



Biomass Pre-treatment Methods and Their Economic Viability for Efficient Production of Biofuel

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Authors' contributions

This work was carried out in collaboration between all authors. Author SB prepared the first draft of the manuscript. Authors SB and SR both searched literature. The manuscript has been finalized by author AK. Author AK also designed the study and managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Development of sustainable technology for bioenergy/biofuel generation using lignocellulosic biomass is of prime importance in the present day research. An economically viable technology for production of ethanol from lignocellulosic biomass will certainly provide opportunity to the non-oil producing countries to refrain from deprivation of fuel and explore the concept of bio-refineries for complete utilization of the feedstock. Biomass feedstock is abundant in the form of network of cellulose, hemicelluloses and lignin and hence the name lignocellulose. The structure and composition of lignocellulosic biomass is a major hindrance in its complete digestibility into fermentable monomer sugars. Reports are in the literature indicating that pre-treatment of biomass from wood and grasses can increase the yield of sugars upto 90%. Here, we have reviewed various technologies and methods for the pre-treatment of biomass with emphasis on their advantages and disadvantages with respect to the production of biofuel. Also, capital expense (CAPEX) and operational expense (OPEX) is considered to assess the economic viability of the technology.

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1. INTRODUCTION

The continuous depletion of fossil fuel resources and their increased demand has changed the outlook from ancient resources of fuel to new resources especially biomasses of plant origin. Plant biomass is a promising raw material for fuel generation to sustain fuel requirements in the modern age. Reduction of carbohydrates into fuel is an area of active research which includes co-fermentation of pentose and hexose sugars into cellulosic ethanol [1]. Several other sources of bio-fuel are also being explored including algal fuel, bio-diesel, and metabolic engineering of microbes for synthesis of fungible fuels. However, in terms of availability of feedstock and productivity, lignocellulosic biomass to bio-ethanol is the most viable option in the present scenario. In 2011, National Renewable Energy Laboratory, USA (NREL) reported that approximately 5 billion gallons ethanol / annum was produced using food grains in the year 2010, and production of more than 8 billion gallons ethanol / annum is expected in 2015 with the use of lignocellulosic biomass [2]. Lignocellulosic biomass contains sugars polymerized into cellulose and hemicelluloses as structural components of the plant cell wall along with lignin, a component that acts as binding cement in the cross linking. The composition of various plant cell walls varies with the ratio of, and interactions between these three polymers. Hardwood contains highest cellulosic content ranging from 40 to 55% of dry matter (DM) followed by softwood having 34 to 50% DM while hemicellulosic content varies from 19 to 40% and 21 to 35% in hardwood and softwood, respectively [3-5]. Lynd et al. [6] stated the importance of cellulosic material as an attractive feedstock for biofuel production due to its low cost, recoverable supply and great abundance.

Hemicelluloses include heteropolysaccharides such as xylans, arabinoxylans, glucuronoxylans, glucomannans and xyloglucans; with xylans being the most abundant. These are highly substituted polysaccharides and their structure varies from one biomass type to another [7]. Interactions between hemicelluloses and lignin inhibit the release of sugar moieties from the complex polymers. The structural complexity and interactions between hemicelluloses and lignin are considered to contribute significantly to recalcitrance of cell walls, a property of cell wall to resist the action of hydrolysing enzymes.

Therefore, it is necessary to separate the three components in order to make the sugar polymers accessible to the hydrolysing enzymes. This is achieved by various pre-treatment methods which can be grouped into physical, biochemical, thermo-chemical or biological depending upon the pre-treatment conditions.

The pre-treatment methods are trailed by the treatment with enzymes to convert complex lignocelluloses into linear moieties of sugars and other carbohydrates into monomers, and fermentation of the monomers by microorganisms. The enzymes depolymerise the composite cell wall of the plants and work on solid- liquid interface.

In this review, we have presented an overview of various conventionally used physical, chemical, biological and thermo-chemical methods of pre-treatment of lignocellulosic biomass to overcome its recalcitrance, along with an insight into upcoming combined approaches for the better dissolution of cellulose and hemicelluloses. We have also discussed the possibilities of bio-refinery development from the by-products generated from these pre-treatments.

2. METHODS FOR PRE-TREATMENT OF BIOMASS

Primary hurdle in using lignocelluloses (component of biomass) for ethanol production is location of entrapped sugars necessary for fermentation. Other hindrances are physiochemical, structural and compositional factors [8]. To overcome these problems, pre-treatment is necessary before fermentation so that cellulose becomes accessible in the plant fiber. Ideally, key features in pretreatment process include de-polymerization of hemicelluloses, preservation of the structure and de-crystallization of cellulose, production of minimal inhibitors of downstream process, cost-effectiveness and finally, production of valuable by-products [9]. A number of agricultural crops like rice straw, wheat straw, cotton stalk, sugarcane bagasse are being used these days as source of biomass for bio-ethanol production [10]. Enzyme hydrolysis is a milder option of pre-treatment method but often leads to incomplete hydrolysis, therefore other methods such as hydrothermal, chemical, thermo-chemical are being explored widely for fractionation of biomass. Alvira [11] explained fractionation of biomass and effectiveness of pretreatment. He

showed that pretreatment is essential in order to overcome the 3D structure of most complex hemicelluloses and cellulose in order to increase the yield of basic sugars required in the fermentation process, to lower down the possibility of carbohydrate degradation, to avoid inhibitory byproducts of pre-processing of the biomass, to recover lignin and its use in formation of other co-products. However, the foremost requirement is cost effectiveness of the process from source to product generation.

2.1 Physical Pre-treatment

Physical treatment increases the accessibility of cellulose in terms of its surface area, pore size and decreases its crystalline structure. There are number of physical and mechanical treatments available viz. grinding, milling, extrusion and irradiation using electron beam, gamma rays, microwaves and ultrasound waves. The size of the material may vary from 10-30 mm to 0.2-2 mm for chipping and milling, respectively. Cadoche and Lopez [12] have predicted a relationship between energy consumed for size reduction of the biomass as a function of final particle size. It has been predicted that energy input of 30 kWh per ton of biomass is required for the particle size of 3-6 mm. Combination of hot compressed water treatment followed by wet disk milling gave 90% glucose and 79% xylose in rice straw with the enzyme loading of 10 FPU/g biomass [13]. The main advantage of physical pre-treatment lies in the negligible use of chemicals, inhibitors are not produced and it is environment friendly. However, these methods are time consuming and require good mechanical power and skills. Although their results are satisfactory, these methods are more effective if used in combination with chemical treatments [14].

2.2 Biological Pre-treatment

Biological delignification is much cost effective, requires low energy input and is an environment friendly approach for pre-treatment. The technique requires direct use of microorganisms or the enzymes extracted from microbes. Essentially bacteria and fungi are used for the purpose because they are evolved to use biomass for their metabolic needs [15]. Recently, *Lactobacillus fermentum* was found effective in preserving cellulose and hemicellulose from sugar beet pulp by approximately 35% after the removal of lignin [16]. It was also found that majority of white rot fungi are capable of degrading woody polymers and some decay is

caused by brown rot fungus which has evolved from white rot fungi and is found selective in degradation of cellulose and hemicelluloses but leaves lignin-rich residue [17]. Xu et al. [18] suggested solid state fermentation to be the process of choice while dealing with white rot fungi for higher production of lignin hydrolyzing enzymes. Patel et al. [19] emphasised on factors for better enzymatic activity and lignin degradation viz; fungal strain, temperature, moisture content, pH, aeration nutrient composition (nitrogen, manganese and copper) and cultivation time [20]. The *Ceriporiopsis subvermispora*, *Dichomitus squalens*, *Phanerochaete chrysosporium* and *Pinus radiata* are some of the white rot fungi strains producing laccase (Lac) and manganese peroxidase (MnP) enzyme [21]. Enzymes mainly involved in pre-treatment are phenol oxidase (laccase) and peroxidases [22,23]. There are reports for bacterial laccases characterized from *Azospirillum lipoferum* and *Bacillus subtilis* [24]. Studies have shown that *Pleurotes* spp. [25], *Lentinula edodes* [26], *Penicillium camemberti* [27], when grown at 25-35°C for 3 to 22 days results in 45-75% and 65-80% holocellulose and lignin degradation, respectively.

2.3 Acid Hydrolysis

Acid hydrolysis can be employed using acids and can precede enzymatic actions. High reaction rate and improved hydrolysis can be obtained by treatment with dilute sulphuric acid [28]. This treatment results in complete dissolution of hemicelluloses but little of cellulose. The other acids that can be used are hydrochloric acid [29], nitric acid [30], ortho-phosphoric acid and also organic acids viz. oxalic acid and maleic acid. Using dilute acid treatment on corn stover, nearly 82% of total sugar recovery could be achieved with enzyme loading of 15 FPU/g cellulose [31]. Lu et al. [32] optimized the pre-treatment of corn stover using 2% acid at 120°C for 43 min. They showed up to 77% xylose yield where as glucose yield was lesser (nearly 8.4%). Yat et al. [33] reported treatment of four timber species viz. aspen, basswood, red maple and balsam fir with 1% sulphuric acid at 180°C with good results. Idrees et al. [34] reported 79.93, 78.71 and 75.9% of sugars yield when Water Hyacinth biomass was treated with phosphoric acid, maleic acid and sulfuric acid, respectively. Selig et al. [7] showed spherical droplet formation on the surface of corn stover residues upon treatment with acid and high temperature. Now a days, heteropoly acids (HPAs) such as

H₅BW₁₂O₄₀, which give negatively charged ions in solution, are used as homogeneous acid catalysts for pre-treatment. These anions are strong hydrogen acceptors due to external oxygen atoms required for cellulose dissolution at low temperatures (nearly 60°C). However, it is very expensive and time consuming treatment [35]. There is a need of an appropriate acid and optimum incubation time and temperature. Dilute sulfuric acid can be used to hydrolyse hemicellulose into monomer sugars and in this process, commercial furfural can be produced [36]. Galbe and Zacchi [37] reported generation of toxic substances after acid treatment, and showed that this generation of toxic substances makes it harder to ferment monomer sugars. Frederick et al. [38] showed the washing of pre-treated biomass of poplar with water reduced the toxic inhibitory substances and resulted in 5.3 times increase in glucose recovery and significantly increased ethanol yield compared to unwashed biomass.

2.4 Alkaline Hydrolysis

As compared to acid hydrolysis, alkaline treatment causes lesser sugar degradation [39]. The method requires alkaline solution of sodium hydroxide, calcium hydroxide or ammonia for pre-treatment process. Sodium hydroxide is the most studied treatment [40] and lime (calcium hydroxide slurry) is one of the cheapest sources for this purpose. Lime treatment of biomass followed by enzymatic reaction may result in changes in structural features like acetylation, crystallization and lignifications [41]. Switch grass was pre-treated using lime at 120°C for 2 hours to get 26% xylan and 29-33% lignin recovery [42,43]. Chang et al. [44] used poplar wood and treated with peracetic acid, KOH and ball milling to generate a correlation between lignin contents, acetyl contents and crystallinity indices (Crls). They reported that lignin contents and crystallinity indices play major role in enzymatic hydrolysis. There are reports of lime treatment to wheat straw at 85°C for 3 hours [43], switch grass at 100°C for 2 hours [42] and corn stover at 100°C for 13 hours to get theoretical yield of nearly 78% [45,46]. A 0.5 g of calcium hydroxide/g of corn stover biomass when treated in oxidative and non oxidative conditions for 4 days at 55°C yielded 91.3% glucose and 51.8% xylose at 15FPU/g cellulase [47]. On the other hand, lime loading should be 0.075g/g of biomass and water loading 5 g/g of biomass at 120°C for 4 hours. Zhao and co-workers [48] showed that pre-treatment with sodium hydroxide

and sodium hydroxide -urea mixture at low temperatures facilitates disruption of hemicelluloses, cellulose and lignin. Because of disruption, the enzymatic hydrolysis can be increased by sodium hydroxide and sodium hydroxide-urea treatment. This process led to 70% sugar yield at low temperature using 7% sodium hydroxide/12% urea solution. Therefore, treatment can be used followed by enzymatic digestion which will increase glucose and ethanol yield during fermentation [49]. Chosdu et al. [50] used irradiation along with 2% sodium hydroxide for corn stalk, peanut husk and cassava bark. Calcium hydroxide (lime) slurry can be used for spray on biomass of 10 mm particle size along with high temperatures that causes delignification and accessibility of cellulose and hemicelluloses [51]. Radio frequency based dielectric heating can also be used for the hydrolysis of switch grass using sodium hydroxide [52,53]. For this purpose, optimum particle size of biomass should be 0.25-0.50 mm and 0.25 g of sodium hydroxide /g for alkali loading. Sometimes ammonia has been found to be a good option for alkaline hydrolysis. Temperature can be set up to 170°C, ammonia concentration from 2.5 to 20% with reaction time of 1 hour for corn cobs and stover mixture and switch grass [54]. This treatment removes lignin and causes partial digestion of hemicelluloses by removing acetyl and uronic acid groups and increases enzyme accessibility [44]. This treatment also degrades ester and glycosidic side chains, and causes swelling of cellulose and partial disruption of crystalline structure of cellulose [55,56].

2.5 Liquid Hot Water (LHW) Hydrolysis

The LHW hydrolysis is one of the promising pre-treatment technologies devoid of use of harmful chemicals and low generation of inhibitory by-products. Upto 55 to 84% hemicelluloses recovery has been reported by Girio et al. [57]. They suggested that cellulose conversion rate will be 82.59% in reed with 180°C temperature for 20 minutes, 1:10 solid to liquid ratio and enzymatic hydrolysis at 50°C for 72 hours with 30 FPU per gram of cellulase enzyme loading of oven dried water insoluble solids. Leandro et al. [58] suggested the use of LHW combined with high pressure carbon dioxide. They treated depithed bagasse and used 12:1 liquid to solid ratio at 93.8°C to 136.2°C for 17.6 to 102.4 minutes and recovered 97.2% cellulose. When bagasse was pre-treated at 115°C for 1 hour after enzymatic hydrolysis, the cellulose conversion was 41.17%. Corn stover was

converted to ethanol at severity factor from 3.95 to 4.54 and nearly 89% xylan was removed [59]. Ko et al. [60] pre-treated hardwood with LHW at the severity range from 8.25- 12.51 and 80-90% initial lignin was recovered. Hot water treatment on aspen woodchips when treated at 160°C for 2 hours with a liquid to solid ratio of 4.76:1 extracted xylose and xylo-oligomers as main five carbon sugars with 0.016 mol/L and 0.018 mol/L concentration respectively and Rhamnose as main six carbon sugar with 0.0042 mol/L concentration [61]. Some minor monomer sugars were also obtained viz; glucose, mannose, galactose and arabinose. It is a potential green technology for pre-treatment and enhances lignocelluloses digestibility, pentosan recovery and sugar extraction [62].

2.6 Ionic Liquid (IL) Hydrolysis

The IL is a relatively new physico-chemical pre-treatment technique of biomass. These are the solvents consisting of ions (cations and anions) having low melting points ~100°C, high polarity, high viscosity, minimal vapor pressure and high thermal stability [63,64]. Ionic liquids have been reported to dissolve raw lignocellulosic biomass and even regenerate biopolymers from raw biomass [65], and they do so mainly by disrupting the intra and inter-molecular hydrogen bonding of biopolymers of lignocellulosic biomass [66]. Xu et al. [67] suggested that cellulose can be dissolved efficiently by the salts having high hydrogen bond basicity and dipolarity. They also suggested that formic acid anions, imidazolium phosphates and phosphonates exhibit higher basicity than chlorides and therefore exhibit good solubility for cellulose. The criteria that determine solubility of cellulose in ILs include hydrogen bond basicity and dipolarity of anion, cation size, presence or absence of functional group on cation. Besides, cation should be a moderate hydrogen bond donor [67-70]. Viscosity is not an important factor in solubilisation of cellulose although ILs with lower viscosity are easier to handle [67]. A 10:1 ratio (g liquid/g biomass) is commonly used for acid hydrolysis, super critical CO₂ and aqueous ammonia treatments [71,72] with the temperature range of 80-180°C. However, higher range of temperature is only followed according to the desired pre-treatment outcome. The treatment parameters such as time and temperature vary with the selected biomass and ionic liquid. Pezoa et al. [73] reported that 1 hour treatment using 1-ethyl-3-methylimidazolium chloride [C₂ mim] causes 10% cellulose dissolution. Use of

cellulase and β -glucosidase for 72 hours at 47°C temperature is sufficient for higher yields of glucose and xylose in corn stover and for wheat straw 150°C temperature is sufficient to double the yields of glucose and xylose. Corn stover upon treatment with [1-ethyl-3-methylimidazolium]OAc at 70°C for 24 hours achieved 84.9% glucose and 64.8% xylose [67]. The most important advantage of ionic liquids for use in pre-treatment is unaltered structures of the lignocellulosic biopolymers after the treatment. Cellulose dissolved in ILs is easily regenerated by using an anti-solvent thus rendering the ILs reusable to a large extent. A careful selection of the ionic liquid, anti-solvent and treatment parameters offers several advantages over other pre-treatment strategies with respect to use of thermal energy, reduced by-product generation, recyclable solvents, renewable feedstock and process and product safety. It is therefore considered a relatively greener process compared to many other pre-treatment processes such as acid and alkaline treatments. The cellulose regeneration can be achieved using anti-solvents such as water, ethanol or acetone. But their toxicity and hygroscopic nature need to be considered. Other major concerns with several ionic liquid treatments are their toxicity, corrosion property and production of inhibitory by-products that may prove to be toxic for the microbes used in downstream processing [11].

2.7 Organosolvation

This method involves the use of various organic or mixture of aqueous-organic compounds with /without acid as catalysts. The process works at high temperature ranging 100 to 250°C using solvents of low boiling points such as methanol and ethanol, alcohols with high boiling point like ethylene glycol, tetrahydrofurfuryl alcohols and glycerol and other organic compounds like ketone, phenols and ethers [74]. Residual lignin (6.4% - 27.4%) can be prepared from softwood pulp using lignol process based on aqueous ethanol organosolvation [75]. In the process, delignification kinetics varies with different solvents. When wood is treated with aqueous ethanol, delignification occurs in three phases: initial, principal and residual whereas it occurs in principal and residual phase when sugarcane bagasse is used. The results depend upon the extent of lignin distribution in various biomasses [76]. It has been suggested that if pre-treatment is done at high temperature ranging from 185 to 210°C, then use of acids can be avoided as

organic acids released during pre-treatment act as catalysts and rupture lignin- hemicellulose complex [77]. It causes hydrolysis of internal lignin bonds and glycosidic bonds in hemicelluloses. The recovered lignin after this treatment is sulphur free, low molecular weight and of high purity. The prior requirements of this process are the optimization of liquid to solid ratio (LSR) which reduces the capital costs and operating cost. Due to low LSR, the main drawbacks are the chances of re-precipitation of lignin over cellulose and making it inaccessible for further treatments. To overcome the drawbacks, extensive washing is necessary which requires energy consumption. Also, due to volatility and flammability of solvents, efficient digesters are required which further increases the cost.

2.8 Thermo-chemical Hydrolysis

Pyrolysis is a type of thermo-chemical decomposition process of organic material in oxygen deficient environment. It has been used to convert solid biomass into gas, liquid and char in the presence of high temperature upto 500-600°C. At 110°C, moisture of biomass is evaporated and consequently decomposition of hemicelluloses, cellulose and lignin starts at 200 to 260°C, 240 to 340°C and 280 to 500°C, respectively with an ideal particle size of less than or equal to 2 mm. Levoglucosan is the major product after pyrolysis along with cellulose, aliphatic and oxygenated compounds. The whole process involves high pressure, shorter residence time and generation of vapor products. Sometimes, sand bath, molten salt bath, microwaves and infrared rays are used for high temperature. NREL, USA has developed a kind of vortex reactor where particles slide on the walls of hot reactor followed by flash pyrolysis [78]. It is a type of destructive distillation process. On the other hand, torrefaction works at high temperatures ranging from 120°C and 300°C while working under inert conditions. It is a mild form of pyrolysis. It converts hygroscopic biomass to hydrophobic material and makes physical treatment easier to employ. It removes moisture and depolymerises polysaccharides. In a properly designed torrefaction system, energy contained in the gases (torgas) is sufficient to bear drying process and torrefaction. Zwart et al. [79] reported conversion of biomass to syn-gas using Fischer-Tropsch (FT) method. However, there are chances of biomass loss and depletion of hemicellulosic material by high temperatures. Mild torrefaction limits sugar degradation upto 5-

10% and also lowers the likelihood of enzymatic and fermentation inhibition [80]. Vincent et al. [81] concluded that torrefaction decreases grinding energy and particle size distribution. However, this technology has technical challenges in its limited flexibility of feedstock choice. There is linear decrease in particle size distribution with increase in anhydrous weight loss. The moisture content should not be more than 15% and particle size ranges between 5 to 20 mm. Another challenge is scale up process to industrial levels and cost in setting up the commercial plant. The process involves release of various gases and organic compounds such as CO₂, CO, acetic acid, methanol, phenols and furfurals. However, some of these compounds can be diverted for generation of value added products.

2.9 Steam Explosion (SE)

It is a hydrothermal process which requires both physical and chemical methods for biomass processing. There is a requirement of high pressure saturated steam, short pulse of high temperature and rapid depressurization to destroy the fibre structure of biomass resulting in hemicelluloses degradation and transformation of lignin, which in turn increases the digestibility of cellulose and further conversion becomes easier. The typical parameters of an SE reaction are temperature ranging from 160°C to 260°C, pressure between 0.7 to 5 Mpa and residence time between 1 to 10 minutes [82]. High pressure and turbulent flow of the material cause its fragmentation and subsequently exposure of the surface area to enzymatic activity [83]. The key factors affecting this treatment are residence time, chip size, moisture content and temperature [83,84]. Overend and Chornet [85] gave a severity index (R₀) for the optimization of the process:

$$R_0 = \int_0^t \exp\left(\frac{T-100}{14.75}\right) dt$$

Where, R₀ is function of the reaction time (t) and T is temperature. Limitation of the equation lies in absence of moisture content and particle size which have been found to be important factors influencing the treatment. SE is basically used for pulping process [86], pre-treatment of wood for pelletization to increase its properties like bonding, heating value and hydrophobicity [86,87] and also for bioethanol and biogas production using microbes [88,89]. Ballesteros et al. [90] reported 80% theoretical cellulose rich residue yield when acid impregnated straw was steam

exploded at 190°C for 10 minutes or 200°C for 5 minutes. Addition of an acid before SE helps in solubility of hemicelluloses. However, due to this treatment, toxic substances are generated which are removed by washing with water resulting in loss of soluble sugars and it makes saccharification difficult. If SE is used in combination with chemical treatments (mainly dilute acids), there are chances of vessel corrosion and higher amount of degraded components of biomass.

2.10 Ammonia Fibre Explosion (AFEX)

AFEX is similar to steam explosion but here liquid ammonia is used at a concentration of more than 70% and at high temperatures and pressure [91]. The pressure is rapidly released vaporizing ammonia which can be later recovered and recycled for another processing [92]. This can be done using either mild condition which utilizes 1 kg ammonia/kg dry biomass at 100°C for 5 to 10 minutes or severe condition which utilizes 2 kg ammonia/kg dry biomass (early mature grass etc) at 200°C for nearly 30 minutes. Lee et al. [93] compared the effect of auto-hydrolysis and AFEX on coastal Bermuda grass, where the results clearly indicated that the theoretical sugar yield of AFEX was 97-110% and that of auto-hydrolysis was 76-98%. The process recovers almost complete solid mass and increases the digestibility of lignocelluloses. Auto-hydrolysis is a method where no chemical is used and only water as medium with varying temperature range (130-230°C) and time is applied on biomass causing depolymerisation of hemicelluloses and hydrolysis of cellulose [93].

AFEX does not change the chemical composition of the biomass components and the sugar yield is about 94.8% (theoretically) whereas Liquid hot water gives maximum of 55% sugar yield due to increased by-product generation [93]. The highest delignification (nearly 47%) has been achieved when switchgrass biomass was pre-soaked in ammonium hydroxide (30%) with liquid-solid ratios of 5-10 mL/g for varying time (5-10 days) [94]. Kim and Lee [95] reported 93% digestibility with 10 FPU/g-glucan when corn stover biomass was treated with Ammonia Recycled Percolation (ARP). After 168 hours of AFEX pre-treatment followed by enzymatic hydrolysis of reed canary grass using 15FPU Spezyme CP cellulase/g of glucan gave 86% of glucose and 78% of xylose for vegetative stage, and 89% of glucose and 81% of xylose for seed stage [47].

2.11 CO₂ Explosion

This technique uses carbon dioxide as a supercritical fluid (SC-CO₂) [96] and works as liquid hot water hydrolysis. It works at 3100-4000 psi pressure, moisture content of 0-80% and temperature ranges between 110 to 170°C with different residual times. In contrast to steam explosion, this technique is used for lower temperature treatments and has reduced cost. Supercritical carbon dioxide is relatively of low cost, non toxic, non flammable, higher accessibility technique and can be easily recovered in extraction process [95,97,98]. The injected CO₂ is believed to react with the carbonic acid and as a result there is improvement in hydrolysis. Jayawardhana and Van Walsum [99] suggested that the cost using carbonic acid system is lower than acid based systems. It is highly sensitive to pressure and concentration of solids used. Enzymatic hydrolysis following SC-CO₂ treatment with low moisture content for untreated aspen and southern yellow pine gave total reducing sugar content as 14.5% and 12.85%, respectively while increasing the moisture content to 73% gave the yield of 84.7% and 27.3% in aspen and southern yellow pine, respectively at 3100 psi for 30 minutes retention time [100]. Zheng et al. [96] suggested that CO₂ explosion technique is more cost effective than ammonia explosion after working on recycled paper mixed with sugarcane bagasse and re-pulping waste. Kim and Hong [100] suggested SC-CO₂ as better pretreatment method for hydrolysis of both hardwood and softwood. However, this method is not economically viable due to costly high pressure equipments.

2.12 Combined Approaches

It is evident from numerous studies that type of biomass has a strong bearing on the selection of pre-treatment method; and even within a biomass type, there are several factors, such as genetic make-up, growth conditions, storage conditions etc, that affect the structure and composition of the biomass, thus making it necessary to alter the treatment parameters or use combined technique. There are several studies that have reported significant advantage of combined pre-treatment approaches. Alkaline treatment can be used in combination with irradiations like microwaves or radio waves. For the purpose, biomass can be immersed in alkaline solution of proper normality and exposing the slurry to microwaves at 250 watt for

5-20 minutes of residence time. Sodium hydroxide has been found to be efficient for pre-treatment of switchgrass and coastal Bermuda grass [101]. Torrefaction followed by grinding in an electrically heated ball mill has been explored on spruce and wheat straw and showed only 4% loss during experiments [102]. Also acid impregnation of wheat straw trailed by steam explosion at 180°C for 10 minutes has been reported to yield 300 g/kg biomass sugar in filtrate [88]. Li et al. [103] reported use of catalyst for the pre-treatment. In this study, Copper (II) 2, 2'- bipyridine was used as catalyst with alkaline hydrogen peroxide treatment and showed significant increase in glucose and xylose yields in heartwood and sapwood. Nguyen et al. [104] suggested the use of two stage acid impregnated wood tree chips followed by SE that will give 10% increase in sugar yields and 50% reduction in enzyme requirements. Combination of lime pre-treatment (LP) and wet oxidation (WO) treatment is found to be low cost physico-chemical treatment. Oxidising agents such as oxygen, water or hydrogen peroxide can be used in Wet oxidation. It is said to increase hemicelluloses digestibility and cause deacetylation and delignification of the biomass. Table 1 summarises the advantages and disadvantages of various pre-treatment methods discussed in this review. Although each pre-treatment method has some advantages and disadvantages, it is important to consider factors such as biomass type/types that will be used as starting material, budget of the plant, possibility of a bio-refinery, etc. before starting the facility. Use of biological agents viz; enzymes and microorganisms is the safest mode and requires less expertise as compared to dealing with other high pressure and energy consuming pre-treatment methods. Also, they are safe for environment also and release no harmful gases and heat. However, other commonly used and cheaper methods that are well established also have scope for alteration and combination depending upon the type of biomass being used and the available resources. It is necessary to design robust pre-treatment technologies that can handle a range of biomass types. Hence, optimization of pre-treatment methods is a field which requires more systematic research.

3. ECONOMIC VIABILITY

The major goal of economic assessment of any process is to evaluate the cost of production and the income generated through the product sale. There are two major expenses namely capital

expense (CAPEX) and operational expense (OPEX) that have to be considered to assess the economic viability of any technology. CAPEX is a business expense incurred to start up the facility and related infrastructure. On the other hand, operational expense involve raw material, labor cost, utilities, maintenance, administrative and general plant cost. A technology that involves low CAPEX/OPEX and is able to sustain itself to generate profits is generally considered economically viable. To deal with the increasing operating cost of fuel production, there are many ways to distribute intermediate products of fermentation into commercial use. Pretreatment is an essential step for the production of ethanol and leads to fractionation of these components, depending upon the method of pre-treatment and type of biomass. These fractions and their derivatives have the potential to generate a number of products of high commercial value and complement bio-refinery concept. The major advantage of bio-refinery lies in integrating biomass conversion processes and facilities to produce heat, power, fuel and various value added products which in the long run make the whole process of biomass to ethanol conversion economically viable. The use of hemicelluloses and lignin for generation of value added products will be particularly advantageous as cellulose which is devoid of these components, is readily converted to ethanol.

Hemicelluloses obtained from lignocellulosic biomass have many applications apart from ethanol production. These can be transformed into xylitol, butanediol, 5-hydroxy-methylfurfural (HMF) and butanol. Hemicelluloses can also be converted to polyhydroxyalkanoates (PHA) and polylactates (PLA) like biopolymers. Besides, hemicelluloses can be used as food additives, emulsifiers, thickeners, adhesives and adsorbants [105]. Peng et al. [106] reported their medicinal use such as ulcer protective [107], immune-stimulatory [108,109] and anti-tumor property [109]. Mainly arabino-xylans are used as stabilizers and emulsifiers in the food. Glucomannans are used as caviar substitute. Arabinogalactans are used in mining of copper and iron ores and 4-O- methylglu-curonoxylan is used as absorption agent and also has anti-tumor activity [105]. D-Xylose is the major constituent of hemicelluloses which is purified and used in various media preparations. It is also used as artificial sweetener. The selling price of D-xylose is approximately \$185/kg. The 5-hydroxymethyl furfural (HMF) is another

intermediate generated during production of levulinic acid. HMF is used as precursor of synthetic polymers [109]. Furfurals are generated from pentoses present in hemicelluloses. Presently selling price of furfural is approximately \$ 900-1000/ metric ton. Levulinic acid is used as an additive in pharmaceutical industries. It can also be used to

produce methyltetrahydrofuran, ethyl levulinate and valerolactone [110]. Formic acid is the product of hemicelluloses pre-treatment which is used in formation of hydrogen and carbon dioxide by decomposition, production of leather and can be used in place of mineral acids for cleaning purposes. The market price of formic acid is approximately \$ 500-800/ metric ton [111].

Table 1. Summary of various hydrolysis methods along with their advantages and disadvantages

Pretreatment method	Biomass	Reducing sugar recovery	Advantage	Disadvantage	Reference
Biological	Rice husk	~ 44.7%	Inexpensive	Takes time and energy	Potumarthi R et al. 2013 [124]
Mild Acidic	Corn fibre	~ 56.8%	Increase in porosity	Costly and synthesis of toxic materials like furfurals	Noureddini H et al. 2010 [125]
Alkaline					
Lime	Corn stover	~ 91.3%	Lignin and hemicelluloses can be removed	Salts of calcium and magnesium are formed	Chaturvedi V et al. 2013 [47]
NaOH	Spruce	~ 70%	Lignin can be removed	Salts of calcium and magnesium are formed	Chaturvedi V et al. 2013 [47]
KOH	Switch grass	~ 99.26%	Low inhibitor formation	Lignin alteration	Sharma R. et al. 2012 [126]
Ammonia	Corn stover	~ 73.4%	Lignin and hemicelluloses can be removed	Costly	95 Kim TH et al. 2005
AFEX	Reed canary grass	~ 86%	Lignin removal and decrystallization of cellulose	Costly and not recommended for high lignin contents	Chaturvedi V et al. 2013 [47]
Organosolv	Sugarcane baggase	~ 29%	Pure lignin recovers and used further	Inhibit enzymatic reactions for hydrolysis	Mesa L et al. 2011 [127]
Ozonolysis	Sugarcane baggase	~ 89.7%	Lignin damaged but not good for hemicelluloses and cellulose hydrolysis	Costly	Barros Rda R et al. 2013 [128]
CO2 explosion	Corn stover	~ 85%	Cheaper than AFEX and donot cause degardation of sugars	Cost increases due to requirement of high pressure	Brodeur G et al. 2011 [129]

Xylitol is an intermediate product which can be used as diabetic sweetener, medicine in ear infection and in dental care [112]. It is used in toothpaste to enhance sodium fluoride and chlorhexidine action. The market price of xylitol is approximately \$ 3-3.5/ kg [113].

Cellulose on the other hand, generates glucose units which can directly be converted into ethanol during fermentation. The products of fermentation are fuel (ethanol), organic acids (lactic acid) and solvents (acetone and butanol). In the process, sugars are converted into adenosine triphosphate (ATP) for survival of the bacteria and as a result, bacteria release lactic acid as a waste product. Lactic acid is used in the yogurt or sauerkraut preparation. Its pharmaceutical applications are pH regulation, chiral intermediates and metal sequestration. It is main component of biomaterials viz. sutures and resorbable screws. Most importantly, it is a principal building material for Poly Lactic Acid (PLA) which is used in biodegradable polymers and renewable plastics. The commercial price of lactic acid is approximately \$ 5-13/ kg [114]. It can be used as an inhibitor of *Escherichia coli* O157:H7 and *Salmonella enterica* on spinach leaf surface [115]. In the recent advances in bioenergy industry, solvent like butanol has gained much attention as an alternative [116]. Butanol is considered a superior fuel compared to ethanol due to its higher energy density other fuel properties closer to current petroleum-derived fuels. However, production of bio-butanol requires much research in order to make its production cost-effective [117].

Lignin is a non-sugar component of cell wall and is an amorphous hetero-polymer consisting of phenyl propane units and their precursors namely p-coumaryl alcohols, coniferyl alcohols and sinapyl alcohols. Lignin is hydrophobic in nature, heterogeneous, cross linked anionic polymer. It has strong carbon-carbon (C-C) and ether (C-O-C) linkages which affect its chemical disruption. Polymeric lignin is a high molecular weight compound and therefore, it is important to degrade it in order to convert it into value added fuels and other products. Being one of the most important components of cell wall, lignin is produced in large quantities as a by-product during any fermentation based conversion of biomass-to ethanol process. Therefore, making use of the residual lignin for conversion to value added products will greatly enhance the economic viability of the whole process. The thermal properties of lignin such as

high glass transition temperature (T_g) and melting temperature (T_m) make it a lucrative source of energy. Most of the lignin produced in paper and pulp industry is presently burned to generate process heat. However conversion of lignin into gasoline blending agents such as aromatic hydrocarbons and/or ethers could be potentially more productive. The products generated from degradation of lignin vary with the type of biomass as well as the method used for degradation. Lignin pyrolysis produces bio-oil, phenolics and gases. The phenolic compounds can be used as petrochemical substitution options for applications such as wood-adhesives (resins), bio-plastics, chemicals, bio-fuels, etc [118]. Depending upon the type of biomass, degradation of lignin with HNO₃ is known to generate products such as phenols, guaiacols, syringols, eugenol, catechols, vanillin, vanillin acid, syringaldehyde, syringic acid [119,120]. In presence of homogenous catalyst such as sodium hydroxide and heterogenous catalyst such as alkali metals, and at high temperature and pressure, lignin produces diols and triols which can be further converted to linear and cross-linked polyesters, respectively. Mussatto et al. [121] reported that brewing industries produce sufficient by products and wastes which can be commercially used. Activated carbon, p-coumaric and ferulic acids are some of them which can be extracted from lignin [122]. Also, black liquor remained after extraction can be treated with sulphuric acid (98% w/w) to precipitate lignin [123]. The lignin obtained is neutralised with water and supernatant is dried at 40°C and blended with 85% w/v phosphoric acid to get activated carbon. It can be used to trap impurities of water and air. Lignin can also be used as a bio-sorbent in water purifiers. Lignin base ion-exchangers can be used for heavy metal sorption and also to remove organic pollution.

Therefore, a well planned bio-refinery based on the by-products and their derivatives of biomass to ethanol conversion holds tremendous potential for not only making the process cost-effective but may also aid in better management of waste disposal and other environmental issues related to the process.

4. CONCLUSION

Pre-treatment is an important step in biomass to ethanol conversion process and helps in overcoming the recalcitrance of biomass. The composition and structure of biomass varies from

one type to another and thus emphasising the need for various types of pre-treatments. The goal of the present review is to discuss various hydrolysis methods used to pre-treat lignocellulosic biomass so that it is accessible for fermentation and its simultaneous conversion into biofuel. Besides, the efficient utilization of biomass provides an opportunity to reduce the cost of bio-fuel. In addition to ethanol, several other products of high commercial value can be obtained from the by-products and their derivatives generated in this process. There are reports for many hydrolysis methods and their advantages and disadvantages for industrial applications. These reports in general imply that factors like energy balance, solvent recycling and its degradation must be taken into consideration before selecting the appropriate method for pre-treatment and hydrolysis which is followed by fermentation process. Precautions must be taken so that there is minimum production of inhibitors and if possible, un-hydrolyzed cellulose must be recovered. Current efforts are being made on the use of green solvents to increase the volumetric productivity. Other bio-alcohols such as butanol, methanol etc are also being explored to be used as alternate energy sources. However, while these sources require much more research, production of ethanol from biomass is by far the most viable option owing to the abundance of raw material and relatively established technologies. Although the existing technologies have delivered reasonable results, there is still much scope for improvement which can be achieved by further fine tuning of various steps involved in the whole process.

5. FUTURE SCOPE

Pre-treatment of biomass and thereby converting it into biofuel is the most in-demand and upcoming technology to replace conventional fuel options. Biofuel makes up approximately 3% of total demand for fuel and this will increase upto 7% in near future. There is a need to make a cost-effective technology for biofuel production in order to make biofuels a competitive fuel of choice. Cellulosic ethanol that is derived from lignocellulosic material is one such option owing to abundance and low cost of the raw materials. Several by-products like furfurals, acids and aldehydes, polymers, adhesives and adsorbents are generated during the process. These products if converted into value added products have the potential to lower the overall capital and operational cost of the process. The intermediate sugars obtained after hydrolysing biomass can

be used for other value added products. Use of plants that grow on marginal and wasteland such as *Jatropha* and Elephant grass as raw material could further lower the cost of the process. Novel technologies such as consolidated bio-processing (CBP) and simultaneous saccharification and fermentation (SSF) seek to overcome the high cost of enzymes. Careful selection of biomass, microbes and pre-treatment methods is the key to a robust and commercially viable technology of cellulosic ethanol production. It is a multi-step process and there is scope for more systematic studies and research in order to address the shortcomings of each step.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Himmel M, Ding S, Johnson D, Adney W, Nimlos M. Biomass recalcitrance: engineering plants and enzymes for biofuels production. *Science*. 2007;315: 804–807.
2. Energy Information Administration (2010-06-04), Available:<http://www.eia.gov/forecasts/aeo/> on 2/12/2013.
3. Howard R. Lignocellulose biotechnology: Issues of bioconversion and enzyme production. *Afr. J. Biotechnol.* 2003;2: 602–619.
4. Kim SB, Yum DM, Park SC. Step-change variation of acid concentration in a percolation reactor for hydrolysis of hardwood hemicellulose. *Bioresour. Technol.* 2000;72:289–294.
5. Miller S, Hester R. Concentrated acid conversion of pine softwood to sugars. Part 1: Use of a twin-screw reactor for hydrolysis pretreatment. *Chem. Eng. Commun.* 2007;194:85–102.
6. Lynd LR, Weimer PJ. Microbial cellulose utilization: Fundamentals and biotechnology. *Microbio. and Mol. Biol. Rev.* 2002;66:506-577.
7. Selig MJ, Viamajala S, Decker SR, Toker MP, Himmel ME. Deposition of lignin droplets produced during dilute acid pretreatment of maize stems retards enzymatic hydrolysis of cellulose. *Biotechnol. Prog.* 2007;23:1333–1339.

8. Hsu TA, Ladisch MR, Tsao GT. Alcohol from cellulose. *Chem. Eng. Technol.* 1980; 10:315–319.
9. Agbor VB, Cicek N, Sparling R, Berlin A, Levin DB. Biomass pretreatment: Fundamentals toward application. *Biotechnol. Adv.* 2011;29:675–685.
10. Pandey A, Biswas S, Sukumaran RK, Kaushik N. Study on the availability of Indian Biomass Resources for Exploitation: A Report Based on Nationwide Survey. TIFAC; 2009.
11. Alvira P, Thomas-Pejo E, Ballesteros M, Negro MJ. Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review. *Bioresour. Technol.* 2010;101:4851–4861.
12. Cadoche L, Lopez GD. Assessment of size reduction as a preliminary step in the production of ethanol from lignocellulosic wastes. *Biol. Wastes.* 1989;30:153–157.
13. Hiden A, Inoue H, Yanagida T, Tsukahara K, Endo T, Sawayama S. Combination of hot compressed water treatment and wet disk milling for high sugar recovery yield in enzymatic hydrolysis of rice straw. *Bioresour. Technol.* 2011;11:743-748.
14. Hendriks ATWM, Zeeman G. Pretreatments to enhance the digestibility of lignocellulosic biomass. *Bioresour. Technol.* 2009;100:10-18.
15. Canam T, Town J, Iroba K, Tabil L, Dumonceaux T. Pretreatment of Lignocellulosic Biomass Using Microorganisms: Approaches, Advantages, and Limitations. In: Chandel AK, Silva SSD editors. *Sustainable degradation of lignocellulosic biomass-Techniques, Applications and Commercialization.* In Tech publisher. DOI: 10.5772/55088.
16. Zheng Y, Yu C, Cheng YS, Zhang R, Jenkins B, Vander Gheynst JS. Effects of ensilage on storage and enzymatic degradability of sugar beet pulp. *Biores. Technol.* 2011;102:1489-1495.
17. Eastwood DC, Floudas D, Binder M, Majcherczyk A, Schneider P, Aerts A, et al. The plant cell wall-decomposing machinery underlies the functional diversity of forest fungi. *Science.* 2011;333:762-765.
18. Xu C, Ma F, Zhang X. Lignocellulose degradation and enzyme production by *Irpex lacteus* CD2 during solid-state fermentation of corn stover. *J. Biosci Bioeng.* 2009;108:372–375.
19. Patel H, Gupte A, Gupte S. "Effect of different culture conditions and inducers on production of Laccase by a Basidiomycetes fungal isolate *Pleurotus ostreatus* HP-1 under solid state fermentation," *BioResources* 2009b;4:268-284.
20. Hatakka A, Hammel KE. Fungal biodegradation of lignocelluloses, In: Esser K, Hofrichter M editors. *The Mycota, A Comprehensive Treatise on Fungi as Experimental Systems for Basic and Applied Research- Industrial Applications,* 2nd Edition, Springer Berlin Heidelberg. 2010;319-340.
21. Isroi, Millati R, Syamsiah S, Niklasson C, Cahyanto MN, Lundquist K, Taherzadeh MJ, Biological pretreatment of lignocelluloses with white-rot fungi and its applications: A review. *Bio Resources.* 2011;6:5224-5259.
22. Krause DO, Denman SE, Mackie RI, Morrison M, Rae AL, Attwood GT, McSweeney CS Opportunities to improve fiber degradation in the rumen: microbiology, ecology, and genomics. *FEMS Microbiol. Rev.* 2003;27:663-693.
23. Malherbe S, Cloete TE. Lignocellulosic biodegradation: Fundamentals and applications: A review. *Environ. Sci. Biotechnol.* 2003;1:105–114. DOI: 10.1023/A:1020858910646.
24. Kunamneni A, Ballesteros A, Plou FJ, Alcalde M. Fungal laccase—a versatile enzyme for biotechnological applications. In: Mendez-Vilas a editor. *Communicating current research and educational topics and trends in applied microbiology.* Badajoz: Formatex Research Center. 2007;223–245.
25. Ragunathan R, Swaminathan K. Bioconversion of agro-wastes by fungus, *Pleurotus* spp. *Biol Membr. J.* 2004;30:1–6.
26. Songulashvili G, Elisashvili V, Penninckx M, Metreveli E, Hadar Y, Aladashvili N, Asatiani M. Bioconversion of plant raw materials in value-added products by *Lentinus edodes* (Berk.) Singer and *Pleurotus* spp. *Int. J. Med. Mushrooms.* 2005;7:467–468.
27. Taseli BK. Fungal treatment of hemp-based pulp and paper mill wastes. *Afr. J. Biotechnol.* 2008;7:286–289.
28. Esteghlalian A, Hashimoto AG, Fenske JJ, Penner MH. Modeling, optimization of the dilute-sulfuric-acid pretreatment of corn stover, poplar and switchgrass. *Bioresour. Technol.* 1997;59:129–136.

29. Goldstein IS, Pereira H, Pittman JL, Strouse BA, Scaringelli FP. The hydrolysis of cellulose with super concentrated hydrochloric acid. *Biotechnol. Bioeng.* 1983;13:17–25.
30. Brink DL. Method of treating biomass material. U.S. Patent No. 5221357; 1993.
31. Zhu Z, Sathitsuksanoh N, Vinzant T, Schell DJ, McMillan JD, Zhang YHP. Comparative study of corn stover pretreated by dilute acid and cellulose solvent-based lignocellulose fractionation: Enzymatic hydrolysis, supramolecular structure, and substrate accessibility. *Biotechnol. Bioeng.* 2009;103:715–724.
32. Lu XB, Zhang YM, Yang J, Liang Y. Enzymatic hydrolysis of corn stover after pretreatment with dilute sulfuric acid. *Chem. Eng. Technol.* 2007;30:938-944.
33. Yat SC, Berger A, Shonnard DR. Kinetic characterization of dilute surface acid hydrolysis of timber varieties and switchgrass. *Bioresour. Technol.* 2008;99: 3855–3863.
34. Idrees M, Adnan A, Sheikh S, Qureshi FA. Optimization of dilute acid pretreatment of water Hyacinth biomass for enzymatic hydrolysis and Ethanol production. *EXCLI J.* 2013;12:30-40.
35. Ogasawara Y, Itagaki S, Yamaguchi K, Mizuno N. Saccharification of natural lignocellulose biomass and polysaccharides by highly negatively charged heteropolyacids in concentrated aqueous solution. *Chem Sus Chem.* 2011; 4:519-525.
36. Zeitsch KJ. The Chemistry and Technology of Furfural and Its many By-products. Volume 13 of Sugar series. Elsevier Publisher; 2000.
37. Galbe M, Zacchi G. Pretreatment of lignocellulosic materials for efficient bioethanol production. *Adv. Biochem. Eng. Biotechnol.* 2007;108:41–65.
38. Frederick N, Zhang N, Djioleu A, Ge X, Xu J, Carrier DJ. The Effect of Washing Dilute Acid Pretreated Poplar Biomass on Ethanol Yields. InTech Publisher; 2013. Available:<http://creativecommons.org/licenses/by/3.0>
39. Sharma R, Palled V, Sharma-Shivappa RR, Osborne J. Potential of potassium hydroxide pretreatment of Switch grass for fermentable sugar production. *Appl. Biochem. Biotechnol.* 2012;169:761–772.
40. MacDonald DG, Bakhshi NN, Mathews JF, Roychowdhury A, Bajpai P, Moo-Young M. Alkali treatment of corn stover to improve sugar production by enzymatic hydrolysis. *Biotechnol.- Bioeng.* 1983;25:2067–2076.
41. Kim S, Holtzaple MT. Effect of structural features on enzyme digestibility of corn stover. *Bioresour. Technol.* 2006;97:583–591.
42. Chang VS, Burr B, Holtzaple M. T. Lime pretreatment of switchgrass. *Appl. Biochem. Biotechnol.* 1997;63:3–19.
43. Chang VS, Nagwani M, Holtzaple MT. Lime pretreatment of crop residues bagasse and wheat straw. *Appl. Biochem. Biotechnol.* 1998;74:135–159.
44. Chang VS, Holtzaple MT. Fundamental factors affecting biomass enzymatic reactivity. *Appl. Biochem. Biotechnol.* 2000; 84:5–37.
45. Karr WE, Holtzaple M.T. The multiple benefits of adding non-ionic surfactant during the enzymatic hydrolysis of corn stover. *Biotechnol. Bioeng.* 1998;59:419–427.
46. Karr WE, Holtzaple T. Using lime pre treatment to facilitate the enzymatic hydrolysis of corn stover. *Biomass Bioenergy.* 2000;18:189–199.
47. Chaturvedi V, Verma P. An overview of key pretreatment processes employed for bioconversion of lignocellulosic biomass into biofuels and value added products. *3 Biotech.* 2013;3:415–431.
48. Zhao Y, Wang Y, Zhu JY, Ragauskas A, Deng Y. Enhanced enzymatic hydrolysis of spruce by alkaline pretreatment at low temperature. *Biotechnol. Bioeng.* 2008;99: 1320–1328.
49. Teater C, Yue Z, MacLellan J, Liu Y, Liao W. Assessing solid digestate from anaerobic digestion as feedstock for ethanol production. *Bioresour. Technol.* 2011;102:1856–1862.
50. Chosdu R, Hilmy NE, Erlinda TB, Abbas B. Radiation and chemical pretreatment of cellulosic waste. *Radiation Phys. Chem.* 1993;42:695–698.
51. Nachiappan B, Fu Z, Holtzaple MT. Ammonium carboxylate production from sugarcane trash using long-term air-lime pretreatment followed by mixed-culture fermentation. *Bioresour. Technol.* 2011; 102: 4210–4217.
52. Hu Z, Wang Y, Wen Z. Alkali (NaOH) Pretreatment of switchgrass by radio frequency-based dielectric heating. *Appl. Biochem. Biotechnol.* 2008;148:71–81.

53. Hu Z, Wen Z. Enhancing enzymatic digestibility of switchgrass by microwave-assisted alkali pretreatment. *Biochem. Eng J.* 2008;38:369–378.
54. Iyer V, Wu ZW, Kim SB, Lee YY. Ammonia recycled percolation process for pretreatment of herbaceous biomass. *App. Biochem. Biotechnol.* 1996;57:121–132.
55. Ibrahim MM, El-Zawawy WK, Abdel-Fattah YR, Soliman NA, Agblevor FA. Chemical and physicochemical pretreatment of lignocellulosic biomass: A review. *Carbohydr. Polymer.* 2011;83:720–726.
56. Sills DL, Gossett JM. Assessment of commercial hemicellulases for saccharification of alkaline pretreated perennial biomass. *Technol.* 2011;102:1389–1398.
57. Gírio FM, Fonseca C, Carvalheiro F, Duarte LC, Marques S, Bogel-Lukasik R. Hemicelluloses for fuel ethanol: A review. *Bioresour. Technol.* 2010;101:4775–4800.
58. Gurgel LVA, Pimenta MTB, Curvelo AADS. Enhancing liquid hot water (LHW) pretreatment of sugarcane bagasse by high pressure carbon dioxide (HP-CO₂) Industrial Crops Products. 2014;57:141–149.
59. Li X, Lu J, Zhao J, Qu Y. Characteristics of Corn Stover Pretreated with Liquid Hot Water and Fed-Batch Semi-Simultaneous Saccharification and Fermentation for Bioethanol Production. *PLoS One* 2014;9:e95455 (PMCID 3998958). DOI: 10.1371/journal.pone.0095455 .
60. Kyong KJ, Kim Y, Ximenes E, Ladisch MR. Effect of liquid hot water pretreatment severity on properties of hardwood lignin and enzymatic hydrolysis of cellulose. *Biotechnol. Bioeng.* 2015;112:252–262.
61. Yan J, Liu S. Hot water pretreatment of boreal aspen woodchips in a pilot scale digester. *Energies.* 2015; 8:1166-1180.
62. Lu J, Li XZ, Zhao J, Qu Y. Enzymatic saccharification and ethanol fermentation of reed pretreated with liquid hot water. *J. Biomed. Biotechnol.* 2012. Article ID 276278. (PMCID: PMC3362088).
63. Wasserscheid P, Keim W. Ionic liquids—New solutions for transition metal catalyst. *Angewandte Chemie International Edition* 2000;39:3773–3789.
64. Zavrel M, Bross D, Funke M, Buchs J, Spiess AC. High-throughput screening for ionic liquids dissolving (ligno-)cellulose. *Bioresour. Technol.* 2009;100:2580–2587.
65. Fort DA, Remsing RC, Swatoski RP, Moyna P, Moyna G, Rogers RD. Can ionic liquids dissolve wood? Processing and analysis of lignocellulosic materials with 1-n-butyl-3-methylimidazolium chloride. *Green Chem.* 2007;9:63-69.
66. Moulthrop JS, Swatoski RP, Moyna G, Rogers RD. High resolution ¹³C NMR studies of cellulose and cellulose oligomers in ionic liquid solutions. *Chem. Commun.* 2005;1557–1559.
67. Xu F, Shi YC, Wang D. Enhanced production of glucose and xylose with partial dissolution of corn stover in ionic liquid, 1-Ethyl-3-methylimidazolium acetate. *Bioresour. Technol.* 2012;114 :720–724.
68. Fukaya Y, Hayashi K, Wada M, Ohno H. Cellulose dissolution with polar ionic liquids under mild conditions: Required factors for anions. *Green Chem.* 2008;10:44–46.
69. Mäki-Arvela P, Anugwom I, Virtanen P, Sjöholm R, Mikkola JP. Dissolution of lignocellulosic materials and its constituents using ionic liquids—A review. *Indian Crops Prod.* 2010; 32:175–201.
70. Feng L, Chen Z. Research progress on dissolution and functional modification of cellulose in ionic liquids. *J. Mol. Liq.* 2008; 142:1–5.
71. Narayanaswamy N. Supercritical carbon dioxide pretreatment of various lignocellulosic biomasses. MS Thesis, Ohio University, Athens; 2010.
72. Garrote G, Dominguez H, Parajo JC. Hydrothermal processing of lignocellulosic materials. *Eur. J. Wood Wood Products.* 1999;57:191–202.
73. Pezoa R, Cortinez V, Hyvarinen S, Reunanen M, Hemming J, Lienqueo ME, Salazar O, Carmona R, Garcia A, Murzin DY, Mikkola JP. Use of ionic liquids in the pretreatment of forest and agricultural residues for the production of bioethanol. *Cell Chem Technol.* 2010;44:165–172.
74. Thring RW, Chorent E, Overend R. Recovery of a solvolytic lignin: Effects of spent liquor/acid volume ratio, acid concentration and temperature. *Biomass.* 1990;23:289–305.
75. Pan X, Arato C, Gilkes N, Gregg D, Mabee W, Pye K, Xiao Z, Zhang X, Saddler J. Biorefining of softwoods using ethanol organosolv pulping: Preliminary evaluation of process streams for manufacture of fuel-grade ethanol and co-products. *Biotechnol. Bioeng.* 2005;90:473–481.

76. Curvelo AAS, Pereira R. Kinetics of ethanol-water of sugar cane bagasse. The 8th International Symposium on Wood and Pulping Chemistry Proc, Helsinki, Finland. 1995;2:473-478.
77. Zhao X, Cheng K, Liu D. Organosolv pretreatment of lignocellulosic biomass for enzymatic hydrolysis. *Appl. Microbiol. Biotechnol.* 2009;82:815-827.
78. Miura M, Kaga H, Sakurai A, Takahashi K. Rapid pyrolysis of wood block by microwave heating. *J. Anal. Appl. Pyro.* 2004;71:187-199.
79. Zwart RWR, Boerrigter H, Van der Drift A. The impact of biomass pretreatment on the feasibility of overseas biomass conversion to fischer-tropsch products. *Energy Fuels.* 2006;20:2192-2197.
80. Chiamonti D, Rizzo AM, Prussi M, Tedeschi SS, Zimbardi F, Braccio G, Viola E, Pardelli PT. 2nd generation lignocellulosic bioethanol: Is torrefaction a possible approach to biomass pretreatment? *Biomass Conversion Biorefineries.* 2011;1:9-15. DOI 10.1007/s13399-010-0001-z.
81. Vincent R, Alexandre G, Mathieu R, René G. Energy requirement for fine grinding of torrefied wood. *Biomass Bioenergy.* 2010; 34:923-930.
82. Sun Y, Cheng J. Hydrolysis of lignocellulosic materials for ethanol production: A review. *Bioresour. Technol.* 2002;83:1-11.
83. Duff SJB, Murray WD. Bioconversion of forest products industry waste cellulose to fuel ethanol: A review. *Bioresour. Technol.* 1996;55:1-33.
84. Wright JD. Ethanol from biomass by enzymatic hydrolysis. *Chem. Eng. Progr.* 1998;84:62-74.
85. Overend RP, Chornet E. Fractionation of lignocellulose by steam-aqueous pretreatments. *Philosophical Transactions Royal Soc.* 1987;321:523-536.
86. Kokta BV, Ahmed A. Steam Explosion Pulping. In: Young RA, Akhtar M editors. *Environmentally Friendly Technologies for the Pulp and Paper Industry.* John Wiley & Sons, Inc; 1998.
87. Adapa P, Tabil L, Schoenau G, Opoku A. Pelleting characteristics of selected biomass with and without steam explosion pretreatment. *Intl. J. Agr. Biol. Eng.* 2010; 3:62-79.
88. Cara C, Ruiz E, Ballesteros I, Negro MJ, Castro E. Enhanced enzymatic hydrolysis of olive tree wood by steam explosion and alkaline peroxide delignification. *Process Biochem.* 2006;41:423-429.
89. Dererie DY, Trobro S, Momeni MH, Hansson H, Blomqvist J, Passoth V, Schnürer A, Sandgren M, Ståhlberg J. Improved bio-energy yields via sequential ethanol fermentation and biogas digestion of steam exploded oat straw. *Bioresour. Technol.* 2011;102:4449-4454.
90. Ballesteros I, Negro MJ, Cabañas LA, Manzanares P, Ballesteros M. Ethanol production from steam-explosion pretreated wheat straw. *Appl. Biochem. Biotechnol.* 2006;129-132:496-508.
91. Teymouri F, Perez LL, Alizadeh H, Dale BE. Ammonia fiber explosion treatment of corn stover. *Appl. Biochem. Biotechnol.* 2004;113:951-963.
92. Bals B. Evaluating the impact of ammonia fiber expansion (AFEX) pretreatment conditions on the cost of ethanol production. *Bioresour. Technol.* 2011;102: 1277-1283.
93. Lee JM, Jameel H, Venditti RA. A comparison of the autohydrolysis and ammonia fiber explosion (AFEX) pretreatments on the subsequent enzymatic hydrolysis of coastal Bermuda grass. *Bioresour. Technol.* 2010;101: 5449-5458.
94. Isci A, Himmelsbach JN, Pometto AL, Raman R, Anex RP. Aqueous ammonia soaking of switchgrass followed by simultaneous saccharification and fermentation. *Appl. Biochem. Biotechnol.* 2008;144:69-77.
95. Kim TH, Lee YY. Pretreatment and fractionation of corn stover by ammonia recycle percolation process. *Bioresour. Technol.* 2005;96:2007-2013.
96. Zheng YZ, Lin HM, Tsao GT. Pretreatment for cellulose hydrolysis by carbon dioxide explosion. *Biotechnol Progr.* 1998;14:890-896.
97. Srinivasan N, Ju LK. Pretreatment of guayule biomass using supercritical carbon dioxide-based method. *Bioresour. Technol.* 2010;101:9785-9791.
98. Luterbacher JS, Tester JW, Walker LP. High-solids biphasic CO₂-H₂O pretreatment of lignocellulosic biomass. *Biotechnol. Bioeng.* 2010;107:451-460.
99. Jayawardhana K, Van-Walsum GP. Modeling of carbonic acid pretreatment process using ASPEN-Plus. *Appl. Biochem. Biotechnol.* 2004;113:1087-1102.

100. Kim KH, and Hong J. Supercritical CO₂ pretreatment of lignocellulose enhances enzymatic cellulose hydrolysis. *Bioresour. Technol.* 2001;77:139-144.
101. Keshwani DR, Cheng JJ. Microwave-based alkali pretreatment of switchgrass and coastal bermudagrass for bioethanol production. *Biotechnol Progr.* 2010;26: 644–652.
102. Saleh SB, Hansen BB, Jensen PA, and Dam-Johansen K. Efficient fuel pretreatment: Simultaneous torrefaction and grinding of biomass. *Energy Fuels.* 2013;27:7531–7540.
103. Li Z, Chen CH, Hegg EL, Hodge DB. Rapid and effective oxidative pretreatment of woody biomass at mild reaction conditions and low oxidant loadings. *Biotechnol. Biofuels.* 2013;6:119-127.
104. Nguyen QA, Tucker MP, Keller FA, Eddy FP. Two-stage dilute-acid pretreatment of softwoods. *Appl. Biochem. Biotechnol.* 2000;84-86:561-576.
105. Spirodon I, Popa VI. Hemicelluloses: structure and properties. In: Dimitriu S, editor. *Polysaccharides: Structural Diversity and Functional Versatility.* New York: Marcel Dekker. 2005;1204.
106. Peng F, Peng P, Xu F, Sun RC. Fractional purification and bioconversion of hemicelluloses. *Biotechnol. Adv.* 2012;30: 879-903.
107. Cipriani TR, Mellinger CG, De Souza LM, Baggio CH, Freitas CS, Marques MCA, Gorin PAJ, Sasaki GL, Lacomini M. A polysaccharide from a tea (infusion) of *Maytenus ilicifolia* leaves with anti-ulcerprotective effects. *J. Nature Products.* 2006;69:1018–1021.
108. Kulicke WM, Lettau AI, Thielking H. Correlation between immunological activity, molar mass, and molecular structure of different (1→3)-β-D-glucans. *Carbohydr. Res.* 1997;297:135–143.
109. Kitamura S, Hori T, Kurita K, Takeo K, Hara C, Itoh W, Tabata K, Elgsaeter A, Stokke BT. An antitumor, branched (1→3)-β-D-glucan from a water extract of fruiting bodies of *Cryptoporus volvatus*. *Carbohydr. Res.* 1994;263:111–121.
110. Klingler FD, Ebertz W. Oxocarboxylic Acids In: *Ullmann's Encyclopedia of Industrial Chemistry.* Wiley-VCH, Weinheim; 2005. DOI:10.1002/14356007.a18313.
111. Zheng zhou Mahaco Trading Co., Ltd. Available:<http://www.mahaco.cn/about.asp> on 10/07/2014.
112. Ritter AV, Bader JD, Leo MC, Preisser JS, Shugars DA, Vollmer WM, Amaechi BT, Holland JC. Tooth-surface-specific Effects of Xylitol: Randomized Trial Results. *J. Dental Res.* 2013;92:512–517.
113. Anhui Leafchem Co., Ltd. Available:<http://www.leafchem.com/> on 10/07/2014
114. Suzhou Houjin Chemical Co., Ltd. Available:<http://www.cnhoujin.com/English/> on 10/07/2014.
115. Cálix-Lara TF, Rajendran M, Talcott ST, Smith SB, Miller RK, Castillo A, Sturino JM, Taylor TM. Inhibition of *Escherichia coli* O157:H7 and *Salmonella enterica* on spinach and identification of antimicrobial substances produced by a commercial Lactic Acid Bacteria food safety intervention. *Food Microbiol.* 2014;38:192-200.
116. Zheng J, Tashiro Y, Wang Q, Sonomoto K. Recent advances to improve fermentative butanol production: Genetic engineering and fermentation technology. *J. Biosci. Bioeng.* 2015;119:1-9. Doi: 10.1016/j.jbiosc.2014.05.023.
117. Green EM. Fermentative production of butanol—the industrial perspective. *Curr. Opin. Biotechnol.* 2011;22:337-347.
118. Somleva MN, Peoples OP, Snell KD. PHA Bioplastics, Biochemicals, and Energy from Crops. *Plant Biotechnol. J.* 2013;11: 233-252.
119. Varanasi P, Singh P, Auer M, Adams PD, Simmons BA, Singh S. Survey of renewable chemicals produced from lignocellulosic biomass during ionic liquid pretreatment. *Biotechnol for Biofuels.* 2013; 6:14. Doi:10.1186/1754-6834-6-14.
120. Zilnik LF, Jazbinsek A. Recovery of renewable phenolic fractions from pyrolysis oil. *Separation and Purification Technol.* 2012;86:157-170.
121. Mussatto SI, Moncada J, Roberto IC, Carlos A. Cardona Techno-economic analysis for brewer's spent grains use on a biorefinery concept: The Brazilian case. *Bioresour. Technol.* 2013;148:302–310.
122. Holladay JE, Bozell JJ, White JF, Johnson D. Top Value-Added Chemicals from Biomass Volume II—Results of Screening for Potential Candidates from Biorefinery Lignin. Pacific Northwest National Laboratory, U.S. Department of Energy

- under Contract DE-AC05-76RL01830; 2007.
123. Gosselink RJA. Lignin as a renewable aromatic resource for the chemical industry, Ph.D. Thesis, Wageningen University, Wageningen, NL; 2011.
 124. Potumarthi R, Baadhe RR, Nayak P, Jetty A. Simultaneous pretreatment and saccharification of rice husk by *Phanerochete chrysosporium* for improved production of reducing sugars. *Bioresour. Technol.* 2013;128:113–117.
 125. Noureddini H, Byun J. Dilute-Acid Pretreatment of Distillers' Dried Grain with Solubles (DDGS) and corn fiber. *Bioresour. Technol.* 2010;101:1060-1067.
 126. Sharma R, Palled V, Sharma-Shivappa RR, Osborne J. Potential of potassium hydroxide pretreatment of switch grass for fermentable sugar production. *Appl. Biochem. Biotechnol.* 2012;169:761–772.
 127. Mesa L, González E, Cara C, González M, Castro E, Mussatto SI. The effect of organosolv pretreatment variables on enzymatic hydrolysis of sugarcane bagasse. *Chem. Eng. J.* 2011;168:1157–1162.
 128. Barros Rda R, Paredes Rde S, Endo T, Bon EP, Lee SH. Association of wet disk milling and ozonolysis as pretreatment for enzymatic saccharification of sugarcane bagasse and straw. *Bioresour. Technol.* 2013;136:288–29. DOI: 10.1016/j.biortech.2013.03.009.
 129. Brodeur G, Yau E, Badal K, Collier J, Ramachandran KB, Ramakrishnan S. Chemical and physicochemical pretreatment of lignocellulosic biomass: A review. *Enz Res.* 2011; Article ID 787532; DOI: 10.4061/2011/787532 .

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