

## Review Article

# Chemical Pretreatment Methods for the Production of Cellulosic Ethanol: Technologies and Innovations

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Pretreatment of lignocellulose has received considerable research globally due to its influence on the technical, economic and environmental sustainability of cellulosic ethanol production. Some of the most promising pretreatment methods require the application of chemicals such as acids, alkali, salts, oxidants, and solvents. Thus, advances in research have enabled the development and integration of chemical-based pretreatment into proprietary ethanol production technologies in several pilot and demonstration plants globally, with potential to scale-up to commercial levels. This paper reviews known and emerging chemical pretreatment methods, highlighting recent findings and process innovations developed to offset inherent challenges via a range of interventions, notably, the combination of chemical pretreatment with other methods to improve carbohydrate preservation, reduce formation of degradation products, achieve high sugar yields at mild reaction conditions, reduce solvent loads and enzyme dose, reduce waste generation, and improve recovery of biomass components in pure forms. The use of chemicals such as ionic liquids, NMMO, and sulphite are promising once challenges in solvent recovery are overcome. For developing countries, alkali-based methods are relatively easy to deploy in decentralized, low-tech systems owing to advantages such as the requirement of simple reactors and the ease of operation.

## 1. Introduction

Cellulosic or second generation (2G) bioethanol is produced from lignocellulosic biomass (LB) in three main steps: pretreatment, hydrolysis, and fermentation. Pretreatment involves the use of physical processes (e.g., size reduction, steaming/boiling, ultrasonication, and popping), chemical methods (e.g., acids, bases, salts, and solvents), physico-chemical processes (e.g., liquid hot water and ammonium fibre explosion or AFEX), biological methods (e.g., white-rot/brown-rot fungi and bacteria), and several combinations thereof to fractionate the lignocellulose into its components. It results in the disruption of the lignin seal to increase enzyme access to holocellulose [1, 2], reduction of cellulose crystallinity [3, 4], and increase in the surface area [5, 6] and porosity [7, 8] of pretreated substrates, resulting in increased

hydrolysis rate. In hydrolysis, cellulose and hemicelluloses are broken down into monomeric sugars via addition of acids or enzymes such as cellulase. Enzymatic hydrolysis offers advantages over acids such as low energy consumption due to the mild process requirements, high sugar yields, and no unwanted wastes. Enzymatic hydrolysis of cellulose is affected by properties of the substrate such as porosity, cellulose fibre crystallinity, and degree of polymerization, as well as lignin and hemicellulose content [9, 10]; optimum mixing [11]; substrate and end-product concentration; enzyme activity; reaction conditions such as pH and temperature [12, 13]. The cost of commercial enzymes is a major economic headache in 2G bioethanol production and such pretreatment methods that support low enzyme dosages per unit biomass while optimizing ethanol yields (in addition to other favourable factors) are of interest in cellulosic ethanol production. It is

known that cellulase loadings of less than 10 FPU/gram-cellulose are essential for economic production of cellulosic ethanol [14].

In fermentation, sugars are converted into ethanol under liquid- or solid-state using yeast or bacteria. The process economics is improved significantly if both C5 and C6 sugars are utilized, though the fermentation efficiency of C5 sugars is very low. Further, the yeasts cannot endure low pH as well as high ethanol and byproduct concentrations [15]. Additional challenges with xylose fermenting yeasts include long fermentation periods, low productivity, high viscosity of fermentation broth, and byproduct formation [15]. For both enzymatic hydrolysis and fermentation, the presence of degradation compounds such as furfural and hydroxymethyl-furfural (HMF) produced during pretreatment inhibits the smooth functioning of enzymes. Thus, ongoing research under pretreatment has focussed on the development of innovative methods requiring the use of mild conditions that significantly reduce inhibitor formation while maintaining high sugar yields.

In general, pretreatment presents the most practical and economic challenges in the attempt to commercialize cellulosic bioethanol [16–18] since it may affect upstream [14] as well as downstream processes by determining fermentation toxicity, enzymatic hydrolysis rates, enzyme loadings, product concentrations and purification, waste treatment demands, and power generation [19]. The results from many studies have shown that pretreatment is relatively costly among the various operations and processes involved in cellulosic ethanol production [20–25], representing about 20% of the total cost [14]. While pretreatment introduces additional cost, the consequence of hydrolysing lignocellulose without pretreatment is far less favourable since only about 20% of native biomass is hydrolysed [26].

It is generally accepted that efficient pretreatment should avoid size reduction and use of costly chemicals [19, 27], improve fibre reactivity and maximize formation/recovery of sugars [28], avoid loss of carbohydrate [29], avoid formation of enzyme-inhibiting byproducts [30, 31], preserve cellulose and hemicellulose fractions that are easily digestible by hydrolytic enzymes [32, 33], generate high-value lignin coproduct [18, 26], minimize energy requirement [22, 34], and achieve high sugar yields under high biomass loads [35]. However, no perfect pretreatment method has been discovered since there are variations in terms of suitability of one method for various materials, which may be further compounded by factors such as maturity, mode of harvest, extent of drying, and storage conditions of the feedstock [36–39]. The chemical methods of pretreating lignocellulosic materials are widely employed in many pilot and demonstration plants since they are ideal for low lignin materials. This paper reviews the various chemical methods that have received significant attention globally, with emphasis on process innovations and interesting findings from the work of researchers in the field. It is the second of two papers (with the first article addressing physical and physicochemical methods) that is expected to serve as a reference for researchers in both academia and industry. The methods discussed are centred on the use of acids, bases, oxidants, and solvents.

## 2. Chemical Pretreatment of Lignocellulosic Biomass

**2.1. Acid Hydrolysis.** In this method, dried biomass is milled, (occasionally) presoaked in water, and submerged in acidic solution under specific temperatures for a period of time. Pretreated content is filtered to separate the liquor from the unhydrolysed solid substrate which undergoes washing (to extract sugars and remove acids) and/or neutralization before saccharification. Generally, the hydrogen ion concentration is directly correlated with the hydrolysis reaction constant; thus, the more negative the pKa value of the acid, the more effective the hydrolysis process [40]. Sulphuric ( $\text{H}_2\text{SO}_4$ ) and phosphoric ( $\text{H}_3\text{PO}_4$ ) acids are widely used since they are relatively cheap and efficient in hydrolysing lignocellulose, though the latter gives a milder effect and is more benign to the environment. Hydrochloric (HCl) acid is more volatile and easier to recover and attacks biomass better than  $\text{H}_2\text{SO}_4$  [41]; similarly, nitric acid ( $\text{HNO}_3$ ) possesses good cellulose-to-sugar conversion rates [42]. However, both acids are expensive compared to sulphuric acid.

In acidic media, the amorphous hemicelluloses in LB hydrolyse quicker (than cellulose) to soluble sugars [43, 44] and some oligomers especially in mild conditions [21] through the disruption of xylosidic bonds and cleavage of acetyl ester groups [45, 46], and the lignin seal is degraded through substitution reactions and broken links accompanied by condensation reactions that prevent dissolution [47, 48]. Cellulose undergoes preferential degradation of amorphous regions leading to enlarged cellulose fibrils and fibril aggregates [44] and an increase in the crystallinity index of the pretreated material [38, 43].

The process is generally affected by particle size, temperature, reaction time, acid concentration, and liquid-to-solid ratio. The combined severity factor ( $R'_0$ ) is an index used to assess the effect of pretreatment temperature, time, and pH on the efficiency of the process as shown below [49]:

$$\log R'_0 = \log \left( t \cdot e^{(T-100)/14.75} \right) - \text{pH}, \quad (1)$$

where  $t$  is the reaction time in minutes and  $T$  is the hydrolysis temperature in °C. Acid pretreatment comes under two main variations—dilute and concentrated acid hydrolysis, each applied under further process variations.

**2.1.1. Dilute Acid Pretreatment.** Under dilute acid (0.2–2.5% w/w) processes, high temperatures (120–210°C) and pressures are used to achieve reaction times in seconds or minutes and are thus suitable for continuous operations [21, 50]. The low acid consumption is a major advantage in terms of cost and process severity [51]. Moreover, low acid concentrations (<1% w/v sulphuric/phosphoric) release essential nutrients (S and P) that enhance downstream fermentation [52]. A variation of the process involves two stages of pretreatment: in the first stage, most hemicelluloses in the biomass substrate are solubilised in the presence of a more dilute acid, while the second stage involves the use of a higher acid concentration to hydrolyse the cellulose and the remaining hemicellulose [53, 54].

Increasing pretreatment severity generally leads to higher rates of cellulose conversion to glucose at shorter reaction times, though conditions that yield high glucose do not necessarily translate to high xylose yields. Pretreatment of municipal solid waste (carrot and potato peelings, grass, newspaper, and crap paper) with dilute acid  $\text{H}_2\text{SO}_4$ ,  $\text{HNO}_3$ , and  $\text{HCl}$  was undertaken by Li et al. [55] who found the glucose yield of pretreated substrates to depend more on acid concentration and enzyme loading than reaction temperature. For feedstock mixtures such as aspen and switchgrass, and aspen and balsam, the process was observed to have no synergistic or antagonistic effect on enzymatic hydrolysis, indicating the likelihood of predicting such combined systems based on models for pure species yields [56]. A comparatively low number of researchers have investigated the nitric acid pretreatment of LB. Dilute  $\text{HNO}_3$  pretreatment was found to give the highest glucose concentration (compared to dilute  $\text{H}_2\text{SO}_4$ ) in the pretreatment of rye straw [57]. However, byproducts from nitric acid pretreatment are difficult to remove by washing of the pretreated substrates [42].

Also, dilute  $\text{H}_3\text{PO}_4$  is frequently used; the acid was applied on potato peels with overall sugar yield reaching 82.5% of the theoretical even though arabinose conversion was found to be low due to its thermal instability [58]. Its application to bamboo and corn cob also yielded high sugars at  $170^\circ\text{C}$  for 45 minutes [59] and  $140^\circ\text{C}$  for 10 minutes, respectively [60]. In another work, Avci et al. [61] achieved 85% glucose and 91.4% xylose yields on corn stover at 0.5% (v/v)/ $180^\circ\text{C}/15$  min and 1% (v/v)/ $160^\circ\text{C}/10$ , respectively, at low concentrations of degradation products. In an attempt to improve process performance, researchers have tried combinations of acids and other compounds with mixed results. Heredia-Olea et al. [62] combined  $\text{HCl}$  and  $\text{H}_2\text{SO}_4$  in pretreating sweet sorghum bagasse but did not record any significant improvement over acid treatment. However, Zhang et al. [52] recorded higher xylose yields during combined ( $\text{H}_2\text{SO}_4/\text{H}_3\text{PO}_4$ ) pretreatment of oil palm empty fruit bunch compared to single acid application. Anaerobic storage of pretreated substrates was found to improve enzymatic degradability of reed canarygrass and switchgrass in a pilot-scale plant [63].

Aside mineral acids, organic acids such as maleic, fumaric [8], and oxalic [64–67] have been found to be useful substitutes to mineral acids. Oxalic and maleic acids have higher solution potential and degrade hemicelluloses more efficiently than sulphuric acid [8]. In addition, they have two pKa values which favour efficient hydrolysis over a range of temperatures and pH values [66, 68]. The use of maleic and fumaric generally results in lower degradation products at similar conditions compared to sulphuric acid [69]. Oxalic acid application to corn stover gave best results at  $160^\circ\text{C}$  for 10 minutes at a concentration of 200 mM [70]. On maple wood, oxalic pretreatment resulted in equivalent glucose (87.7%), xylose (86.9%), and total sugar yields (87.4%) compared to dilute sulphuric and hydrochloric acids [71].

*Main Disadvantages.* Though dilute acid pretreatment has received wide attention from researchers due to its advantages

such as high cellulose content of pretreated substrates and low requirement of enzymes, it comes with some drawbacks. A major demerit of this process is its requirement of special corrosion-resistant reactors which are usually expensive both in investment and operation [72], compared to other chemical (e.g., dilute alkali) and physicochemical (e.g., steam explosion and AFEX) methods [13, 73]. The energy consumption of the process and the cost of the acid [74] as well as performance limitations based on particle size (a few millimetres) and solids concentration ( $\leq 30\%$ ) contribute significantly to the overall cost [14]. Dilute acids are less effective in removing lignin compared to alkaline methods. Neutralization of pretreated contents creates solid waste [75], though it is necessary for improving the downstream fermentation process.

The sugar yield is reduced because a portion of the sugar is degraded into enzyme-inhibiting byproducts such as furfural (2-furaldehyde), 5-hydroxymethylfurfural (5-HMF), acetic acid, gypsum, vanillin, and aldehydes (4-hydroxybenzaldehyde, syringaldehyde, etc.) as a result of the conditions that cause cellulose to rapidly break into sugars [76–79]. It is pertinent that inhibitors are removed by filtering off hydroxylate liquor followed by washing and drying of cellulose-rich residues [80] or by using reverse osmosis to exclude acetic acid, furfural 5-HMF, and other compounds before fermentation [81, 82]. The use of membranes to detoxify hydroxylates could be exploited at the industrial level due to the ease of scaling up for large-scale operations. Further, employing simultaneous saccharification and fermentation (SSF) ensures rapid conversion of glucose into ethanol and the continuous removal of ethanol during fermentation [83]. Other options pertain to the use of agents such as activated carbon to selectively absorb inhibitors [84] or the use of yeast strains that tolerate inhibitors at significant high levels [85].

Another compound known to cause inhibition of enzymatic hydrolysis of cellulose is pseudolignin—an acid-insoluble substance that is formed from repolymerization of degradation products with/without lignin. Surface lignin reduces cellulase affinity for biomass substrate and lowers the hydrolysis rate [86]. High acid concentrations, temperatures, and treatment time generally create conditions for increased degradation products and pseudolignin, resulting in reduced lignin recovery in the hydroxylate. Inhibitor concentrations may be reduced by combining mild physical-chemical conditions and optimised enzyme loadings [87], by using a two-stage process [53] or by performing deacetylation prior to pretreatment to reduce hydroxylate toxicity [45]. Losses in C5 sugars solubilised into the liquid stream are avoided if it is further processed [33] or if unfiltered hydroxylates are wholly used in enzymatic hydrolysis, where neutralization of the hydroxylate can be achieved by the use of a novel single-step neutralization and buffering procedure to adjust the pH [87, 88].

*2.1.2. Concentrated Acid Pretreatment.* This pretreatment variation uses concentrated sulphuric (65–86% w/w), hydrochloric (41%), or phosphoric (85% w/w) acids to pretreat dried (5–10% moisture), pulverized biomass at low temperatures ( $30$ – $60^\circ\text{C}$ ) and pressures. Pretreated contents

are diluted with deionized water for saccharification to take place at moderate temperatures (70–121°C), separated into solid and liquid fractions, followed by washing and neutralization of the solid substrates [89, 90]. Another variation involves the addition of organic solvents such as acetone to pretreated contents followed by agitation of the mixture to stop the reaction and to separate the solids from the supernatant (containing lignin) which undergo further washing before enzymatic hydrolysis [91].

The efficiency of the process is affected by acid concentration, acid/biomass ratio, process temperature, and time. Two patented process configurations—the Arkenol and the Biosulfurol processes—which are based on the concentrated  $\text{H}_2\text{SO}_4$  platform have shown potential commercially. The Arkenol process involves pretreatment (decrystallization of biomass) at temperatures below 50°C at acid (70–77%)/biomass (10% moisture, <1 mm particle size) ratio of 1.25 [92]. In the Biosulfurol process, the biomass is trickled in the acid (70%) in the presence of dry  $\text{CO}_2$  from the fermenter followed by dilution of the pretreated slurry with water. The acid is partly separated from the biomass slurry by the use of membranes before fermentation and partly in an anaerobic digester after fermentation. The relative merits of the Biosulfurol process, according to van Groenestijn et al. [92], include the nonrequirement of enzymes, low temperature treatment, low production of degradation products, and the capacity to fractionate various biomass with high ethanol yields.

Concentrated  $\text{H}_3\text{PO}_4$  is effective at low temperatures, dissolves cellulose in the presence of water, possesses no inhibitory effects on hydrolysis and fermentation, and gives high sugars [93] compared to dilute acid pretreatment [89]. Pretreated biomass substrates are left with uneven and rough molecular surfaces that enhance enzyme adsorption rates and thereby accelerate hydrolysis [94]. Combinations of acid and *p*-cresol, a phenol derivative, enabled complete separation of lignin and carbohydrates in the pretreatment of oil palm empty fruit bunch [90]. In cellulose solvent- and organic solvent-based lignocelluloses fractionation (COSLIF), the biomass feedstock is fractionated by adding concentrated  $\text{H}_3\text{PO}_4$  under mild conditions (50°C, 1 atm, and 60 min) followed by the addition of an organic solvent such as ethanol (95% v/v) or acetone under room temperature for 10 min [95, 96]. The main advantages of COSLIF include high enzyme accessibility to cellulose in pretreated substrates [96] and effective fractionation of diverse biomass with good sugar yields [95]. High glucan digestibility at low enzyme dose is usually achieved with COSLIF [96, 97] and in combination with other agents such as ionic liquids [97].

Despite the aforementioned merits, COSLIF (like other concentrated acid methods) is slow and uses high loads of solvent. Further, depolymerisation and sugar degradation increase at temperatures above 50°C [95]. Other challenges with concentrated acid pretreatment include corrosion of equipment, acid recovery, and neutralization waste when acid is not recovered.

## 2.2. Alkali Pretreatment

**2.2.1. Process Background.** In alkaline pretreatment, lignocellulosic materials are mixed with bases such as sodium,

potassium, calcium, and ammonia [50, 98] at specific temperatures and pressures in order to degrade ester and glycosidic side chains of the material [18], leading to lignin structure disruption [99, 100], cellulose swelling, and decrystallization [74]. Alkaline treatment extracts hemicelluloses from polysaccharides and produces organic acids that lower the pH. Two streams are formed comprising a wet solid fraction composed of mainly cellulose and a liquid fraction containing dissolved hemicelluloses, lignin, and some unreacted inorganic chemicals. The solids are separated and washed in warm/hot water until neutrality before they are hydrolysed. Washing removes enzyme inhibitors and residual unreacted reagents and improves the release of sugars from pretreated solids.

The process is influenced by NaOH loading, liquid-to-solid ratio, temperature, and time, among others. In general, pretreatment is less severe since it can be carried out under atmospheric conditions though at the cost of longer retention times [50, 101]. Low alkali concentrations (<4% w/w) are mostly used at high temperatures and pressures. Mild alkali pretreatment of biomass favours enzymatic hydrolysis especially for materials that have relatively low lignin content. Sugar degradation and corrosion problems are less severe in alkali processes than in acid pretreatment [57, 102] and mild conditions (55°C) may not require posttreatment washing since enzyme inhibiting compounds are generally low [103].

Bases that have been used widely include hydroxides of potassium, sodium, and calcium, as well as sodium carbonate ( $\text{Na}_2\text{CO}_3$ ). KOH selectively removes xylan [104] as was observed on peashrub at 25°C for 10 h with high efficiency [105]. Application of KOH pretreatment on rye straw gave lower sugar yields than dilute acid [57]. It was, however, found to give high fermentation efficiency relative to other methods [42]. Regarding  $\text{Na}_2\text{CO}_3$ , efficient delignification of biomass is realized which makes the carbonate a promising chemical for pretreatment. Application of the carbonate [106] as well as combined  $\text{Na}_2\text{CO}_3$ - $\text{Na}_2\text{SO}_3$  [107] on rice straw recorded good carbohydrate preservation and sugar yields.

The bases—NaOH and  $\text{Ca}(\text{OH})_2$ —have been extensively investigated as agents for pretreatment and are discussed in the sections below.

**2.2.2. Sodium Hydroxide Pretreatment.** Though expensive, sodium hydroxide (NaOH) is used widely due to its relative high alkalinity for the fractionation of various materials including agricultural residues and wood. Dilute NaOH application loosens the biomass structure, separate the bonds between the lignin and the carbohydrates, increases the internal surface area, decreases the degree of polymerization and crystallinity, and disrupts the lignin structure [108]. Homogenization of pretreated substrates enhances glucose yields by increasing the surface area and porosity of the biomass [6]. High alkaline concentrations generally cause increased biomass delignification [109], though severe concentrations (6–20% w/w) result in cellulose dissolution and reduced lignin removal [110]. Pedersen et al. [33] observed that increasing the pH from 10 to 13 increased the removal of lignin from 40 to 80% w/w dry wheat straw at 140°C. Also, by varying the NaOH loading rate from 3 to 9% based on

initial dry bagasse, Zhao et al. [111] recorded an increase in delignification, from 52.3% to 75.5%.

Compared to acid hydrolysis, NaOH pretreatment appears to improve enzymatic biodegradability due to the higher delignification ability of alkali. Evidence of this assertion, for example, can be found in the work of Ioelovich and Morag [112] who applied both dilute  $\text{H}_2\text{SO}_4$  and NaOH to four materials and found the alkali to be more efficient in terms of sugar yields, delignification, and biomass utilization rate.

In many situations, positive results have come out from combined NaOH and other agents such as peracetic acid [111], polyelectrolyte [113], and hydrogen peroxide. In addition, the environmental impact is low and no special reactors are required. As a pretreatment agent, alkaline peroxide favours enzymatic hydrolysis as it removes lignin efficiently [114] and produces insignificant inhibitors [115, 116]. Its application to corn stover was effective in producing good glucose yields at low (0.125 g  $\text{H}_2\text{O}_2/\text{g}$  biomass) peroxide doses [117]. Further, higher sugar yields from grass stovers were obtained using peroxide relative to dilute NaOH and AFEX [118]. Another positive aspect about peroxide is its use in recovering lignin and other components from substrates pretreated by other methods which results in higher sugar yields [116].

**2.2.3. Lime Pretreatment.** Lime—in the form of quick lime, CaO, and slaked lime,  $\text{Ca}(\text{OH})_2$ —has also been extensively investigated as a pretreatment agent due to its low cost, safety in handling, availability in many countries, and ease of recovery. In pretreatment, lime and water are added to the feedstock at temperatures ranging from ambient to  $130^\circ\text{C}$ , sometimes in the presence of oxygen to enhance delignification [21, 119]. A loading of 0.1 gram of slake lime per gram of biogas is common and process time varies from hours to weeks [26]. Lime improves hydrolysis rates of biomass by removing acetyl groups and a considerable portion of the lignin fraction [120], reducing counter-productive cellulase adsorption [14] and formation of degradation byproducts [121], and promoting cellulose accessibility [122].

Lime has been applied on various feedstocks with encouraging results. Lignin was selectively removed at low carbohydrate losses in the treatment of sugarcane bagasse at optimised conditions of  $90^\circ\text{C}$  for 90 h at lime loading of 0.4 g/g bagasse [123]. Lime application to feedstocks such as, *inter alia*, corn stover [124], switchgrass [125], and sugarcane bagasse [126] gave high carbohydrate conversions to simple sugars. Combined lime and oxidants so far have shown positive results [119] and further investigations are needed. The ease with which lime can be recovered—via precipitation to  $\text{CaCO}_3$  using  $\text{CO}_2$ —is a major advantage. The carbonate may be combusted alongside with lignin and other residues in a boiler to ash and CaO which can be mixed with water and slaked to form  $\text{Ca}(\text{OH})_2$  [21].

Despite the merits of lime pretreatment, its use faces drawbacks such as longer reaction period (influenced by the reaction temperature) compared to NaOH under similar conditions. Moreover, it dissolves in water at a slower rate and thus requires higher volumes of water for pretreatment. In

some instances, lime pretreatment produced substrates with less favourable characteristics; for example, lime pretreated sugarcane grass was less amenable to cellulose hydrolysis compared to dilute acid [78].

**2.2.4. General Drawbacks of Alkaline Methods.** Alkaline pretreatment is generally unsuitable for woody biomass due to the requirement of severe conditions needed to fractionate recalcitrant wood. Thus, lignin removal may be improved via methods that include oxygenation [19] and the addition of chemicals such as urea [108]. Other drawbacks are the loss of hemicelluloses and the formation of inhibitors at harsh conditions. Further, the formation of salts upon neutralization of pretreated contents may present challenges with disposal. Salts hamper the purification of pretreated hydroxylates [127], while posttreatment washing results in sugar losses [103]. Higher catalyst loadings are generally used and thus require recovery and reuse to improve process economics in an industrial scale plant [128].

### 2.3. Wet Oxidation (WO)

**2.3.1. Process Description.** In WO, biomass undergoes oxidation in an aqueous (acidic, neutral, and alkaline conditions) solution via reaction with oxygen (air) at elevated temperatures ( $125\text{--}315^\circ\text{C}$ ) and pressures (0.5–5 MPa) [54, 129]. The pretreated suspension is filtered to separate the cellulose-rich solid from the hemicellulose-rich filtrate, and the solid component is washed with deionized water before undergoing enzymatic hydrolysis. WO pretreatment oxidizes the hemicellulose fractions of materials into intermediates such as carboxylic acids—via peeling reactions and chain cleavage, and from the phenolic structures of lignin [130], acetaldehydes, and alcohols, and finally to  $\text{CO}_2$  and  $\text{H}_2\text{O}$  [131]. The degree of fractionation, in most cases, is influenced by the reaction temperature more than the time and oxygen dose [138]. High temperatures, pressures, pH, and catalysts favour rapid oxidation [129]; the catalysts further cause increases in the acid (formic and oxalic) concentration regardless of the reaction temperature [130].

Generally, alkaline WO reduces the formation of enzyme-inhibiting compounds such as furfural and HMF compared to acidic and neutral conditions [132, 133]. High phenolic concentrations reduce the volumetric productivity of the enzyme [134] by causing partition and loss of integrity of cell membranes of the fermenting organisms and are thus more toxic than HMF and furfural [7]. Reports from several researchers indicate that wet oxidation achieves good hydrolysis and fermentation yields from various LB, notably, spruce [130, 131], wheat straw [134], rape straw [135], and rice husk [136]. Under optimized conditions of  $185^\circ\text{C}$ , 5 bar, and 15 min, about 67% of cellulose in the solid fraction of rice husk was obtained, while 89 and 70% of lignin and hemicelluloses were removed [136]. WO pretreatment ( $195^\circ\text{C}$ , 15 min, 12 bar, 2 g/L  $\text{Na}_2\text{CO}_3$ ) and subsequent enzymatic hydrolysis of winter rye, oilseed rape, and faba bean produced ethanol yields of 66%, 70%, and 52% of the theoretical, respectively [137].

**2.3.2. Drawbacks.** WO is costly to operate owing to the need to supply high pressure oxygen and chemicals such as sodium carbonate [138]. It appears that alkaline WO does not favour woods as was observed in the pretreatment of spruce [131] and willow [133], where optimized conditions were found at 12 bar and 200°C in 10 min; and 185°C, 12 bar O<sub>2</sub>, 15 min, respectively, under neutral conditions. One potential option for reducing cost is to use air instead of oxygen in a modified process known as wet air oxidation (WAO) as was employed for the pretreatment of shea-tree sawdust [139], resulting in maximum sugar yield of 263.5 mg glucose/g dry biomass at optimum conditions of 150°C/45 min/1% H<sub>2</sub>O<sub>2</sub>/10 bar air [140].

#### 2.4. Organosolv Pretreatment

**2.4.1. Process Description.** The organosolv process involves the addition of an (aqueous) organic solvent mixture with/without a catalyst—such as an acid (HCl, H<sub>2</sub>SO<sub>4</sub>, etc.), a base (e.g., NaOH), or a salt (MgCl<sub>2</sub>, Fe<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub>, etc.)—to the biomass under specific temperatures and pressures [13, 141, 142]. The process produces three main fractions—a high purity lignin, a hemicellulosic syrup containing C5 and C6 sugars, and a relatively pure cellulose fraction. The pretreated solid residues are separated by filtration and washed with distilled water to remove solvents and degradation products which may possess inhibitory characteristics to downstream process such as enzymatic hydrolysis and fermentation. Pretreatment conditions lead to simultaneous hydrolysis and lignin removal [74] via the disruption of internal bonds in lignin, lignin-hemicellulose bonds, and glycosidic bonds in hemicelluloses and to a smaller extent in cellulose [142]. Other changes include the formation of droplets of lignin on the surface of pretreated biomass, a situation that inhibits hydrolysis by adsorption on the surface of the cellulose [143]. Process variables such as temperature, reaction time, solvent concentration, and acid dose affect the physical characteristics (crystallinity, degree of polymerization of cellulose, and fibre length) of the pretreated substrates. In most situations, high temperatures and acid concentrations as well as elongated reaction times cause considerable degradation of sugar into fermentation inhibitors.

Though sulphuric acid has been used extensively as a catalyst due to its strong reactivity, it is toxic and corrosive and possesses inhibitory characteristics [144]. Park et al. [144] evaluated the effectiveness of acidic (H<sub>2</sub>SO<sub>4</sub>), basic (NaOH), and neutral (MgCl<sub>2</sub>) catalysts on pine and found the acid as the most efficient in terms of the ethanol yield; however, an increase in the concentration of the base from 1 to 2% had a positive effect on digestibility. Organic solvents/acids that have been used as catalysts include formic, oxalic, acetylsalicylic, salicylic acid, methanol, ethanol, acetone, ethylene glycol, triethylene glycol, and tetrahydrofurfuryl alcohol [13, 145–147]. The use of CO<sub>2</sub> as a catalyst did not improve process yields on pretreatment of willow wood [151].

**2.4.2. Process Variations.** Organosolv pretreatment comes in several variations based on the solvent type, catalyst, and

process conditions. The Battelle organosolv method involves the use of a ternary mixture of phenol, water, and HCl to fractionate the biomass at about 100°C and at 1 atm [148]. The acid depolymerizes lignin and hydrolyses the hemicellulose fraction and the lignin dissolves in the organic phase (phenol), while the monosaccharides are attracted to the aqueous phase upon cooling of the fractionated biomass.

Similarly, the formic acid organosolv process (formasolv) involves the application of formic acid, water, and HCl to depolymerize, oxidize, and dissolve lignin, hemicellulose, and extractives in the biomass, and the precipitation of the lignin is achieved by the addition of water [148]. Formic acid has a good lignin solvency and the process can be undertaken under low temperatures and at atmospheric pressure [147]. However, formic acid may cause formylation of the pretreated substrates which could reduce cellulose digestibility. Pretreated substrates can be deformylated in alkaline solution as was observed on bagasse at 120°C [147]. Formic acid (5–10% w/w; no catalysts) was used by Kupiainen et al. [149] on delignified wheat straw pulp at 180–220°C, yielding a maximum glucose yield of 40%.

Organosolv pretreatment with acetic acid (acetosolv) produces higher yields than formic since less mass is dissolved for a given time. In addition, acetosolv achieves higher cellulose viscosity in smaller time periods [148].

The use of ethanol in organosolv pretreatment (ethanosolv) enables the recovery of high value products including cellulose, sulphur- and chlorine-free lignin, enriched hemicelluloses, and extractives. Further purification may be achieved via the use of solvents such as ionic liquids [127]. Unlike formasolv, the ethanosolv process is usually operated under higher pressures and temperatures. In addition, reprecipitation of lignin occurs due to lower lignin solubility [147]. The reduced toxicity of ethanol—compared to solvents such as methanol—to the downstream fermentation process and the fact that ethanol is the final product are additional benefits [142, 150]. Generally, lower ethanol/water ratios favour hemicellulose hydrolysis and enzymatic degradability of pretreated substrates [151] since ethanol is an inhibitor to the performance of hydrolytic enzymes. Ethanosolv has been explored for the development of proprietary technologies such as the Alcell process which is a sustainable alternative to kraft pulping [152], and the Lignol process—a biorefinery platform that uses aqueous ethanol (50% w/w) for pretreating LB at 200°C and 400 psi to separate the various components in woody biomass [153, 154]. High sugar yields and product recovery have been observed on ethanosolv pretreatment of various materials including hybrid poplar [155] and Japanese cypress [156]. A major advantage is the potential to recover much of the ethanol [143, 157] and water [141] which reduces the operating cost. Ethanosolv coproducts such as hemicellulose syrup and lignin can serve as feedstocks for the production of high value biochemicals. Moreover, ethanosolv lignins whose functional groups and molecular weight depend on process conditions are known to possess antioxidant properties [158].

In several situations, presoaking materials, for example, in acidic medium [159] or in bioslurry [160], positively affect the process in terms of sugar yields and lignin removal, among

others. Other variations involve the combined use of acid and basic catalysts [146], microwave-assisted organosolv [161], and the avoidance of catalysts [162]. In another variation, the biomass is treated with the inclusion of ferric sulphate and sodium hydroxide to the biomass/liquor (formic acid and hydrogen peroxide) mixture. Formic acid reacts with hydrogen peroxide to produce peroxyformic acid and its application in organosolv pretreatment (Milox) of biomass produced good results on both hardwoods and softwoods [148].

**2.4.3. Drawbacks.** Though organosolv is promising due to the potential to obtain byproducts in pure forms for the manufacture of high-value biochemicals, the process is generally costly to operate due to the requirement of high temperatures and pressures. The use of mineral acids in the organosolv process is an environmental concern, and corrosion due to the use of organic acids is a challenge. In addition, pretreated substrates need washing to prevent lignin from precipitating, and recovery of expensive volatile organic solvents needs very efficient control systems and additional energy requirements [163].

## 2.5. Ionic Liquids (Green Solvents)

**2.5.1. Properties of Ionic Liquids.** Ionic liquids (ILs) are salts consisting of large cations (mostly organic) and small anions (mostly inorganic), with a low degree of cationic symmetry and a melting point below 100°C. ILs are nonflammable [14], are liquid at room temperature [164], and are known to improve antielectrostatic and fire-proof properties of wood [165]. They have low volatility and high thermal stability [14] up to temperatures of about 300°C [166], high electrical conductivity, high solvating properties, and wide electrical window [167]. Other favourable aspects involve characteristics such as water stability, polarity, refractive index, and density [168].

Ionic liquids exist in two main forms—simple salts comprising single cations and anions, and those where equilibrium is involved [50]. The most common forms contain the imidazolium cation which can pair with anions such as chloride, bromide, acetate, sulphate, nitrate, methanoate, and triflate. ILs could be designed and developed to pretreat specific biomass under optimal conditions by combining cations and anions which can result in an estimated formulation of  $10^9$  ILs [169]. For example, the ILs 1-ethyl-3-methylimidazolium glycinate (Emim-Gly) and 1-allyl-3-methylimidazolium chloride (Amim-Cl) were synthesized from various compounds for the dissolution of bamboo [170] and wood [71], respectively. In addition, the properties of ILs could be altered by varying the length and branching of the alkyl groups that are integrated into the cation [171].

Not all characteristics of ionic liquids are favourable as solvents in pretreatment. For example, chloride-based ILs such as 1-butyl-3-methylimidazolium chloride (Bmim-Cl) are toxic, corrosive, and very hygroscopic, while others such as Amim-Cl are viscous with reactive side chains [172]. Also, ILs with long alkyl chains have the tendency to obstruct

nonpolar active sites of enzymes due to their hydrophobic nature [173]. Others have favourable properties and have thus been under intense investigation as promising solvents. For example, phosphate-based solvents possess higher thermal stability and lower viscosity and toxicity than chloride-based ones [167]. Positive outcomes have also been recorded with the use of 1-ethyl-3-methylimidazolium acetate (Emim-Ac) since it is favourable to in situ enzymatic saccharification due to its biocompatibility and enzymatic activity [174].

**2.5.2. Process Description and Mechanism.** In IL pretreatment, a mixture of the biomass (0.1–0.5 mm) and the solvent—sometimes in the presence of water and acid—is incubated at temperatures ranging from 80 to 160°C for 10 minutes to 24 hours, followed by the addition of an antisolvent to precipitate the cellulose fraction. In acidic conditions, biomass dissolution is followed by acid hydrolysis of dissolved cellulose [175]. The pretreated supernatant is removed via centrifugation or filtration, and the cellulose is washed with distilled water, lyophilized (freeze-dried), and saccharified. The antisolvent is separated from the IL by processes such as flash distillation as the IL is recovered for reuse. ILs convert carbohydrates in lignocellulosic materials into fermentable sugars via two main pathways: one is the pretreatment of the biomass to improve its enzymatic hydrolysis efficiency, and the other focuses on the transformation of the hydrolysis process from a heterogeneous to a homogeneous reaction system by dissolution in the solvent [176].

Both the cation and the anion function differently in the dissolution of biomass [169]. The effectiveness of pretreatment is predicted using the Kamlet-Taft hydrogen bond acceptor ability,  $\beta$ : usually, the higher values of  $\beta$  translate to higher lignin removal and vice versa [177]. It is known that component ions in ILs influence enzyme activity [178] and stability [173], and the anion—as a result of its hydrogen basicity—attacks and breaks the hydrogen bonds of cellulose structure [179] by forming hydrogen bonds with the cellulose [180]. Thus, the cellulose is solubilised and the crystalline nature is reduced [181] via cell wall swelling resulting from dislocation of hydrogen bonds between cellulose fibrils and lignin [182], partial removal of hemicellulose, and biomass delignification [183].

The dissolved cellulose in IL-pretreated hydroxylate is precipitated (regenerated) into cellulose II on addition of anti-solvents such as water [182, 184], methanol [184, 185], acetone [186], and ethanol [166] via preferential solute-displacement mechanism. The type of antisolvent was found to have no effect on the structure of regenerated cellulose in the pretreatment of Avicel, according to Dadi et al. [185]. Regenerated cellulose is more amorphous and has additional sites for enzyme adsorption which is favourable to saccharification. Addition of water in the cause of IL-biomass reaction improves cellulose hydrolysis by increasing selectivity to glucose and cellobiose [187]. If antisolvent mixtures such as water/acetone are used, then cellulose and lignin are distinctly separated through dissolution in water and acetone, respectively [170, 188].

The temperature is a key parameter that influences sugar release pattern, saccharification kinetics, and sugar yields

[189]. Higher temperatures and pretreatment times are more efficient in solubilising lignin [190]. From the work of Zhi-Guo and Hong-Zhang [191], nearly 100% increase in glucose yield was recorded on pretreatment of wheat straw with Amim-Cl when the temperature was increased from 125 to 150 °C at a reaction time of 2 h. At ambient conditions, a fraction cellulose I may remain and recrystallize to microfibrils of cellulose I upon expulsion of the solvent [192].

**2.5.3. Application.** ILs are increasingly being used to dissolve various LB as shown in Table 1. The effectiveness of the solvent depends partly on the type of biomass being pretreated and the final application of pretreated substrates including the regenerated cellulose. Carbohydrate losses are generally low and degradation products are significant only at severe conditions. Through the work of authors such as Cheng et al. [193], Xie et al. [172], Dadi et al. [166], Hou et al. [194], and others, it is known that significant sugar yields are achieved without the elimination of crystallinity.

**2.5.4. Drawbacks and Process Modifications.** Though the use of ionic liquids is under consideration for large scale applications, several challenges such as high solvent cost, high solvent loading, technical challenges and cost of solvent regeneration, and inadequate knowledge on the impact of ionic liquids on the environmental [38, 165, 167]. ILs with high viscosities have low potential in terms of mass and phase transfer which presents challenges in engineering applications [165, 175]. Moreover, the separation of hydrophilic ILs and monomeric sugars in water is difficult [178, 204]. Some ILs also exhibit tendencies to denature enzymes [168] and the active sites of enzymes could be blocked by layers of hydrophilic ILs, decreasing or destroying the aqueous phase surrounding enzyme surface [173]. Washing of regenerated cellulose as well as recycling of ILs via processes such as evaporation and reverse osmosis is practically costly which presents challenges in the development of process technologies for the efficient use of ILs within a biorefinery. While delignification increases with high temperatures, such conditions also cause hemicellulose losses as was observed in the pretreatment of switchgrass and agave bagasse at 120 and 160 °C [190].

In order to address some of the shortcomings of conventional IL pretreatment approach, several process routes and configurations are being developed. The use of lower IL concentrations is realized by using aqueous ILs [175] or by undertaking both pretreatment and saccharification in a single unit followed by direct extraction of sugars which avoids the need to wash regenerated cellulose. Aqueous ILs have lower viscosities, reduce recycling demands in terms of cost, and effectively deal with high biomass loadings [175]. Emerging green solvents such as cholinium-based ILs have been found to be more biocompatible and renewable and lower in cost, and with yields comparable to imidazolium-based ILs [198].

Economic improvements have been reported via interventions such as the use of thermophilic cellulase in high solvent concentrations [205]; application of acid catalysts

such as sulfonated carbon materials (sugar catalysts) [206], HCl [175, 187], and Nafion NR50 [207]; inclusion of chemicals such as  $\text{NH}_4\text{OH-H}_2\text{O}_2$  [208] and electrolyte solution [181]; direct (without cellulase) conversion of pretreated substrates to biofuels in consolidated bioprocessing [209]; the reuse of solvent in several batches [169]. Notwithstanding other process enhancements, it is necessary that lignin-derived products are efficiently utilized to improve process economics.

**2.6. Oxidizing Agents.** Unlike the methods discussed above, pretreatment involving the application of oxidizing agents has received less attention among researchers partly due to the high cost of the oxidants such as ozone and hydrogen peroxide. The sections below discuss oxidant-based methods, highlighting recent advances in research and process development.

**2.6.1. Ozonolysis.** In ozonolysis, ozone is sparged into a mixture of biomass and water at room temperature and specific time periods leading to the solubilisation of lignin and hemicelluloses. The focus of attack is on the aromatic ring of lignin [7], and the process is affected by ozone concentration, biomass type and moisture content, and air/ozone flow rate [210]. Though the process is relatively expensive due to large requirements of ozone, the process comes with benefits as follows: high dry matter concentrations (45–60%), effective removal of lignin, very low production of inhibitory products, and reactions performed at atmospheric conditions [211, 212]. Ozone, thus, barely attacks carbohydrates [212]. Lignin degradation products such as carboxylic acids that may form can be eliminated by washing with water at room temperature even though it comes at the expense of some carbohydrates losses.

In some instances, ozone application to specific biomass resulted in low sugar yields. In basic medium, ozone application was found to be inefficient on wheat and rye straw [210]. Ozone treatment of cotton stalk (10% w/v) at 4 °C for 30–90 minutes reduced lignin by 11.97–16.6%, at xylan and glucan solubilisation of 1.9–16.7% and 7.2–16.6%, respectively; comparatively, NaOH treatment achieved higher delignification of 65.63% [109]. In another work, high delignification and low carbohydrate loss were observed when a two-step method comprising ozone and ethanosolv was applied to Sweetgum, *Miscanthus*, and Loblolly pine [213]. In addition, combined ozonolysis and autohydrolysis offer benefits such as high hemicellulose solubilisation, high glucose and ethanol yields, low use of chemicals, and low waste production [213].

Recently, the use of plasma-generated ozone (from air or oxygen-enriched air) at atmospheric conditions has attracted interest among researchers including Schultz-Jensen and team at the Risø National Laboratory for Sustainable Energy in Denmark [211]. Employing a fixed-bed reactor, a  $\text{CO}_2$  detector, and a technique for continuous determination of ozone consumption, lignin degradation of ozone pretreatment of wheat straw was monitored in real time with respect to ozone consumption and  $\text{CO}_2$  emission. Lignin degradation of 1 mm particles was found to be almost complete while that



TABLE I: Application of ILs to selected biomass resources.

Biomass	Solvent	Temperature (°C), time (min)	Reference
Eucalyptus	Emim-Ac	150, 3	[183]
	Amim-Cl	120, 5	[184]
Poplar	Emim-Ac	120, 1	[195]
Pine	Amim-Cl	120, 5	[184]
Spruce	Amim-Cl	120, 5	[184]
Energy cane bagasse	Emim-Ac	120, 0.5	[196]
		160, 3	[38]
Switchgrass	Emim-Ac	120–160, 6	[190]
		120, 8	[170]
Bamboo	Emim-Gly	120, 8	[170]
Wheat straw	Amim-Cl	100–150, 2–6	[191]
	Bmim-Ac	100–150, 0.17–1	[164]
Water hyacinth	Bmim-Ac	100–150, 0.17–1	[164]
Rice husk	Bmim-Cl, Emim-Ac	100, 10	[197]
Rice straw	Ch-Aa	90, 2	[194]
Kenaf powder	Ch-Ac	110, 16 h	[198]
Cassava pulp	Emim-Ac, Dmim-SO <sub>4</sub> , Emim-DePO <sub>4</sub>	25–120, 24	[188]

Dmim-SO<sub>4</sub>: 1, 3-Dimethylimidazolium methyl sulphate. Emim-DePO<sub>4</sub>: 1-Ethyl-3-methylimidazolium diethyl phosphate. Ch-Aa: Cholinium amino acids; Ch-Ac: Cholinium acetate.

of 2 mm particles was less than 80%, leaving a solid fraction mainly composed of carbohydrates. Maximum glucose and ethanol yields of 78% and 52% were observed after enzymatic hydrolysis and SSF (based on glucan), respectively, based on optimal ozonisation for 1 h [214]. The ethanol yield was relatively low and the process economics has the potential to be improved via the recovery and use of lignin byproducts and hemicellulose, as well as developing schemes to reduce ozone consumption for similar yields.

Aside high delignification and carbohydrate preservation, pretreated substrates have shown potential for use in the production of enzymes by fungi such as *Trichoderma reesei*, with lower titres of cellulases and higher amount of xylanases recorded from autoclave sterilization of pretreated materials, compared to nonsterilized substrates with antibiotics added [215].

**2.6.2. Other Oxidizing Agents.** When exposed to biomass, oxidants such as hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), peracetic acid (C<sub>2</sub>H<sub>4</sub>O<sub>3</sub>), sulphur trioxide (SO<sub>3</sub>), and chlorine dioxide (ClO<sub>2</sub>) solubilise hemicellulose and lignin under mild alkaline or neutral conditions. The pretreated biomass undergoes oxidative delignification due to the reaction of the aromatic ring of lignin with the oxidizing agents leading to improved digestibility compared to alkaline pretreatment only [7]. Biomass degradability is affected by the type of biomass, oxidant dose, reaction temperature, and time. Among the oxidants, hydrogen peroxide is the most studied. Hydrogen peroxide degrades into hydrogen and oxygen and does not leave residues in the biomass [216]. By pretreating water hyacinth and lettuce with NaOH followed by H<sub>2</sub>O<sub>2</sub>, Mishima et al. [217] recorded higher sugar yields compared to NaOH, H<sub>2</sub>SO<sub>4</sub>, and hot water pretreatments under similar conditions. Process variations that have also produced high sugar yields include the addition of catalysts such as manganese

acetate [218], postpretreatment acid saccharification [216], and alkaline-peroxide application without postpretreatment washing [219].

Generally, H<sub>2</sub>O<sub>2</sub> permits fractionation of biomass at ambient pressures and low temperatures, allowing the use of low cost reactors. Unfortunately, the application of oxidizing agents produces soluble lignin compounds that inhibit the conversion of hemicelluloses and cellulose to ethanol. There is also loss of sugar due to the occurrence of nonselective oxidation [7, 104]. In addition, the high cost of oxidants is a major limitation for scaling up to industrial levels.

Recently, the use of sulphur trioxide in a process called sulphur trioxide microthermal explosion (STEX) has been explored to pretreat biomass such as rice straw [220]. Biomass is hanged above a solution of oleum (50% SO<sub>3</sub>) and NaOH (1% w/v) and swirled in a test tube at 50°C/1 atm for 7 h, followed by washing to obtain the solids [220]. The internal explosion is believed to occur due to heat released from SO<sub>3</sub>-straw reaction that causes rapid expansion of air, and water from the interior of the biomass, resulting in enhanced structural changes and pore volume [220] and partial removal of lignin and hemicellulose [221]. Pretreatment of rice straw at the aforementioned conditions resulted in saccharification yield of 91% [220]. The efficient handling of SO<sub>3</sub> will be a challenge due its corrosiveness regarding this emerging pretreatment. Another novel process involves the use of chlorine dioxide (in the presence of aqueous ethanol) on biomass which results in high fraction of glucose in pretreated biomass and low formation of inhibiting compounds [222].

**2.7. Sulphite Pretreatment.** The sulphite process is a matured technology that has been used in the pulp and paper industry for decades and has been adapted to the pretreatment of LB for enhanced enzymatic hydrolysis and fermentation to ethanol. In this method, milled biomass (<6 mm) is

mixed in sulphite ( $\text{Na}_2\text{SO}_3$ ,  $\text{NaHSO}_3$ , etc.) solution (1–10% w/w) in acidic, basic, or neutral environments at selected temperatures (80–200°C) and reaction times (30–180 min). Pretreated solids are separated from the spent liquor by filtration and the solids are washed with distilled water and dried before undergoing saccharification. The process partially degrades and sulphonates lignin and enhances glucose yields due to the formation of sulphonic and weak acid groups which improves the hydrophilicity of pretreated substrates [223]. Sulphonation is enhanced in the presence of volatile organic solvents such as ethanol which reduces the surface tension thereby allowing effective solution penetration of the biomass. In addition, the lignin is hydrolysed and dissolved in the organic phase and is easily recovered in pure forms [223]. Lignin removal can be accelerated further by the presence of sulphomethyl groups produced from combined action of sulphite and formaldehyde on lignin, resulting in high sugar yields [224].

Sulphite application is emerging as a promising pretreatment due to positive results recorded from several materials. Pretreatment of corn stover with alkaline  $\text{Na}_2\text{SO}_3$  at 140°C resulted in 92% lignin removal and 78.2% total sugar yield (0.48 g/g raw biomass) after enzymatic hydrolysis, which was higher than four other alkali-based methods under similar conditions [225]. About 79.3% of total glucan was converted to glucose and cellobiose during corn cob pretreatment at 156°C, 1.4 h, 7.1% charge, and solids loading of 1:7.6 w/w; and subsequent SSF gave 72.2% theoretical ethanol yield [226]. In another work, pretreatment of corn cob residues produced the highest glucose yields (81.2%) in the presence of ethanol compared to acidic, basic, and neutral conditions [223].

At the pilot level, sulphite-based methods have been investigated and demonstrated good results. In a proprietary process, known as sulphite pretreatment to overcome recalcitrance of lignocellulose (SPORL), the biomass material is first pretreated with dilute solutions of sulphuric acid and sodium bisulphite ( $\text{NaHSO}_3$ ) after which the residual solids are separated from the hydroxylate. The solids are then dismilled and pressed to obtain solids moisture content of about 30% [227]. Lignin is sulphonated and partially removed, and hemicellulose is nearly removed completely which favours subsequent size reduction and enzymatic hydrolysis [228]. The SPORL process has been effectively used to pretreat (180°C, 25 min, liquor/wood = 3:1 v/w) lodgepole pine for subsequent conversion to ethanol at a yield of 276 L/ton wood and at a net energy output of 4.55 GJ/ton wood [227]. SPORL pretreatment of switchgrass was superior to dilute acid [228, 229] and alkali [228] in terms of the digestibility of the pretreated substrates. Similarly, higher sugar yields and lower inhibitor concentration were found with SPORL-pretreated agave stalk relative to dilute acid and NaOH [230]. It was also found superior to the organosolv and steam explosion pretreatments based on the evaluation of total sugar recovery and energy consumption [23]. The main advantages of sulphite pretreatment are high sugar yields, effective lignin removal, and recovery of biomass components in less chemically transformed forms. The drawbacks include sugar degradation at severe conditions, large volumes of process water used in postpretreatment washing, and the high

costs of recovering pretreatment chemicals. It has, however, been shown through the work of Liu and Zhu [231] that the negative effects of soluble inhibitors and liginosulphonate on enzymatic hydrolyses could be counteracted by adding metal salts to the pretreated contents, making it possible to avoid the costly washing process.

**2.8. Glycerol.** Crude glycerol—a byproduct in biodiesel production—has attracted interest as a substrate for fermentation into ethanol and other biochemicals [232] and also as a solvent for fractionating biomass in order to improve the economics of cellulosic ethanol as well as the upstream biodiesel production. Glycerol pretreatment causes efficient delignification of biomass. Guragain et al. [164] investigated the best conditions in the use of crude glycerol (water: glycerol = 1:1) in pretreating wheat straw and water hyacinth and arrived at 230°C for 4 h for wheat straw and 230°C for 1 h for water hyacinth. Enzymatic hydrolysis of pretreated wheat straw produced reducing sugar yields (mg/g of sample) of 423 and 487 for crude and pure glycerol, respectively, compared to a low figure of 223 for dilute acid. In addition, hydrolysis tests on water hyacinth gave yields of 705, 719, and 714 for crude glycerol, pure glycerol, and dilute acid, respectively. Similarly, Ungurean et al. [186] performed glycerol pretreatment of wood (poplar, acacia, oak, and fir) and recorded higher cellulose conversion rates compared to dilute acid application. However, combinations of glycerol and acid/IL pretreatment yielded higher sugar levels compared to glycerol pretreatment alone.

There are wide variations in the composition of crude glycerol which usually contains methanol, ash, soap, catalysts, salts, and nonglycerol organic matter, among others, in varied proportions [233]. While the potential for exploring crude glycerol application together with other methods is high, there is the need to assess the quality of crude glycerol and its effects on sugar and ethanol yields of promising feedstocks.

**2.9. Aqueous *N*-Methylmorpholine-*N*-Oxide (NMMO).** NMMO is a well-known industrial solvent used in the Lyocell process for the production of fibres and has attracted interest for use as a pretreatment solvent. Cellulose dissolves (without derivatization) in NMMO/ $\text{H}_2\text{O}$  system and the hydrogen bonds in cellulose are disrupted in favour of new bonds between cellulose and solvent molecules [234], leading to swelling and increased porosity [235], as well as reduced degree of polymerization and crystallinity which improves enzymatic saccharification. Addition of boiled distilled water to pretreatment slurry containing dissolved biomass causes cellulose I to precipitate into cellulose II which is more reactive. Regenerated solids are filtered and washed with warm/boiling water until the filtrate is clear.

Like ionic liquids, NMMO dissolves biomass with no/less chemical modification at low/moderate temperatures (80–130°C). Additional favourable characteristics of NMMO pretreatment include high sugar yields, formation of low degradation products, high solvent recovery, and no adverse effect on the environment. Further, cellulase activity is not negatively affected by low concentrations (15–20% w/w) of

TABLE 2: Selected large-scale cellulosic ethanol plants based on chemical pretreatment.

Company/ institution	Location	Capacity*	Pretreatment	Feedstock	Status	Reference
SEKAB	Örnsköldsvik, Sweden	100 t/y	Two stage dilute $H_2SO_4/SO_2$	Pine chips	Operational since 2004; scale-up projects planned	[199] Updated
Abengoa bioenergy	Salamanca, Spain	5 m L/y		Wheat and barley straw, and so forth	Operational since 2009	<a href="http://www.kcc.state.ks.us/energy/kwrec-09/presentations/B1_Robb.pdf">http://www.kcc.state.ks.us/energy/kwrec-09/presentations/B1_Robb.pdf</a> (accessed 11/02/13)
	York, NE, USA	0.4 m L/y	Acid impregnation + steam explosion	Corn stover	Operational since 2008	
	Kansas, USA	25 m gal/y		Corn stover, wheat straw, switchgrass, and so forth	Under construction, startup 2014	
BioGasol	Ballerup, Denmark	4 t/h	Dilute acid/ steam explosion or wet explosion	Wheat straw and bran, corn stover, and so forth, garden wastes, energy crops, and green wastes	Under development	<a href="http://www.biogasol.com/Home-3.aspx">http://www.biogasol.com/Home-3.aspx</a> (accessed 11/02/13)
Procethol 2G, FuturoI	Pomacle, France	180 m <sup>3</sup> /y 3.5 m L/y 180 m L/y	Under development	Wheat straw, switchgrass, green waste, <i>Miscanthus</i> , Vinnasses, and so forth	Operational since 2011 Planned, 2015 Planned, 2016	<a href="http://www.projet-futuroI.com/index-uk.php">http://www.projet-futuroI.com/index-uk.php</a> (accessed 13/02/13)
Izumi Biorefinery	Japan	300 L/day	Arkenol	Cedar, pine, and hemlock	Operational since 2002	[200]; <a href="http://bfreinc.com/docs/IZUMI_Status_2004_for_BlueFire_051606.pdf">http://bfreinc.com/docs/IZUMI_Status_2004_for_BlueFire_051606.pdf</a> (accessed 13/02/13)
INEOS	Florida, USA	8 m gal/yr	Thermochemical	Municipal solid waste and so forth	Operational	<a href="http://www.ineosbio.com/60-Technology_platform.htm">http://www.ineosbio.com/60-Technology_platform.htm</a> (accessed 11/02/13)
ZeaChem	Oregon, USA	250,000 gal/y	Chemical	Hybrid poplar, corn stover, and cob	Construction completed	<a href="http://www.zeachem.com/index.php">http://www.zeachem.com/index.php</a> (accessed 12/02/13)
Logos Technologies	California, USA	50,000 gal/y	Colloid milling	Corn stover, switchgrass, and wood chips	Operational	<a href="http://www.logos-technologies.com/">http://www.logos-technologies.com/</a> (accessed 13/02/13)
BlueFire	Mississippi, USA	19 m gal/y	Concentrated acid (Arkenol)	Wood waste, municipal solid waste	Under construction	<a href="http://bfreinc.com/">http://bfreinc.com/</a> (accessed 15/02/13)
Weyland AS	Norway	200 m <sup>3</sup> /y	Concentrated acid	Corn stover, sawdust, paper pulp, switchgrass, and so forth	Operational since 2010	<a href="http://weyland.no/">http://weyland.no/</a> (accessed 15/02/13)
Borregaard	Norway	20 m L/y	Acidic/neutral sulphite	Wheat straw, eucalyptus, spruce, and so forth	Operational	[201]; <a href="http://www.borregaard.com/">http://www.borregaard.com/</a> (accessed 11/02/13)

TABLE 2: Continued.

Company/ institution	Location	Capacity*	Pretreatment	Feedstock	Status	Reference
Queensland University of Technology	Queensland, Australia	Pilot	Acid, alkaline, steam explosion, ionic liquid, and so forth	Sugarcane bagasse	Operational, since 2010	[202]
Praj industries	India	10 m L/y	Thermochemical	Corn cob, sugarcane bagasse, and so forth	Under development	[200] (Updated)
Lignol Energy Corporation	Canada	100 m <sup>3</sup> /y 40–200 m L/y	Organosolv	Wood, agricultural residues, and so forth	Operational Planning	<a href="http://www.lignol.ca/index.html">http://www.lignol.ca/index.html</a> (accessed 06/02/13)
Blue sugars	Wyoming, USA	1.4 m gal/y	Acid, thermomechanical	Pine and so forth	Operational	<a href="http://bluesugars.com/">http://bluesugars.com/</a> (accessed 13/02/13)
Petrobras/Blue sugars Dupont	Brazil	10 m gal/y	Acid, thermomechanical	Sugarcane bagasse	Under development	<a href="http://bluesugars.com/company-partnerwith.htm">http://bluesugars.com/company-partnerwith.htm</a> (accessed 16/02/13)
Danisco Cellulosic Ethanol (DDCE)	Iowa, USA	30 m gal/y	NH <sub>3</sub> Steam recycled	Corn stover	Under construction	[200]; <a href="http://biofuels.dupont.com/">http://biofuels.dupont.com/</a> (accessed 13/02/13)
COFCO /SINOPEC /Novozyme	Zhaodong, China	62 m L/y	Steam explosion (with acid impregnation)	Corn stover	Under construction	[203]

\* Pilot (<2000 t/y or 2.5 m<sup>3</sup>/y), demo (2000–50,000 t/y or 2.5–65 m<sup>3</sup>/y), commercial (>50,000 t/y or 65 m<sup>3</sup>/y).

NMMO, indicating the potential of application in continuous processes [235].

Aqueous NMMO has already been used on a host of biomass as a sole pretreatment method or in combination with others. Almost total conversion of cellulose to ethanol at ethanol yields of up to 85.4% and 89% for pretreatment (130°C/3 h) of oak and spruce, respectively, was observed [236]. High saccharification yields (>90%) were observed for ultrasound-assisted NMMO treatment of sugarcane bagasse [233], as well as pretreatment of birch [237]. Poornejad et al. [238] compared the effectiveness of NMMO and the ionic liquid (Bmim-Ac) treatment on rice straw at 120°C/5 h. Glucan conversion was complete with the ionic acid, while 96% conversion was realized with the use of NMMO. Upon SSF, the yield of ethanol was higher with NMMO (93.3%) than Bmim-Ac (79.7%).

At the pilot scale, concentrated NMMO pretreatment (85% w/w, 130°C, 5 h) has been investigated on spruce and birch by Lennartsson et al. [239]. For wood chips below 2 mm, maximum hydrolysis yields (mg/g wood) ranged from 195 to 128 for spruce and 136 to 175 for birch depending on the scale of the pilot reactor using nonisothermal SSF. A techno-economic analysis of NMMO pretreatment of spruce for ethanol and biogas production was undertaken by Shafiei et al. [240] who observed relatively high process energy efficiency of 79%.

**2.10. Inorganic Salts.** Recently, salts such as iron (III) chloride [241] and calcium chloride [242] have found use in the pretreatment of biomass. The salt hydrolyses in water to form a strong acidic solution which causes rapid removal of hemicelluloses from biomass during pretreatment. Application of the salt (0.26 M) to olive tree residues at 152.6°C for 30 min resulted in 100% removal of hemicellulose, and subsequent saccharification produced a yield of 36.6 g glucose/100 g of glucose in the original biomass [241]. Combined CaCl<sub>2</sub>-microwave treatment was investigated on corn stover and under optimum conditions (162.1°C, 12 min); glucose recovery reached 65.5% which was higher than that of steam-exploded (1.5 MPa, 5 min) corn stover [242].

### 3. Future Outlook and Conclusion

Due to its high reactivity at mild conditions, chemical pretreatment of lignocellulosic biomass forms the basics of several proprietary cellulosic ethanol production configuration and technologies that have been developed by various research groups and companies for development at various levels, usually with financial support from national governments and public bodies (e.g., Swedish Energy Agency, Danish Ministry of Energy, US Department of Energy/Agriculture, and Canadian Sustainable Development Technology Canada) and multinational institutions such as the European Union. Table 2 gives profiles of some of the main projects undertaken or under construction/development underpinned by breakthrough pretreatment, hydrolysis, and fermentation technologies, as well as process integration and optimization.

The pretreatment of feedstocks to enhance biodegradability to simple sugars has been the subject of intensive research globally with a focus on maximizing sugar yields at high solid loads and at the lowest economic and environmental costs. This paper has reviewed widely known and emerging chemical pretreatment methods with regard to process description, advantages, drawbacks, and recent innovations employed to offset inherent challenges. Though cellulosic ethanol is close to commercialization, there are still technical, economic, and environmental challenges associated with biomass pretreatment, hydrolysis, and fermentation. No solvent has been found to work best for all biomass and such optimized methods and process conditions for various materials need to be investigated and developed further.

Some major challenges of the chemical pretreatment include the requirement of extensive size reduction; handling biomass at high solids concentration, corrosion, solvent costs, and recovery; environmental pollution from solvents, byproducts, and waste from reactions. Nonetheless, the aforementioned challenges are being dealt with via several interventions, notably, the application of novel solvents and the combination of different chemical methods with physico-chemical and biological pretreatments to achieve higher sugar yields, milder process conditions, lower use of costly solvents, low enzyme loads, recovery, and use of biomass components in pristine forms, and improvements in environmental sustainability.

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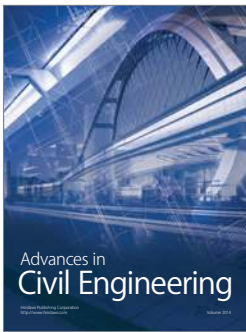
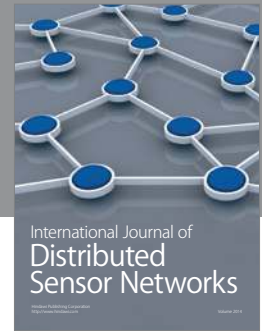
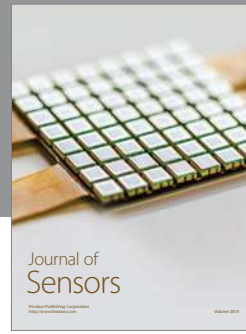
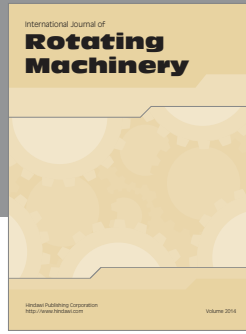
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