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Emerging Physico-Chemical Methods for Biomass Pretreatment

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Abstract

A major challenge to commercial production of cellulosic ethanol pertains to the cost-effective breakdown of the complex and recalcitrant structure of lignocellulose into its components by pretreatment methods—physical, chemical, physico-chemical, biological and various combinations thereof. The type and conditions of a pretreatment impacts both upstream processes such as size reduction as well as downstream processes such as enzymatic hydrolysis and enzyme loadings, and as such the choice of a pretreatment method for a specific biomass (or mix of materials) is influenced by several factors such as carbohydrate preservation and digestibility, sugar and ethanol yields, energy consumption, equipment and solvent costs, lignin removal and quality, formation of sugar/lignin degradation products, waste production, and water usage, among others. This chapter reviews both well-known and emerging physico-chemical methods of biomass fractionation with regards to process description and applications, advantages and disadvantages, as well as recent innovations employed to improve sugar yields, environmental sustainability and process economics.

Keywords: lignocellulose, ethanol, pretreatment, physico-chemical pretreatment

1. Introduction

Pretreatment and enzymatic hydrolysis present the most practical challenges (technical, environmental and economic) in the attempt to commercialize cellulosic bioethanol. Pretreatment is costly since it represents about 20% of total cost [1]. However, without pretreatment, enzymatic degradation of native biomass is generally below 20% yield [2], making pretreatment a crucial process of bioethanol production. In general, the selection of a pretreatment method

for a material or mix of feedstocks is influenced by factors that include carbohydrate preservation and digestibility, sugar and ethanol yields, energy consumption, equipment and solvent costs, lignin removal and quality, formation of degradation products from sugars and lignin, waste production, and water usage.

Pretreatment may be categorized as physical (e.g., size reduction, autoclaving, irradiation, popping, ultrasonication, steaming and extrusion), chemical (use of acids, alkali, solvents, etc.), biological (white-rot fungi, brown rot fungi, etc.), and physico-chemical which combine both physical and chemical processes to ensure the digestibility of the lignocellulosic material. Physical pretreatment usually demands high-energy consumption (e.g., side reduction) and is undertaken before chemical or biological pretreatment. While chemical methods offer benefits such as efficient fractionation of biomass and good sugar yields during enzymatic hydrolysis, their environmental impacts are higher than physical and biological methods due to biomass degradation into enzyme-inhibiting compounds, corrosion of reactors, solvent recycling issues, and generation of waste [3, 4].

The well-known physico-chemical pretreatment include liquid-hot water (LHW), steam explosion (SE), ammonium fiber explosion (AFEX), soaking in aqueous ammonia (SAA) and irradiation-chemical method. This chapter reviews and present novel findings as well as process innovations in physico-chemical processing of recalcitrant biomass to sugars and ethanol.

2. Liquid hot-water (LHW) pretreatment

2.1. Description

This hydrothermal process involves cooking of biomass in liquid water at high temperatures (150–240°C) and short times (≤ 50 min). Pretreatment causes pressurized water to rupture and penetrate the cell structure, resulting in fractionation of biomass into two product streams—liquid hydroxylate containing hemicelluloses sugars, minerals, and degradation products such as furfural and acetic acid, and a solid fraction comprising most of the cellulose and lignin and some residual hemicellulose. Pretreated solid substrates have increased surface area and pore volume, and consist of separated individual cellulose fibers, with large particles of repolymerized lignin on the surfaces of the cellulose matrix [5]. The harshness of the process is described by a severity factor (R_o) that allows for the determination of combined effects of temperature and reaction time on sugar yields and degradation products. It is expressed as: $R_o = t \times exp[(T - 100)/14.75]$, where t = reaction time (min), and T = temperature (°C) [6].

Generally, the carbohydrate content of pretreated substrates increases with temperature until a maximum temperature is reached where further temperature increase result in substantial degradation. Thus, high temperatures greater than 230°C disrupt pretreated particles and reduce the surface area and pore volume, which in turn limit enzymatic digestibility [5].

Based on the work of Mosier et al. [2], it is observed that at reaction conditions of 200–230°C and ≤ 15 min, biomass dissolution ranged from 40 to 60%, comprising 4–22, 35–60, and 100% of cellulose, lignin, and hemicellulose, respectively. Much of the hemicellulose dissolve into

poly- and oligosaccharides even at high severities which is beneficial since the minimization of monomer formation reduces the chance of further degradation into aldehydes (2-furaldehyde, 5-hydroxymethylfurfural, etc.), which are known inhibitors of enzymatic hydrolysis. However, oligomers also possess inhibitory tendencies to cellulase activity [7]. It has been established that digestibility of pretreated substrates depended more on partial removal of hemicellulose and relocalization of lignin than the modification of crystallinity and rupture of the cell wall [5, 8].

2.2. Applications

LHW pretreatment has been applied to pretreat various feedstocks including agricultural residues, woods and industrial waste. Archambault-Leger et al. [9] applied both batch and flowthrough pretreatment to corn stover, bagasse, and poplar and observed higher hemicellulose recovery, removal of non-carbohydrate carbon, and glucan conversion under simultaneous saccharification and fermentation (SSF) in the flowthrough reactor. Some authors have included additives to positively influence pretreatment outcomes. For example, by adding $AlCl_3$ to pretreatment solution of microcrystalline cellulose, low concentration of degradation products and high glucose yields were obtained [10]. Optimum glucose yield of 80% was also observed when solid carbon dioxide was used in hydrothermal fractionation of *Eucalyptus* [11]. In another study, an alcohol-water mixture was used to overcome challenges due to deposition of lignin particles on pretreated materials, and thus achieved increased pore volume and higher sugar yields [12].

Combinations of LHW and other methods have also been employed to overcome inherent drawbacks and to improve sugar yields. Low degradation products and higher sugar yields—xylose (91.62%) and glucose (88.12%)—was observed when LHW treatment (180°C, 20 min) of *Eucalyptus* was followed by wet disk milling before saccharification [13]. Alkaline-assisted LHW treatment of rice straw was found to improve glucose recovery and yield under enzymatic hydrolysis, caused by increased removal of hemicellulose and lignin [14].

At demonstration and industrial scale, one notable application of LWH is in the Integrated Biomass Utilization System (IBUS) platform where biomass is converted into ethanol, C5 molasses, and lignin pellets using uncatalyzed steam in an energy efficient manner underscored by high dry matter content in all process routes [15]. It was developed as a three-stage, pilot-scale process for treating wheat straw—by soaking at 80°C for 20 min, hemicellulose recovery at 170–180°C for 7.5–15 min, and cellulose hydrolysis at 195°C for 3 min. Under these conditions both ethanol production and lignin recovery for power production are maximized [16]. High glucose yield required the avoidance of water addition to the third stage while high hemicellulose yield (83%) required water addition. It was improved by Petersen and team [17] who used a two-stage procedure to achieve high cellulose recovery (over 90%) on wheat straw: soaking of biomass at 80°C for 5–10 min followed by pretreating at temperatures and residence times ranging from 185 to 198°C and 6–12 min respectively. The optimum pretreatment temperature was observed at 195°C at which cellulose and hemicellulose recovery reached 93–94 and 70% respectively at lower water/biomass ratio compared to the three-stage process. However, the two-step process was found to present economic challenges in

the recovery of C5 sugars after the first pretreatment in a commercial-scale plant, prompting Inbicon to settle for a simpler, one-stage treatment processs [15]. Currently, the Inbicon demonstration plant, which is based in Kalundborg (Denmark), processes about 4 tonnes straw/h and at yields greater than 198 L ethanol/tonne of wheat straw.

2.3. Positive attributes and drawbacks

LHW offers improved digestibility of cellulose by enzymes due to the solubilization of hemicelluloses and avoidance of inhibitors. Compared to steam explosion, LHW gives lower concentrations of solubilized hemicellulose and lignin products due to higher water input as well as higher pentosan recovery. Generally, catalysts/chemicals are avoided resulting in no/low neutralization demands and byproduct/precipitate generation, with additional benefits such as reduced risk of reactor corrosion and explosion. Reactor cost is lower compared to methods such as AFEX [18]. The effect of particle size reduction on hydrolysis is low, thus, large biomass flowrates can be handled effectively.

There are however drawbacks in LHW related to hemicellulose fractionation into large fractions of oligomers, and xylose yields are generally low, which affect sugar and ethanol yields. There is a risk of sugar degradation into byproducts such as carboxylic acids and furans at severe conditions [19, 20]. A major cost involved in LHW pertains to high energy used to generate saturated liquid water. Consequently, solid loadings are restricted to about 20% [21].

3. Steam explosion (SP)

3.1. Description

In steam explosion, biomass is exposed to saturated steam at high pressure (0.5–4.8 MPa) for a maximum period of 60 min followed by sudden reduction of pressure to atmospheric or lower, resulting in explosive decompression of biomass into component fiber and fiber bundles. The explosion is triggered by evaporation within biomass cells and sudden drop of pressure around the biomass. Exploded materials experience increase in water retention and pore size and specific surface area. Consequently, the bulk density is decreased. To improve penetration efficiency and swelling, biomass is pre-soaked before pretreatment. While the buffering effects of free moisture reduce heat transfer and increase energy demand, bound moisture softens fibers and increase pretreatment efficiency [22]. Thus, by carefully regulating water content of feedstock, substantial gains in sugar yield can be obtained during enzymatic hydrolysis, with collateral benefits in reduced energy demand [23].

The pretreated solids comprise unhydrolyzed cellulose, chemically-transformed lignin, and residual hemicelluloses. The liquid hydrolysate, on the other hand, contains solubilized hemicelluloses in oligomeric forms, with concentrations of monomers usually exceeding similar situations under LHW. Hemicellulose is hydrolyzed via the breakdown of both glycosidic and hemicellulose-lignin bonds. Hydrolysis of parts (acetyl groups and uronic acid substitutions) of hemicelluloses—via the catalytic actions of protons generated from the autoionization of

water—occurs to form acetic and other acids which enhance further fractionation of hemi-cellulose [24], and trigger the release of carbonium ions from benzyl alcohol structures in lignin which cause the breakdown of some of the β-O-4 structures in lignin leading to reduced molecular weight [25]. Simultaneously, condensation reactions may take place in the presence of electron-rich carbon atoms, resulting in lignin repolymerization [25, 26], with the composition affected by pretreatment severity [27].

The process is affected by temperature, reaction time, material size, moisture content and efficient mixing of biomass. The explosion mechanism and time which are independent of the severity factor are also known to affect yields [28]. Increasing reaction time and temperature decreases the degree of polymerization of cellulose [29]. Though severe conditions contribute to reduction in crystallinity and increase in moisture retention, they do not necessarily lead to increased hydrolysis rates due to possibility of thermal degradation of cellulose. Similarly, xylose recovery is reduced for longer pretreatment times due to formation of degradation products. Further, severe conditions increase the intensity of repolymerization and condensation reactions from byproducts of lignin, hemicellulose, and extractives leading to increased molecular weights of lignin [30]. This development reduces substrate amenability to enzymatic hydrolysis caused by the covering of cellulose surface with the repolymerised lignin-like materials (pseudo-lignin). The problem of lignin repolymerization was overcome by Li et al. [31] who used a carbonium ion scavenger (2-napththol) to achieve solubilize lignin, resulting in improved recovery (91%) as against 51% for steam pretreated aspen wood without the additive.

3.2. Applications

SE has been applied in combination with additives and pretreatment methods to improve yields and overall process economics. The major variations include the use of acids and bases as catalysts.

3.3. Acid-catalyzed steam explosion (ACSE)

In this process, SE is undertaken after the biomass is soaked with dilute acid or impregnated with SO_2 or CO_2 at low or atmospheric pressures for 0.5–25 h depending on the temperature (5–100°C). It favors solubilization of hemicelluloses into monomer units, making substrates more reactive while improving enzymatic hydrolysis of cellulose. Compared to dilute acid, SO_2 impregnates biomass substrates better and more uniformly but requires harsher conditions to remove hemicellulose [32]. Both SO_2- and CO_2-based SE create the formation of pores of different sizes and shapes in the outer region of the cell wall of pretreated substrates, with the effect more noticeable in SO_2-based applications due to its higher combined severities under similar conditions [33]. Though CO_2 has a lower solubility compared to SO_2, CO_2 is highly available, less toxic and corrosive, and thus safer to apply.

A major positive attribute about ACSE is that most glucan and lignin are untouched and remain in solid form after pretreatment [34] though lignin presence hinders enzymatic hydrolysis [35]. Nonetheless, high sugar yields are generally obtained. Yields obtained by some investigators are given in **Table 1**.

Agent/catalyst	T (°C), t (min)	Biomass	Observation	Reference
CO_2	205, 15	Sugar cane bagasse and leaves	High glucose yield of 86.6%	[36]
	220, 5		High glucose yield of 97.2%	
SO_2	190, 5	Sugarcane bagasse	Moderately high glucose yield of 79.7%	
		Sugarcane leaves	High glucose yield of 91.9%	
SO_2	205–225, 5–10	Spruce, pine, birch and aspen	High fractionation efficiency of alkaline extractable lignin for hard woods, but low for softwoods.	[31]
H_2SO_4	185, 2	Rice straw	Overall saccharification yield of 73% in a pilot plant	[37]
H_2SO_4	190, 10	Wheat straw	Glucose and xylose yields of 102 and 96% of theoretical. Ethanol yield of 67% based on glucose content of raw material in SSF.	[38]
Acetic/ethanol	180–225, 3–60	Wheat straw	Sugar yield after enzymatic conversion was found higher than treatment without additive, with maximum yield of 264 g/kg DS obtained for ethanol/SE.	[39]

Table 1. Results of acid-catalyzed SE of selected biomass.

The main disadvantages include the toxicity of SO_2 in SO_2-catalyzed applications and the unavoidable release of degradation products. The acidic nature of pretreatment requires expensive reactors that can withstand corrosion. SO_2 may be costly and as such on-site production could be an alternative for improving the financial viability [18]. The efficient use of co-products such as lignin and hemicellulose in process integration improves the economic health of the process considerably.

3.4. Alkaline-catalyzed steam explosion

Alkaline-catalyzed SE has received less attention compared to acid-based SE. The alkaline solution improves delignification of biomass, giving higher enzymatic degradability. Park et al. [40] pretreated *Eucalyptus* under alkaline environment and observed enzymatic digestibility (relative to uncatalyzed SE), leading to a maximum glucose recovery of 66.55%.

3.5. Double-stage pretreatment involving SE

The major target of the two-step process is to achieve higher delignification and increase biomass digestibility. In many cases, significant increase in glucose yields relative to SE application only, have been observed as outlined in **Table 2**.

3.6. Industrial application

SE is among leading pretreatment methods in terms of cost effectiveness and has been implemented at demonstration (e.g., BioGasol plant in Denmark; Green Plains's plants in USA) and industrial scale (e.g., Crescentino, Italy; Raízen and Iogen's plant in São Paulo, Brazil).

First stage	Second stage	Biomass	Results	Reference
SE	Organosolv	Poplar	Improved lignin removal; over 98% recovery of cellulose; glucan digestibility >88%	[32]
SE	O_2 in alkaline solution	Douglas-fir	84% removal of lignin left in exploded substrates	[41]
SE	H_2O_2 + stabilizers	Douglas-fir	Effective lignin removal	[42]
SE	Laccase	Wheat straw	Effective removal of lignin phenols; high ethanol yields	[43, 44]
SE	Fungi	Wheat straw	75% of lignin degraded	[45]
SE	WO	Pine	96% cellulose yield; ~100% hemicellulose yield	[46]
Dilute acid	SE	Rice straw	Reduced inhibitor formation; enhanced xylose yield degradability	[47]
SE	Alkaline	Sugarcane straw	Enzymatic conversion of 85% in an industrial (SE) reactor	[48]

Table 2. Examples of combined pretreatment including SE.

3.7. Positive attributes and drawbacks

SE is among the most cost-effective methods for and agricultural residues and hardwoods since it does not require external catalysts. It offers the possibility of pretreatment at high solids loading due to the high-energy content of steam and low water requirements which reduce capital expenditure. Moreover, excessive dilution of sugars in pretreated liquor is reduced while the downstream processing of waste solution is minimized or eliminated. Another advantage relates to the possibility of using large biomass sizes which can lead to lower energy intensity. Though particles smaller than 2 cm are usually used, a recent study using larger biomass size (2.5 cm) was found to improve saccharification yield and overall process economics more than smaller sizes (0.5–1 cm); however, smaller particles recorded higher pretreated sugar recovery [49]. Corrosion is reduced due to the non-usage/low-use of chemicals.

Despite the advantages, there are inherent drawbacks associated with SE. The formation of inhibitory products, especially furan derivatives, weak acids and phenolic compounds, negatively affect enzymatic hydrolysis and fermentation [50]. Severe conditions cause increased degradation of cellulose and hemicellulose. There is also a risk of condensation and precipitation of soluble lignin components which leads to reduced digestibility of the biomass substrates [41, 51], while disrupting the lignin structure. SE is less effective on softwood and unexploded materials are common. Further, pretreatment at high temperatures and pressures creates additional challenges in material handling, reactor operation, energy management and heat recovery [52]. Thus, scaling-up is a challenge since large volumes of biomass must be heated to high temperatures in short times.

4. Ammonium fiber explosion (AFEX)

4.1. Description

In AFEX, liquid (anhydrous) ammonia at moderate-to-high temperatures (60–200°C) and pressures (6.5–45 bar) is mixed with moist biomass for about 5–30 min, followed by a sudden drop in pressure to atmospheric. Ammonia is usually fed at less than 2 kg/kg of dry biomass. AFEX leads to the removal of lignin and some hemicelluloses, in addition to the decrystallization of cellulose, partly due to the strong affinity of ammonia for such biomass components. According to Chundawat et al. [53], pretreatment causes morphological and physicochemical changes to cell walls of the material, by creating nanoscale network of interconnected tunnels within the cell wall structure through the cleaving of lignin-carbohydrate ester bonds, and the partial removal and subsequent deposition of extractives on cell wall surfaces, leading to enhanced enzymatic access to cellulose. Further, Maillard reactions between ammonia and carbonyl-based aldehydic groups give rise to several intermediate products [54].

AFEX is generally affected by the moisture content and particle size of biomass, ammonia loading and process conditions including temperature and residence time. Higher temperatures cause more ammonia to flash causing greater disruption of the fibrous structure. Both glucan and xylan conversion (at fixed temperature and ammonia loading) was found to increase with moisture content of switchgrass [55]. In another study, particle size reduction increased the conversion of cellulose and xylan during pretreatment of corn stover [56].

4.2. Applications

AFEX has been widely applied to various class of lignocellulosic materials. Some results obtained from AFEX pretreatment of some biomass are given in **Table 3**.

4.3. Positive attributes and drawbacks

AFEX is a dry-to-dry process since no liquid stream is produced, making it potentially less costly compared to steam explosion [63] and dilute acid methods [64]. The process is simple as it reduces requirements of post-pretreatment washing, stream separation and nutrient supplementation, and produces intermediates that are of value in developing advanced bio-products. Reaction temperatures are moderate and energy requirements are low. Large solids (up to 5 cm) can be fractionated with good yields. Moreover, desired solid loadings are easily obtained, and high solid loadings are easier to implement due to low water demands. High glucose and xylose yields are both obtained under similar process conditions which simplify the optimization of process parameters. Moreover, except for some phenolic fragments of lignin and cell wall extractives that may form on the surface of pretreated solids, no enzyme-inhibitors are produced [50]. AFEX give high sugar yields at low enzyme loadings of 1–10 FPU cellulase/g of dry biomass [1]. Klason lignin and carbohydrates are preserved and pretreated substrates possess high fermentability. Recently, process improvements bordering on ammonia loading and recovery, ammonia recycle concentration, and enzyme loadings have been developed and shown to reduce the cost of operation of AFEX-based biorefinery [65].

Reaction conditions	NH$_3$ loading, g/g dry mass	Biomass	Results	Reference
102°C, 30 min, 2.24 MPa	2[a]	Agave bagasse	~100% carbohydrate preservation; 42.5 g glucose and xylose/100 g native biomass	[57]
40–110°C, 1.4 MPa	1	Rice and wheat straw, sorghum and maize stovers	60–85% glucose recovery, 50–85% xylose recovery	[58]
165.1°C, 69.8 min, 14.3% NH$_3$, 2.2 MPa of CO$_2$		Rice straw	93.6% glucose yield; 97% theoretical ethanol yield	[59]
170°C, 10 min	5	Giant weed	94.2% glucan conversion; 84.4% xylan conversion	[60]
150°C for 30 min	1.5	Switchgrass	98% xylose yield	[61]
70°C, 350–430 psi, 14–18 min	0.8	Dry distillers' grains	90% cellulose conversion to glucose	[62]

[a]Wet-basis.

Table 3. Results of AFEX pretreated biomass.

Its main demerit is its unsuitability for handling materials with high lignin content such as wood. Much of the hemicellulose is fractionated to oligomers making it more challenging during fermentation. High pressures are usually required due to high ammonia loadings and high vapor pressure of ammonia. Moreover, ammonia is expensive and recovery of all feed ammonia for reuse is challenging. Safety issues arising from the corrosive and toxic nature of ammonia present additional challenges in process operation at industrial level. Compared to soaking in aqueous ammonia (SAA), AFEX requires expensive reactors and equipment.

5. Soaking in aqueous ammonia (SAA)

5.1. Description

SAA involves treatment of biomass with aqueous ammonia (5–50%w/w) at low temperatures (25–90°C) under ambient pressure in a batch reactor. Pretreatment is undertaken for residence times ranging from about 1 h to 3 months. Pretreatment efficiency is depended on variables such as temperature, reaction time and ammonia concentration. Lignin dissolves in the aqueous solution without appreciable decrease in the carbohydrate content, and high levels of solubilization are observed with high temperatures and times. In addition, severe conditions also cause release of acetyl groups, hemicelluloses, extractives and ash into pretreatment liquor [66]. In other aqueous ammonia treatment, moderate temperatures (≥100°C) are used to achieve high delignification of biomass using pressure vessels [67]. Higher temperatures are compensated using lower reaction times.

Biomass	Optimal pretreatment		DL, %	X/H, %	Hydrolysis	Yield, %		Reference
						Glu	Eth	
Rice straw	27 wt% NH₃, 25°C, 2 wk		42			71	44–49	[75]
Rice straw	21 wt% NH₃, 69°C, 10 h		60.6[a]		15 FPU/g-glucan, 30 CBU/g-glucan	71.1	83.1	[76]
	15 wt% NH₃, 130°C, 325 psig, 20 min	No acid treatment	69.8	77	50°C, 15 FPU/g-glucan, 15 CBU/g-glucan	83.2		[77]
		+ acid treatment				90.8		
	60°C, 15 wt% NH₃, 24 h	PBI: 3 kGy, 45 MeV			50°C, 60 FPU/g-glucan, 10 CBU/g-glucan	90		[78]
Corn fiber (destarched)	15 wt% NH₃, 65°C, 8 h			76–78	50°C, 72 h, 15.57 FPU/g-glucan, 30 CBU/g-glucan	85.4		[79]
Corn stover	29.5 wt% NH₃, 10–60 days, RT		56–74	85	50°C, 72 h, 15 FPU/g-glucan, 30 CBU/g-glucan	86–89	73–77	[80]
	15 wt% NH₃, 60°C, 12 h		62	85	15 FPU/g-glucan	85	77	[81]
	50 wt% NH₃, 30°C, 4 weeks		55		15 FPU/g-glucan, 30 CBU/g-glucan	86.5	73	[82]
	15 wt%NH₃, 69°C, 12 h			>80			84	[70]
	15 wt% NH₃, 60°C, 8 h	Hot water, 10 min	68		50°C, 24 h, 15 FPU/g-glucan, 30 CBU/g-glucan	96		[83]
	12.5 wt% NH₃, 60°C, 24 h, O₂	+ TiO₂, UV	70		50°C, 24 h, 15 FPU/g-glucan, 30 CBU/g-glucan	85		[84]
		+ ZnO, UV				82		
Switchgrass	29.5 wt% NH₃, 10 days, RT		40–50	50			72	[85]
	30 wt% NH₃, 5 days (pilot-scale)	Aseptic conditions					73	[86]
		Semi-aseptic					52–74	
	15 wt% NH₃, 40°C/24 h, 60°C/8 h		40.8–46.9		50°C, 72 h, 22–25 FPU/g-glucan, 44–50 CBU/g-glucan, + xylanase	>85		[87]
	15 wt% NH₃, 120°C, 24 h	No H₂O₂	65		15 FPU/g-glucan, 30 CBU/g-glucan	53.7		[88]
		+ 5% H₂O₂	77			74.3		

Biomass	Optimal pretreatment	DL, %	X/H, %	Hydrolysis	Yield, %		Reference
					Glu	Eth	
Oil palm trunk	80°C, 8 h and 7 wt% NH$_3$	40–50		50°C, 96 h, 60 FPU/g-glucan	95.4	78.3	[89]
Oil palm empty fruit bunch	60°C, 12 h, and 21 wt% NH$_3$	40.9		60 FPU/g-glucan, 96 h	41.4	65.6	[90]
Miscanthus	150°C/30 wt% NH$_3$, 180°C/10 wt% NH$_3$, 1 h (not optimum)	>65	39.3–77.1	50°C, 96 h, 20 FPU/g-glucan	53.4		[91]
News paper	4 wt% NH$_3$ + 2 wt% H$_2$O$_2$, 40°C, 3 h			50°C, 72 h, 60 FPU/g-glucan	90		[92]

DL: delignification; RT: room temperature; PBI: proton beam irradiation; X/H: percentage of xylan/hemicellulose retained in the solids after pretreatment; Glu: maximum theoretical glucose yield after enzymatic hydrolysis; Eth: ethanol yield after fermentation, SSF, SSCF, etc.

[a]Conditions: 70°C, 10 h, 20 wt% NH$_3$.

Table 4. Sugar and ethanol yields from selected SAA pretreated biomass.

5.2. Applications

Chen et al. [68] used aqueous ammonia to pretreat silvergrass, napiergrass and rice straw at room temperature, resulting in over 90% of cellulose recovery in 4 weeks. On destarched barley hull, SAA pretreatment (15w/w NH$_3$, 75°C, 48 h) produced zero glucan loss and 83% saccharification yield using 15 FPU/g-glucan; and with the addition of a xylanase in simultaneous saccharification and co-fermentation (SSCF), a high ethanol yield of 89.4% of the maximum theoretical was obtained [69]. High ethanol concentration and yields from SAA-pretreated corn stover followed the use of a two-phase SSF involving pentose and hexose conversion with the help of *S. cerevisiae* and a recombinant bacterium, respectively [70]. Recently, the addition of surfactants such as Tween 80 and PEG 400 was found to improve sugar and ethanol yields [71]. In a similar study Raj and Krishnan [72] obtained high sugar yield by adding laccase and a mediator to enhance enzymatic hydrolysis of pretreated biomass. Nahar and Pryor [73] also found out that pelleting of samples before SAA application required less harsh pretreatment conditions and lower costs.

Two-stage processes targeting separate removal of hemicelluloses and lignin have also been investigated. Kim et al. [74] employed acetic acid medium to remove hemicelluloses followed by aqueous ammonia at elevated temperatures. Results obtained from other studies are given in **Table 4**.

5.3. Positive attributes and drawbacks

SAA retains most of the hemicelluloses in the solid, eliminating the need to separately process hemicellulose and cellulose sugars. It leads to efficient delignification, producing low levels of enzyme inhibitory compounds. The reactor configuration is simpler and less costly, while ammonia recovery is easier compared to AFEX [18]. It can be adapted to small-scale production. Further, neutralized salts from liquid hydrolysates could be used as nutrient source in fermentation.

There are few disadvantages associated with SAA pretreatment. Since pretreated solids contain high fractions of hemicellulose, a high demand for C5 conversion enzymes is needed to produce xylose and other pentose monomers [18]. Post-treatment washing usually result in carbohydrate losses.

6. Irradiation-chemical pretreatment

6.1. Description

In irradiation-chemical pretreatment, the biomass is typically soaked in a solvent (water, acid, or alkali) before undergoing irradiation via microwaves, gamma radiation, proton and electron beam, or radio frequency. In some cases, irradiation is performed before the chemical or other pretreatment, with advantages that include solubilization of lignin and hemicellulose, minimization of cellulose degradation, use of lower doses of chemical and less severe conditions. Further, undertaking irradiation before milling of biomass can reduce energy consumption (from size reduction) significantly [93].

Dielectric heating of biomass causes more energy absorption by the more polar part which creates a hot spot, resulting in generation of high internal steam pressure that induces an explosive effect, disrupting the biomass structure [94, 95]. The disruption is underpinned by radiolytic reactions that cause release of free radicals, triggering cross-linking and chain scission [96]. Cross-linking reactions are believed to happen within the cellulose structure and as such when they predominate over chain scission reactions, sugar yields are not affected.

In general, pretreatment results in degradation of hemicellulose and lignin, and the alteration of cellulose structure. There is an increase in the specific surface area and a reduction in the degree of polymerization [97], as well as a change in the crystallinity of cellulose to amorphous pattern [98]. In general, higher radiation intensities and lower biomass moisture content lead to higher rates of increase in final temperatures; however longer radiation time causes higher average final temperature and lower rate of temperature increase [99]. Increases in irradiation strength have been found to affect hemicellulose more than lignin or cellulose [96, 100].

6.2. Applications

Microwave-assisted pretreatment has been applied to various materials. In a comparative study of the efficacy of mild sulfuric acid (5% v/v) application in combination with various heating modes—hot plate (100°C, 30 min), autoclave (121°C, 30 min), and microwave (200°C, 700 W, 15 min) on the biodegradability of garden biomass, microwave heat treatment was found to produce 53.95% cellulose recovery, leading to reducing sugar yield of 46.97%, which was about 10% higher than the other two modes [101]. Application of microwaves on alkali pretreated wheat straw [102] and coconut husk fiber [103] was found to produce

higher ethanol concentration and yield than substrates that did not receive any radiation. In another study, yields of 25.3, 21.2, and 46.5 g/100 g biomass, respectively, was obtained during radio frequency-assisted NaOH pretreatment (27.12 MHz, 0.20–0.25 g NaOH/g biomass; 90°C) of switchgrass at solids content of 20% [94]. In an investigation to ascertain the effects of microwave chemical pretreatment on sweet sorghum bagasse (12% moisture, 1–2 mm), lime was found to enhance lignin removal, with sugar yields reaching 23.2 g/100 g biomass (38% of theoretical yield) for lime concentration of 0.1 g/10 ml of water. Microwave has also been used in conjunction with eutectic solvent, with enhanced lignin and hemicellulose removal and improved cellulose digestibility [104].

Under electron beam application, Karthika et al. [105] obtained 79% sugar yield from the saccharification (30 FPU/g-biomass, 144 h) of a hybrid grass exposed to 250 kGy of radiation, while Bak et al. [106] realized 52.1% from rice straw when it was exposed to 80 kGy and saccharified using 60 FPU/g-glucan for 132 h. Prior removal of hemicellulose using dilute acid and alkaline before irradiation exposes cellulase to enzymatic action during hydrolysis, and culminates in higher sugar yields [107]. Electron beam has also been applied together with other physico-chemical methods such as SE with good results [108]. The main challenge regarding the use of electron beam pertains to its low energy and as such some interest are focusing on proton beam.

6.3. Positive attributes and drawbacks

The mode of heating is uniform, energy efficient and offers rapid processing of biomass. Pretreatment is performed at low temperatures and at shorter period. It has the potential to be used for effective isolation of hemicelluloses. Irradiation generates no/low levels of inhibitors and by carefully controlling the chemical pretreatment, inhibitor levels are reduced.

Irradiation-chemical methods do not come without disadvantages. Microwave-assisted pretreatment comes with the risk of causing extensive degradation of hemicelluloses and contamination of dissolved lignin at severe conditions, releasing toxic compounds that inhibit enzymatic hydrolysis. Hu and team [94] argue that practical issues with scaling-up is more of a challenge in microwave than in radio frequency which can be used on large quantities of biomass, and at relatively high solids loading (20–50%) with uniform temperature profile when combined with chemical methods.

7. Conclusion

Among the three main stages of cellulosic ethanol production, namely, pretreatment, hydrolysis and fermentation, pretreatment presents the most practical and economic challenges in the attempt to produce ethanol at industrial-scale due its influence on both upstream and downstream processes. Thus, emerging and promising pretreatment methods that rely on physico-chemical fractionation of biomass are discussed, with prominence given to process description, advantages, drawbacks, and innovations employed to counteract inherent

technical, economic and environmental challenges. The methods reviewed include liquid hot-water (LHW), steam explosion, ammonium fiber explosion (AFEX), soaking in aqueous ammonia (SAA), and irradiation-based pretreatment. Size reduction operations have been well integrated with other chemical and physico-chemical methods at the pilot and demonstration levels though energy consumption remains the rain challenge and as such research is shifting in favor of relatively low-energy methods such as wet disc milling as well as post-pretreatment size reduction. Irradiation-based methods have also shown promise at the industrial-level as demonstrated by burgeoning research interest around the world. With regards to physico-chemical methods, steam explosion and LHW-based methods have already been developed for industrial application.

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