technology bottlenecks of the conversion process, novel science and efficient technology are to be applied, so that bioethanol production from agricultural wastes may be successfully developed and optimized in the near future.

References

[1] Uihlein A, Schbek L. Environmental impacts of a lignocellulosic feedstock biorefinery system: an assessment. Biomass and Bioenergy 2009;33:793–802.
[2] Ballesteros I, Negro MJ, Oliva JM, Cabanas A, Manzanares P, Ballesteros M. Ethanol production from steam-explosion pretreated wheat straw. Applied Biochemistry and Biotechnology 2006;130:496–508.
[3] Campbell CH, Laherrere JH. The end of cheap oil. Scientific American; 1998:78–83.
[4] Lynd LR, Wang MQ. A product – nonspecific framework for evaluating the potential of biomass-based products to displace fossil fuels. Journal of Industrial Ecology 2003;7:17–32.
[5] Dhussa A. Biogas in India. Methane to markets partnership. Meeting of agriculture subcommittee; April 22, 2004.
[6] Lime M, Ekstrandh A, Englesson R, Persson E, Bjornsson L, Lantz M. The Swedish biogas potential from domestic raw materials. Swedish, Malmo: Avfall Sverige utveckling. Report No. 2008:02; 2008.
[7] Demirbas A. Bioethanol from cellulosic materials: a renewable motor fuel from biomass. Energy Sources 2005;27:327–33.
[8] Lewis SM. Fermentation alcohol. In: Godfrey T, West S, editors. Industrial enzymology. 2nd ed. New York: Stockton Press; 1996. p. 12–48.
[9] Taherzadeh MJ, Karimi K. Acid based hydrolysis processes for ethanol from lignocellulosic materials: a review. Bioresources 2007;2(3):472–99.
[10] Cadoche L, Lopez GD. Assessment of size reduction as a preliminary step in the production of ethanol from lignocellulosic wastes. Biology of Wastes 1989;30:153–7.
[11] Binod P, Sindhu R, Singhania RR, Vikram S, Devi L, Nagalakshmi S, et al. Bioethanol production from rice straw: an overview. Bioresource Technology 2010;101:4767–74.
[12] Duff SJB, Murray WD. Bioconversion of forest products industry waste cellulosics to fuel ethanol: a review. Bioresource Technology 1996;55:1–33.
[13] Karimi K, Kheradmandinia S, Taherzadeh MJ. Conversion of rice straw to sugars by dilute acid hydrolysis. Biomass and Bioenergy 2006;30:247–53.
[14] Wati L, Kumari S, Kundu BS. Paddy straw as substrate for ethanol production. Indian Journal of Microbiology 2007;47:26–9.
[15] Vlasenko EY, Ding H, Labavitch JM, Shoemaker SP. Enzymatic hydrolysis of pretreated rice straw. Bioresource Technology 1997;59:109–19.
[16] Chen Y, Sharma-Shivappa RR, Chen C. Ensiling agricultural residues for bioethanol production. Applied Biochemistry and Biotechnology 2007;143:80–92.
[17] Ogawa M, Yoshida N. Nitrous oxide emission from the burning of agricultural residue. Atmospheric Environment 2005;39(19):3421–9.
[18] Kim S, Dale BE. Global potential bioethanol production from wasted crops and crop residues. Biomass and Bioenergy 2004;26:361–75.

[19] Berg C, World bioethanol production, the distillery and bioethanol network. Available from: http://www.distill.com/worldethanolproduction.htm; 2001.
[20] Bjerre AB, Olesen AB, Fernqvist T. Pretreatment of wheat straw using combined wet oxidation and alkaline hydrolysis resulting in convertible cellulose and hemicellulose. Biotechnology and Bioengineering 1996;49:568–77.
[21] Balat M, Balat H, Oz C. Progress in bioethanol processing. Progress in Energy and Combustion Science 2008;34:551–73.
[22] Glassner D, Hettenhaus J, Schechinger T. Corn stover potential: recasting the corn sweetner industry. In: Janick J, editor. Perspectives on new crops and new uses. VA: ASHS Press, Alexandria; 1999. p. 74–82.
[23] Banerjee S, Mudliar S, Sen R, Giri B, Satpute D, Chakrabarti T, et al. Commercializing lignocellulosic bioethanol: technology bottlenecks and possible remedies. Biofuels, Bioproducts and Biorefining 2010;4:77–93.
[24] Peiji G, Yinbo Q, Xin Z, Mingtian Z, Yongcheng D. Screening microbial strain for improving the nutritional value of wheat and corn straws as animal feed. Enzyme and Microbial Technology 1997;20:581–4.
[25] Lee J. Biological conversion of lignocellulosic biomass to ethanol. Journal of Biotechnology 1997;56:1–24.
[26] Sanchez ÓJ, Cardona CA. Trends in biotechnological production of fuel ethanol from different feedstocks. Bioresource Technology 2008;99:5270–95.
[27] Mosier N, Wyman C, Dale B, Elander R, Lee YY, Holtazapple M, et al. Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresource Technology 2005;96:673–86.
[28] Sun Y, Cheng J. Hydrolysis of lignocellulosic material for ethanol production: a review. Bioresource Technology 2002;96:673–86.
[29] Talebnia F, Karakashev D, Angelidaki I. Production of bioethanol from wheat straw: an overview on pretreatment, hydrolysis and fermentation. Bioresource Technology 2010;101(13):4744–53.
[30] Pandey A. Handbook of plant-based biofuels. New York: CRC Press; 2009.
[31] da Silva ASA, Inoue H, Endo T, Yano S, Bon EPS. Milling pretreatment of sugarcane bagasse and straw for enzymatic hydrolysis and ethanol fermentation. Bioresource Technology 2010;101:7402–9.
[32] Prasad S, Singh A, Joshi HC. Ethanol as an alternative fuel from agricultural, industrial and urban residues. Resources, Conservation and Recycling 2007;50:1–39.
[33] Mtui GYS. Recent advances in pretreatment of lignocellulosic wastes and production of value added products. African Journal of Biotechnology 2009;8(8):1398–415.
[34] Fan LT, Gharpuray MM, Lee YH. Cellulose hydrolysis biotechnology monographs. Berlin: Springer; 1987. p. 57.
[35] Hu Z, Wen Z. Enhancing enzymatic digestibility of switchgrass by microwave-assisted alkali pretreatment. Biochemical Engineering Journal 2008;38:369–78.
[36] Sridar V. Microwave radiation as a catalyst for chemical reactions. Current Science 1998;74(5):446–50.
[37] Ooshima H, Aso K, Harano Y, Yamamoto T. Microwave treatment of cellulosic materials for their enzymatic hydrolysis. Biotechnology Letters 1984;6(5):289–94.
[38] Kitchaiya P, Intanakul P, Krairiksh M. Enhancement of enzymatic hydrolysis of lignocellulosic wastes by microwave pretreatment under atmospheric pressure. Journal of Wood Chemistry and Technology 2003;23(2):217–25.
[39] Neves MA, Kimura T, Shimizu N, Nakajima M. State of the art and future trends of bioethanol production, dynamic biochemistry, process biotechnology and molecular biology. Global Science Books; 2007:1–13.
[40] Hamelinck CN, Hooijdonk GV, Faaij APC. Ethanol from lignocellulosic biomass: techno-economic performance in short-, middle- and long-term. Biomass and Bioenergy 2005;28:384–410.
[41] Taherzadeh MJ, Karimi K. Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: a review. International Journal of Molecular Sciences 2008;9:1621–51. doi:10.3390/ijms9091621.
[42] Yu Q, Zhuang X, Yuan Z, Wang Q, Qi W, Wanga W, et al. Two-step liquid hot water pretreatment of Eucalyptus grandis to enhance sugar recovery and enzymatic digestibility of cellulose. Bioresource Technology 2010;101:4895–9.
[43] Wana C, Zhou Y, Li Y. Liquid hot water and alkaline pretreatment of soybean straw for improving cellulose digestibility. Bioresource Technology 2011;102:6254–9.
[44] Alvira P, Tomás-Pejó E, Ballesteros M, Negro MJ. Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: a review. Bioresource Technology 2010;101:4851–61.
[45] Wyman CE, Dale BE, Elander RT, Holtzapple M, Ladisch MR, Lee YY. Comparative sugar recovery data from laboratory scale application of leading pretreatment technologies to corn stover. Bioresource Technol 2005;96:2026–32.
[46] Cardona CA, Quintero JA, Paz IC. Production of bioethanol from sugarcane bagasse: status and perspectives. Bioresource Technology 2009;101(13):4754–66.
[47] Mosier N, Hendrickson R, Ho N, Sedlak M, Ladisch MR. Optimization of pH controlled liquid hot water pretreatment of corn stover. Bioresource Technology 2005;96:1986–93.
[48] Pandey A, Soccol CR, Nigam P, Soccol VT. Biotechnological potential of agro-industrial residues. I: sugarcane baggase. Bioresource Technology 2000;74:69–80.
[49] Sun RC, Lawther JM, Banks WB. Influence of alkaline pretreatments on the cell-wall components of wheat-straw. Industrial Crops and Products 1995;4(2):127–45.
[50] Kumar R, Wyman CE. Effects of cellulase and xylanase enzymes on the deconstruction of solids from pretreatment of poplar by leading technologies. Biotechnology Progress 2009;25:302–14.
[51] Saha BC, Iten LB, Cotta MA, Wu YV. Dilute acid pretreatment, enzymatic saccharification and fermentation of wheat straw to ethanol. Process Biochemistry 2005;40:3693–700.
[52] Martín C, Klinke HB, Thomsen AB. Wet oxidation as a pretreatment method for enhancing the enzymatic convertibility of sugarcane bagasse. Enzyme and Microbial Technology 2007;40:426–32.
[53] Banerjee S, Sen R, Pandey RA, Chakrabarti T, Satpute D, Giri BS, et al. Evaluation of wet air oxidation as a pretreatment strategy for bioethanol production from rice husk and process optimization. Biomass and Bioenergy 2009;33:1680–6.
[54] Ahring BK, Licht D, Schmidt AS, Sommer P, Thomsen AB. Production of ethanol from wet oxidised wheat straw by *Thermoanaerobacter mathranii*. Bioresource Technology 1999;68:3–9.
[55] Pedersen M, Meyer AS. Influence of substrate particle size and wet oxidation on physical surface structures and enzymatic hydrolysis of wheat straw. Biotechnology Progress 2009;25(2):399–408.
[56] Zhao X, Cheng K, Liu D. Organosolv pretreatment of lignocellulosic biomass for enzymatic hydrolysis. Applied Microbiology and Biotechnology 2009;82:815–27.
[57] Singh P, Suman A, Tiwari P, Arya N, Gaur A, Shrivastava AK. Biological pretreatment of sugarcane trash for its conversion to fermentable sugars. World Journal of Microbiology and Biotechnology 2008;24:667–73.
[58] Ferreira S, Durate AP, Ribeiro MHL, Queiroz JA, Domingues FC. Response surface optimization of enzymatic hydrolysis of *Cistus ladanifer* and *Cytisus striatus* for bioethanol production. Biochemical Engineering Journal 2009;45:192–200.
[59] Taherzadeh MJ, Karimi K. Enzyme-based hydrolysis processes for ethanol from lignocellulosic materials: a review. Bioresources 2007;2(4):707–38.
[60] Jorgensen H, Kutter JP, Olsson L. Separation and quantification of cellulases and hemicellulases by capillary electrophoresis. Analytical Biochemistry 2003;317(1):85–93.
[61] Rabinovich ML, Melnik MS, Boloboba AV. Microbial cellulases (review). Applied Biochemistry and Microbiology 2002;38(4):305–21.
[62] Xu J, Takakuwa N, Nogawa M, Okada H, Morikawa Y. A third xylanase from *Trichoderma reesei* PC-3-7. Applied Microbiology and Biotechnology 1998;49:18–724.
[63] Sandgren M, Shaw A, Ropp TH, Wu S, Bott R, Cameron AD, et al. The X-ray crystal structure of the *Trichoderma reesei* family12 endoglucanase 3, Cel12A, at 1.9 Å resolution. Journal of Molecular Biology 2001;308(2):295–310.
[64] Kovács K, Szakacs G, Zacchi G. Comperative enzymatic hydrolysis of pretreated spruce by supernatants, whole fermentation broths and washed mycelia of *Trichoderma reesei* and *Trichoderma atroviride*. Bioresource Technology 2009;100:1350–7.
[65] Krishna SH, Reddy TJ, Chowdary GV. Simultaneous saccharification and fermentation of lignocellulosic wastes to ethanol using a thermotolerant yeast. Bioresource Technology 2001;77(2):193–6.
[66] Itoh H, Wada M, Honda Y, Kuwahara M, Watanabe T. Bioorganosolv pretreatments for simultaneous saccharification and fermentation of beech wood by ethanolysis and white rot fungi. Journal of Biotechnology 2003;103:273–80.
[67] Ortega N, Busto MD, Perez-Mateos M. Kinetics of cellulose saccharification by *Trichoderma reesei* cellulases. International Biodeterioration Biodegradation 2001;47(1):7–14.
[68] Chen M, Zhao J, Xia L. Enzymatic hydrolysis of maize straw polysaccharides for the production of reducing sugars. Carbohydrate Polymers 2008;71:411–5.
[69] Olsson L, Hahn-Hägerdal B. Fermentation of lignocellulosic hydrolysates for ethanol production. Enzyme and Microbial Technology 1996;18(5):312–31.
[70] Alkasrawi M, Eriksson T, Börjesson J, Wingren A, Galbe M, Tjerneld F, et al. The effect of Tween-20 on simultaneous saccharification and fermentation of softwood to ethanol. Enzyme and Microbial Technology 2003;33(1):71–8.
[71] Börjesson J, Peterson R, Tjerneld F. Enhanced enzymatic conversion of softwood lignocellulose by poly(ethylene glycol) addition. Enzyme and Microbial Technology 2007;40:754–62.
[72] Eggman T, Elander RT. Process and economic analysis of pretreatment technologies. Bioresource Technology 2005;96:2019–25.
[73] Eriksson T, Börjesson J, Tjerneld F. Mechanism of surfactant effect in enzymatic hydrolysis of lignocellulose. Enzyme and Microbial Technology 2002;31:353–64.
[74] Belkacemi K, Hamoudi S. Enzymatic hydrolysis of dissolved corn stalk hemicelluloses: reaction kinetics and modeling. Journal of Chemical Technology and Biotechnology 2003;78:802–8.
[75] Saha BC, Cotta MA. Ethanol production from alkaline peroxide pretreated enzymatically saccharified wheat straw. Biotechnology Progress 2006;22:449–53.
[76] Sukumaran RK, Surender VJ, Sindhu R, Binod P, Janu KU, Sajna KV, et al. Lignocellulosic ethanol in India: prospects, challenges and feedstock availability. Bioresource Technology 2010;101:4826–33.
[77] Girio FM, Fonseca C, Carvalheiro F, Duarte CL, Marques S, Bogel-Łukasik R. Hemicelluloses for fuel ethanol: a review. Bioresource Technology 2010;101:4775–800.

[78] Buaban B, Inoue H, Yano S, Tanapongpipat S, Ruanglek V, Champreda V, et al. Bioethanol production from ball milled bagasse using an on-site produced fungal enzyme cocktail and xylose-fermenting *Pichia stipitis*. Journal of Bioscience and Bioengineering 2010;110(1):18–25.
[79] Lynd LR, van Zyl WH, Mcbride JE, Laser M. Consolidated bioprocessing of cellulosic biomass: an update. Current Opinion in Biotechnology 2005;16(5): 577–83.
[80] Szczodrak J, Fiedurek J. Technology for conversion of lignocellulosic biomass to ethanol. Biomass Bioenergy 1996;10:367–75.
[81] Moniruzzaman M. Alcohol fermentation of enzymatic hydrolysate of exploded rice straw by *Pichia stipitis*. World Journal of Microbiology and Biotechnology 1995;11:646.
[82] Nigam JN. Ethanol production from wheat straw hemicellulose hydrolysate by *Pichia stipitis*. Journal of Biotechnology 2001;87:17–27.
[83] Takahashi CM, Lima KGC, Takahashi DF, Alterthum F. Fermentation of sugar cane bagasse hemicellulosic hydrolysate and sugar mixtures to ethanol by recombinant *Escherichia coli* KO11. World Journal of Microbiology and Biotechnology 2000;16:829–34.
[84] Abbi M, Kuhad RC, Singh A. Fermentation of xylose and rice straw hydrolysate to ethanol by *Candida shehatae* NCL-3501. Journal of Industrial Microbiology 1996;17:20–3.
[85] Georgieva TI, Mikkelsen MJ, Ahring BK. Ethanol production from wet exploded wheat straw hydrolysate by thermophillic anaerobic bacterium *Thermoanaerobacter* BG1L1 in a continuous immobilized reactor. Applied Biochemistry and Biotechnology 2008;145:99–110.
[86] Erdei B, Barta Z, Sipos B, Réczey K, Galbe M, Zacchi G. Ethanol production from mixtures of wheat straw and wheat meal. Biotechnology for Biofuels 2010;3:16.
[87] Das P, Ganesha A, Wangikar P. Influence of pretreatment for deashing of sugarcane bagasse on pyrolysis products. Biomass and Bioenergy 2004;27: 445–57.
[88] Nguyen TAD, Kim KR, Han SJ, Cho HY, Kim JW, Park SM, et al. Pretreatment of rice straw with ammonia and ionic liquid for lignocellulose conversion to fermentable sugars. Bioresource Technology 2010;101(19):7432–8.
[89] Zhang X, Yu H, Huang H, Liu Y. Evaluation of biological pretreatment with white rot fungi for the enzymatic hydrolysis of bamboo culms. International Biodeterioration and Biodegradation 2007;60:159–64.
[90] Alizadeh H, Teymouri F, Gilbert TI, Dale BE. Pretreatment of switchgrass by ammonia fiber explosion (AFEX). Applied Biochemistry and Biotechnology 2005;121–124:1133–41.
[91] Teymouri F, Laureano-Perez L, Alizadeh H, Dale BE. Ammonia fiber explosion treatment of corn stover. Applied Biochemistry and Biotechnology 2004; 113–116:951–63.
[92] Chundawat SPS, Venkatesh B, Dale BE. Effect of particle size based separation of milled corn stover on AFEX pretreatment and enzymatic digestibility. Biotechnology and Bioengineering 2007;96(2):219–31.
[93] Park YS, Kang SW, Lee JS, Hong SI, Kim SW. Xylanase production in solid state fermentation by *Aspergillus niger* mutant using statistical experimental designs. Applied Microbiology and Biotechnology 2002;58:761–6. doi:10.1007/s00253-002-0965-0.