Hemicellulose biorefineries: a review on biomass pretreatments

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Biomass pretreatment (BP) plays a crucial role in a lignocellulose feedstock-based biorefinery (LCFBR) for processing of three major output streams (cellulose, hemicelluloses and lignin) into chemicals and biofuels. BP includes processing of lignocellulosic material (LCM) under aqueous, dilute acid or alkaline media to obtain a cellulosic fraction, which is then fermented to produce bioethanol. Hemicellulose is usually treated as a secondary stream due to lack of efficient fermentation of hemicellulosic sugars to ethanol. This review provides BPs assuming that hemicellulose stream should be integrated in LCFBR as a primary fraction for converting into value-added compounds other than bioethanol. Different LCM treatments are analyzed foreseeing bio-based products possible to obtain from hemicellulose path.

Introduction

Biomass pretreatments (BPs) technologies[1-4] (chemical, physical or biological) change/remove structural and compositional constraints to improve hydrolysis rate and increase yields of fermentable sugars from cellulose. Therefore, BPs can be divided in two groups: i) Those related to chemical and biochemical constrains such as cellulose recovery, digestibility, by-product/inhibitor formation; and ii) Those related to bioprocess costs, such as, water usage and energy efficiency. This paper reviews main advantages and bottlenecks of currently available BP technologies, Ne:foreseeing potential added-value products possible to be obtained and most relevant factors, which influence both product yield and consistency.

Biorefinery Concept

Biorefinery is an overall concept of an integrated and diversified processing plant where biomass feedstocks are converted into a wide range of valuable products, much likewise to petroleum refineries. Integrated biorefinery is a processing facility that extracts carbohydrates, oils, lignin, and other materials from biomass, converts them into fuels, high value chemicals and other materials, with a zero waste approach[5-10] (Fig. 1). Pulp and paper mills, corn wet and dry mills that produce multiple products from biomass can be categorized as biorefineries. At Mediterranean countries, including Portugal, olive-pomace oil extraction facilities and wine distilleries are examples of *proto-biorefineries* since they produce a wide combination of food, feed and power[11].

In comparison to petroleum refineries, biorefineries utilize a variety of feedstocks and a larger range of processing technologies. Biomass feedstock is bulkier than fossil fuels[12]. By integrating production of higher value bioproducts into biorefinery's fuel and power output, overall profitability and productivity of all energy related products are potentially improved. Increased productivity and efficiency can also be achieved through operations that decrease overall energy intensity of biorefinery's unit operations, maximizing use of all feedstock components, byproducts and waste streams, and using scale-up economies, common processing operations, materials, and equipment to drive down all production costs[5,7]. Biorefinery can be considered as an evolution of concepts like "*Green Chemistry*" or *Chemurgy*[6,13].

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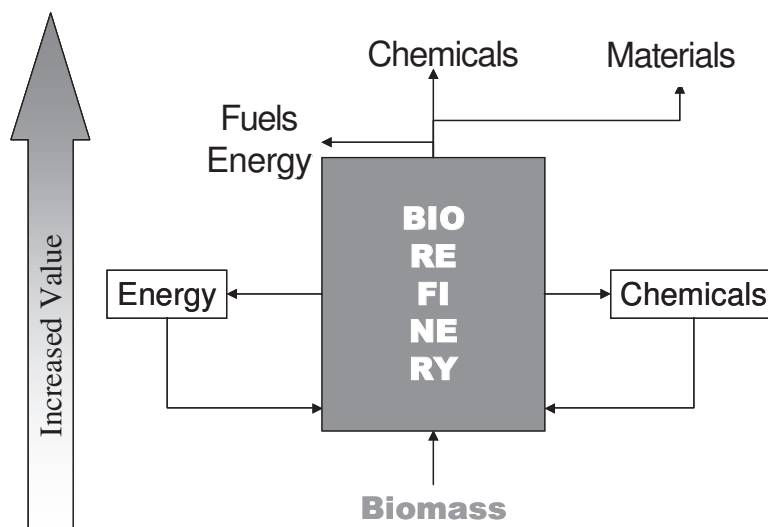Tel: +351 21 092 4721; Fax: +351 21 716 36 36
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Fig. 1—Biorefinery concept

Biorefinery Platforms

Depending on raw materials, technological processes, and products obtained, biorefinery platforms can be distinguished (Table 1) based on sugar (biochemical), syngas (thermochemical), biogas, or carbon-rich chains platforms[7]. Biorefinery platforms may incorporate other processes from other platforms[14] (anaerobic digestion of fermentation effluents[15], or thermochemical transformation of lignin in sugar platform biorefinery). Biorefinery platforms, based only on main raw materials[6,8,16] used, are: i) Lignocellulose feedstock-based biorefinery (LCFBR) that uses nature-dry raw material (wood, straw etc.); ii) Whole crop biorefinery that uses cereals; and iii) Green biorefineries that use nature-wet biomasses such as green grass, alfalfa, immature cereal etc.

Biochemical and Thermochemical Biorefineries

Most promising emerging biorefinery platforms are sugar (biochemical) and syngas (thermochemical) platforms[7]. Biochemical platform focuses on fermentation of sugars extracted from lignocellulosic feedstocks. After feedstock preparation (size reduction), conversion technologies involve three basic steps: i) Convert biomass to sugar or other fermentation feedstock; ii) Bioconversion of these biomass intermediates using biocatalysts; and iii) Process products to yield added value chemicals, fuel-grade ethanol and other fuels, heat and/or electricity. Top value added chemicals that can be produced in a biochemical platform biorefinery have been identified[5] (Table 2). Economic viability of a biochemical biorefinery is highly sensitive to feedstock cost, operating scale and macromolecular biomass composition.
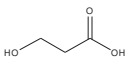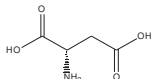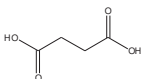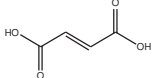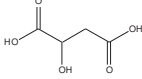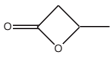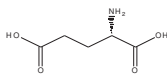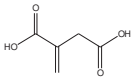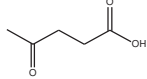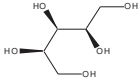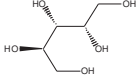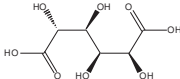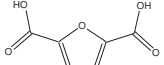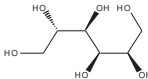
Thermochemical biorefineries focuses on gasification (heating biomass with about one-third of oxygen necessary for complete combustion, producing syngas), and/or pyrolysis (heating biomass in absence of oxygen, producing a pyrolysis oil). Both syngas and pyrolysis oil can be used as cleaner and more efficient fuels than solid biomass, but can also be chemically converted to other valuable fuels and chemicals. After feedstock preparation (drying, size reduction), conversion technologies include feeding, conversion (gasification, pyrolysis), and product clean up and conditioning. Hydrogen and methanol are probably the best short-term options for several biobased commodity chemical production since obtaining simple alcohols, aldehydes, mixed alcohols and Fischer-Tropsch liquids from biomass are not economically viable. Gas cleanliness is key barrier to economic production of syngas from biomass[5]. Economic viability of this type of biorefinery is also highly sensitive to feedstock cost and operating scale, but conversely to biochemical platform, macromolecular biomass composition does not play such an important role[2].

Although biochemical platforms costs can be lower than thermochemical platforms, at least in short to mid-term, there is not yet a clear definition of what is the most appropriate capacity for a biochemical biorefinery. Some studies pointed out to values close to or higher than 350,000 t/y dry feedstock for ethanol-based biorefineries[15,17,18] and a supply region up to 150 km radius[19]. But, if whole material could be upgraded for

Table 1—Most common biorefinery platforms and their major characteristics

Platform	Raw materials	Main processes	Products	Development stage
Sugar (Biochemical)	Lignocellulosic and starch biomass	Chemical and enzymatic hydrolysis, Fermentation, Biotransformation, Chemical and catalytical processes	Added value chemicals (both from sugar and lignin) Building block chemicals Materials (from lignin or lignocellulose) Fuel ethanol. Heat and electricity (from lignin)	Laboratory, large scale pilot plant and commercial (sugarcane and starch based)
Syngas (Thermochemical)	Lignocellulosic biomass but also plastics, rubber etc.	Thermochemical processes : - Gasification - Pyrolysis	Syngas Pyrolysis oil Added value chemicals Gaseous or liquid fuels	Laboratory, large scale pilot plant
Biogas	Liquid effluents Manure	Anaerobic digestion	Methane and carbon dioxide (biogas) Added value chemicals	Large scale pilot plant, commercial
Carbon-rich chains (Oil)	Plant oils such as soybean, rapeseed corn, palm, and canola oils. Animal fat	Transesterification	Fatty acid methyl ester (biodiesel), Glycerin and fatty acids as platform chemicals	Commercial

Table 2—Top value added chemicals derived from sugars in a biochemical biorefinery platform[5]

	Aminoacids	Carboxylic acids	Polyols	Lactones
C3		3-Hydroxy propionic acid	Glycerol	
C4	Aspartic acid	Succinic acid Fumaric acid Malic acid		3-Hydroxy butyrolactone
C5	Glutamic acid	Itaconic acid Levulinic acid	Arabitol Xylitol	
C6		Glucaric acid 2,5 furan dicarboxylic acid	Sorbitol	

several high value co-products (hemicellulose and lignin fractions), total capacity to ensure economic viability can decreases to 33,000 t/y[20], which opens very interesting opportunities to upgrade local agro-industrial byproducts[11,21]. Although biochemical platform is in a more advanced stage as compared to other biorefinery platforms, many improvements[22-27] are still needed.

Pretreatment Processes

Depending on the process and conditions used, during pretreatment, hemicellulose sugars may be degraded to weak acids, furan derivates and phenolics[28]. These compounds may inhibit the later fermentation processes, leading to lower yields and productivities.

Acid Hydrolysis

Acid catalyzed hydrolysis have been described as early as 19th-century, with commercial applications from beginning of 20th century[6,29]. Acid hydrolysis can be divided in two general approaches, based on concentrated acid/low temperature, or dilute acid/high temperature.

Concentrated Acid

Concentrated acid-based processes are very expensive[2] and cause significant operational problems[4,29]. As a catalyst, sulphuric acid (H_2SO_4) is most commonly used although other mineral acids [hydrochloric (HCl), nitric (HNO_3) and trifluoracetic acid (TFA)] have also been assayed. Concentrated acids remove mostly cellulose and hemicellulose, resulting in a lignin-enriched solid phase. Polysaccharide solubilization (soluble phase) is reached using different acid concentrations, like 72% H_2SO_4, 41% HCl or 100% TFA[30]. HCl and TFA are easier to recover than H_2SO_4. Concentrated acids allow to operate at low/medium temperature and pressure leading to formation of low amounts of degradation products[31]. However, degradation products formation rate is severely affected by slight changes in temperature[32], conversely what occurs in case of dilute acid hydrolysis even at high temperatures[33,34]. Acid recovery is a key step for economic viability of concentrated acid pretreatments[35], and these neutralization costs have hampered general use of these pretreatments. Moreover, equipment corrosion problems are an additional disadvantage[36]. Nevertheless, there seems to be a renewable interest in these processes[37].

Dilute Acid

Dilute sulfuric acid processes (0.5-1.5%, temp. 121-160°C) have been most favored for industrial application,

because it achieves reasonably high sugar yields from hemicellulose[2,29]. H_2SO_4 is usual acid employed although HCl, HNO_3, and H_3PO_4 are also employed[3,36,38]. Compared to concentrated acid hydrolysis, this pretreatment generates lower degradation products as well much less corrosion problems in hydrolysis tanks, pipes, etc. Addition of CO_2 to aqueous solutions forms carbonic acid as an alternative of acid addition[39-41]. Carbonic acid behaves better than most of inorganic acids and is specifically suited for hydrolysis conditions under mild pH at high temperatures and pressures[40]. Although, carbonic acid is responsible for an increase in xylan hydrolysis, which is lignocellulosic material (LCM) dependent[39-41].

Based on studies of hemicellulose hydrolysis using percolation reactors[42-44], novel technologies have been developed using counter-current shrinking-bed reactors[45], giving high cellulose hydrolysis yields without significant formation of degradation products[46]. Besides inorganic acids, some weak organic acids, like maleic acid is a potential catalyst to promote cellulose hydrolysis[47,48].

Hydrothermal Pretreatments

Hydrothermal processes use water, steam or both, and heat for BP. Hydrothermolysis[49] started as a pretreatment method before enzymatic hydrolysis. Hydrothermal processes include autohydrolysis[50-55], aqueous liquefaction[56], aqueous extraction[57], aqueous pretreatment[58,59], hydrothermolysis[60-62], aquasolv[63], hydrothermal pretreatment[64], aqueous pre-hydrolysis[65] and pressure cooking in water[66]. Hydrothermal treatments mainly include liquid hot water (autohydrolysis) and steam-explosion between 150°C and 230°C[67]. For temperatures below 100°C, there is no hydrolytic effect on material[68], whereas above 220°C some cellulose degradation takes place[69,70]. Between 240-250°C, pyrolysis reactions become important[71,72]. Reaction time of hydrothermal treatments varies from seconds to hours, which is related with operation temperature.

Solids concentration, usually reported as liquid-to-solid ratio (LSR), may vary between 2 and 100 (w/w)[67,73], although most common values are around 10. Higher LSR are usually associated to continuous reactors[73], whereas lower LSR are, in general, used in BPs that employ steam since specific heat of steam is higher than that of water. LSR may also affect catalyst (acid) concentration in reaction media by two way: i) Decrease of LSR lead to a higher release of acetyl groups favoring

depolymerization[50]; and ii) Lowering LSR lead to increase in buffer capacity at a neutral pH, which mainly related with ash content of feedstock, decreasing hydrolysis rate[74,75].

Influence of particle size is not significant for high LSR, but for low LSR, increase of particle size leads to a decrease in biomass solubilization, probably due to a deficient LCM impregnation[76]. For pretreatments requiring steam, influence of particle size seems to be more important, since for large biomass particles, joint effect slow heating time, that occurs in particle core, and short reaction times may lead to an irregular/ heterogeneous biomass particle heating[77]. For some LCM, particle size reduction is not necessary for occurring a maximal solubilization rate[63], while for other LCM similar size reduction clearly affects amount of solubilized polysaccharide recovered, indicating higher polymer degradation[78].

Autohydrolysis

Autohydrolysis process uses compressed liquid hot water (temp., 200°C; pressure, > saturation point;) to hydrolyze hemicellulose in minutes. Hemicellulose recovery is high, and no catalyst is needed in this process. Lignin is not significantly solubilized; neither sugar largely decomposes, thus yielding a liquid fraction without many potential bioconversion inhibitor compounds. However, solubilized hemicellulose appears mainly in oligosaccharide form[55,79].

Autohydrolysis/hydrothermal processes have a similar mechanism as dilute acid hydrolysis. Both are hydrolysis processes catalyzed by hydronium ions (H_3O^+). In autohydrolysis, where water is the only reactive added to substrate, catalysts of hydrolysis are in a first stage hydronium ions coming from water auto-ionization that lead to depolymerization of hemicelluloses by selective hydrolysis of both glycosidic linkages and acetyl groups. In a second stage, hydronium ions coming from acetic acid also act as catalysts, improving reaction kinetics. The contribution of hydronium ions from acetic acid is higher than that from water autoionization[51,56]. Hydrolysis of uronic acids should also occur in first stages of treatment, simultaneously with acetyl groups[80]. Despite to their resistance to hydrolysis[81], uronic acids may also contribute to formation of hydronium ions[65] but their role in hydrolysis is still not completely understood.

In autohydrolysis[80-83], acids resulting from hydrolysis of acetyl and uronic groups, originally present in hemicelluloses, catalyze hydrolysis of links between hemicelluloses and lignin as well as hydrolysis of carbohydrates. Autohydrolysis may convert hemicelluloses into soluble saccharides with high yield and low byproducts formation[57,63,84,85], rendering a easily extractable[59] solid residue rich in cellulose and lignin. Owing to mild pH, corrosion problems are reduced[57,68], and steps of acid recycling and precipitates removal are no longer necessary. The possibility to reduce both capital and operational costs due to the absence of acid emphasizes both reduced environmental impact[59] and economical advantages of aqueous-based processes compared to other hydrolytic technologies[86]. Autohydrolysis products are a mixture of oligosaccharides, monosaccharides, acetic acid and furan derivatives [furfural (2-furaldehyde) and hydroxymethylfurfural (5-hydroxymethyl-2-furaldehyde, HMF)], which can further undergo decomposition reactions, yielding formic acid[87] and levulinic acid[88], respectively.

Steam Explosion

Steam explosion was developed in 1925 for hardboard production[89]. Use of steam explosion for BP was introduced in early 1980's on aspen wood[90]. In this process, material is heated using high-pressure steam (20-50 bar, 210-290°C) for a short period (from seconds to few minutes). Steam condenses under high pressure, thereby "wetting" the material, which is driven out of reactor through a small nozzle by induced force. Several phenomena occur at this stage. Due to decrease in pressure, condensed moisture evaporates and desegregation of lignocellulosic matrix takes place, braking down inter- and intra-molecular linkages[51]. One study[91] emphasized role of mechanical effects (shear and explosive decompression) in process efficiency, while other studies[77,92] consider that explosive decompression is not essential for its efficiency. Other authors reported that process is fundamentally chemical in nature[90,93]. Chornet & Overend[94] described steam explosion as being a thermomechanochemical process where breakdown of structural components is aided by heat in the form of steam (thermo), shear forces due to expansion of moisture (mechano), and hydrolysis of glycosidic bonds (chemical).

There are several studies applying steam explosion for BP in ethanol process. Schultz *et al*[95] compared effectiveness of steam explosion pretreatment on mixed hardwood chips, rice hulls, corn stalks, and sugarcane bagasse. Steam explosion has also been used as a

pretreatment method for agricultural residues, namely *Onopordum nervosum* and *Cyanara cardunculus*[96], *Brassica carinata*[97], olive tree biomass[98], and sunflower stalks[99]. Some steam explosion processes have been mostly used as pretreatment methods for kraft pulps bleaching in paper and pulp industry. Some well known steam explosion-based pretreatments reported include Masonite[100], Siropulper[101], Iotech[102] and STAKE (www.staketech.com) processes.

Most steam treatments yield high hemicellulose solubility (producing mainly oligosaccharides) along with low lignin solubility. Studies without added catalyst report sugars recoveries between 45-65%[93]. Major operational difference between autohydrolysis and steam explosion processes is that in later higher temperatures along with shorter times are used, being also common the prior LCM impregnation with H_2SO_4[71,77,103-107] or SO_2[103,105-110]. A pretreatment variant of catalyzed steam explosion is CO_2 explosion[31], which acts to steam and ammonia explosion[31]. Glucose yields in later enzymatic hydrolysis are low (75%) compared to steam and ammonia explosion. Overall, CO_2 explosion is more cost effective than ammonia explosion, preventing inhibitors formation[2,4].

Wet Oxidation

In wet oxidation, autohydrolysis occurs in presence of oxygen or air as catalysts[111], which allows reactor operation at reduced temperatures and to shorter reaction times. On the other hand, pretreatment is more expensive mainly due to capital costs associated with pressure equipment. However, Na_2CO_3[112] and not oxygen is the most employed catalyst for wet oxidation pretreatments. Under alkaline medium, it is possible to get high rates of biomass-to-monosaccharides under moderated conditions, short reactions time and low formation of furan aldehydes[112,113] and phenolics aldehydes, but not aliphatic acids[113,114]. During wet oxidation, occurrence of considerable delignificationtm:[112,113], is correlated with the increase of carboxylic acids[113].

Delignification Pretreatments

Delignification methods, mostly employed in pulp and paper kraft and sulphite technologies, discharge huge amount of liquid and gaseous wastes such as sulfur-containing gas emissions. Alternative delignification methods include organosolv treatments where lignin removal is promoted by direct action of an organic solvent (ethanol, methanol, acetone, acetic acid, formic acid, ethyl acetate, etc.) to solubilize lignin and hydrolyze

hemicellulosic fraction[31]. When a catalyst is used (acids, anthraquinone or salts), a more selective delignification can be reached and lignin itself is less degraded and of higher added-value for further technical applications[115,116] (www.lignol-innovations.com). Lignol approach[117] uses a blend of ethanol and water (50:50, w/w), at 200°C and 400 psi, to extract from LCM most of lignin as a fine precipitate by flashing pulping liquor to atmospheric pressure, followed by rapid dilution with water. Water-soluble stream yield hemicellulose sugars (50% in oligomeric form), besides significant amounts of furfural. Biodelignification (biological degradation of lignin by microorganisms) has advantages of low energy use and mild environmental conditions but hydrolysis rate is very low[4]. Sometimes biological treatments are used in combination with chemical treatments[2].

Alkaline Treatments

Alkali pretreatment increases cellulose digestibility. Depending on catalyst used, alkaline pretreatments can be divided into two major groups: i) Pretreatments that use sodium, potassium, or calcium hydroxide; and ii) Pretreatments that use ammonia. Conversely to acid or hydrothermal processes, alkaline-based methods are more effective for lignin solubilization exhibiting only minor cellulose and hemicellulose solubilization, excepting ammonia recycling percolation treatment, which yield biomass solids mostly containing cellulose[2,4,118].

Lime and NaOH Pretreatments

Sodium and calcium hydroxide (lime) are most common catalysts (conc. 0.05-0.15 g alkali/g biomass) applied in batch mode directly to biomass[119-124], or as a subsequent treatment to acid hydrolyzed biomass[125,126]. Use of alkali treatment (temp., 30-130°C; reaction time, 10 min - 18 h) before an acid hydrolysis has also been reported[127]. As a possible way to increase efficiency, ultrasonically assisted extraction has been reported to give a slightly higher yield of hemicelluloses and specially of lignin, than those of classical alkali procedure[128]. From the obtained hydrolyzates, it is possible to easily recover phenolic compounds (ferulic and *p*-coumaric acids from brewery's spent grain) for applications in food, health, cosmetic, and pharmaceutical industries[121,129]. Depending on feedstock and operating conditions, significant increases on glucose recovery are reported from 60% to almost quantitative glucose yields[123,125,126].

In alkaline hydrolysis, alkali induces swelling leading to increase in internal surface area. Next, a decrease in degree of polymerization, and crystallinity occurs with a

consequent separation of structural linkages between lignin and carbohydrates. Then, disruption of lignin structure takes place, followed by saponification of intermolecular ester bonds that crosslink hemicelluloses and other components. This leads to an increase of LCMs porosity with removal of such crosslinks[4]. In addition, removal of acetyl and uronic acid substitutions of hemicelluloses increase accessibility of enzyme to hemicellulose and cellulose surface in a further enzymatic posthydrolysis[1,3].

Main advantage of these processes is the use of low temperature and pressure. Lime as compared to NaOH and KOH has lower cost and less significant safety requirements. It can be recovered from hydrolyzate by reaction with CO_2, so that formed carbonate can then be reconverted to lime[3]. Unlike acid-catalyzed pretreatments, a limitation occurs because some alkali is converted to irrecoverable salts or incorporated as salts into biomass[3].

Alkaline Peroxide

Addition of an oxidant agent (air/oxygen[112] or H_2O_2) to alkaline (lime or NaOH catalyzed) pretreatment can greatly improve performance, by favoring lignin removal.. These treatments are applied in batch mode, either directly to biomass[130], or to steam exploded[131-133] or hydrothermally processed biomass[134]. H_2O_2 treatment (1-3%; reaction time, 45 min to 5 h; temp., 30-80°C; optimal pH, 11.5[131-136]) is not very efficient, presenting a lower effect as compared to NaOH treated biomass, but it gives higher delignification than acid hydrolysis, as seen for cotton stalks[123]. Reaction mechanism is similar as for alkaline hydrolysis, but it is complemented with direct oxidative effect over lignin. H_2O_2 over air/oxygen do not require expensive high-pressure equipments except some safety issues. Alkaline peroxide treatments seem to have a faster kinetics at low temperature, thus presenting technological and economical advantages over lime and NaOH catalyzed pretreatments. Its association with steam explosion, or hydrothermal process seems to be an effective strategy to selectively recover and potentially upgrade lignocellulosic fractions.

Ammonia Fiber Explosion (AFEX)

AFEX can be presented as a combination of steam-explosion and alkaline pretreatments processes[137]. Biomass is treated in batch mode with liquid anhydrous ammonia (1-2 kg ammonia/kg biomass) at moderate temperatures (40-140°C) and high pressure (250-300 psi)

for few minutes, after which pressure is rapidly released[4,138,139]. In AFEX, reaction mechanism produces a combination of chemical and physical effects that induces cleavage of lignin-carbohydrate complex[140], hemicellulose hydrolysis, and cellulose decrystallization, that further lead to an increased surface area. These modifications enable near complete enzymatic conversion of cellulose and hemicelluloses to fermentable sugars at low to moderate enzyme loadings[31,138]. Although, this process can be considered a dry to dry process as dry mass recovery following AFEX treatment is 100%[138], but water washing have greatly improved glucan conversion, due to removal of phenolic extractives from AFEX-treated biomass[140].

AFEX has been applied successfully to herbaceous and agricultural residues[137-143] with approx. 100% of theoretical glucose yield, but it only works moderately well on hardwoods, and is not attractive for softwood derived materials[3,31,141]. Also, it involves high costs associated to ammonia, its recovery, and considerable safety issues regarding practical use of ammonia[31]. On the other hand, economy is positively influenced by high total sugar yields achieved, without the presence of potential inhibitors (sugar degradation products[3]) and possibility to recover feedstock protein[144] as a biorefinery co-product (animal feed).

Ammonia Recycling Percolation (ARP)

In ARP, aqueous ammonia is used in flow-through mode using a column reactor containing biomass. It contains following parameters: ammonia conc., 2.5-20% (typically, 10-15%); reaction time, up to 90 min[3,4]; solid conc., 15-30% (w/w); temp., 140-170°C (up to 210°C[145]); and percolation rate, 5 ml/min[1,145]. This process has already been successfully applied to hardwoods[145,146], corn stover[147-150], and with a slight less efficiency to softwood pulp mill sludges[151], producing highly effective delignification (60-85%)[145,147,148] and cellulose hydrolysis yields close to the theoretical value. ARP process can also solubilize hemicellulose (40-60%, mainly in oligomeric form), but cellulose fraction remains intact (solubilization <10%)[145-148,151].

High enzymatic digestibility by ARP pretreated biomass can be explained by simultaneous removal of lignin and hemicellulose. Aqueous ammonia reaction mechanism is cleavage of ether and ester bonding in lignin carbohydrate complex and swelling. SEM indicated that biomass structure got deformed and its fibers exposed by ARP[147,148]. Crystallinity index increased with

pretreatment reflecting removal of amorphous portion of biomass. Crystalline structure of cellulose in biomass, however, was not changed by ARP treatment[147,148]. ARP is not economical process[1].

Hemicellulose Path: Combined Hydrolysis and Posthydrolysis

Most promising pretreatment option for hemicellulose breakdown is dilute acid, which significantly increases susceptibility to hydrolysis for cellulose. However, it produces fermentation inhibitors, and acid utilization is a critical and expensive issue, resulting in a waste disposal problem, and thereby requiring use of expensive corrosion-resistant equipments. Furthermore, lignin valorization potential is decreased. This may eventually shift balance in favor of a slightly less effective hydrolytic pretreatments, but also less problematic and environment friendly[2]. In this case, autohydrolysis or steam-explosion may lead the run for best pretreatment technology for LCM biorefineries. Moreover, current research efforts may lead to get higher yields eventually similar to diluted acid yields[2]. Although, autohydrolysis process is currently at laboratory/pilot stage, it could become commercially available, in mid-term, with process yields forecasted around 88-98%, this is higher than for dilute acid or steam-explosion.

Many of these pretreatment options render soluble hemicellulose, either totally or in a very significant quantity, in oligomeric form (for autohydrolysis, values up to 60-80% reported[55,58,79,152-155]). Oligosaccharides do have significant market potential either as specialty pharmaceuticals, chemicals or food additives[156-158]. Therefore, to fully upgrade this stream, other products must be targeted, such as bioethanol, organic alcohols such as polyols[5], namely arabitol and more relevant xylitol[159-162], organic acids, yeast extract[163,164], 1,2-propanediol, 2,3-butanediol, and aromatic chemical intermediates[165,166]. Hydrolysis of hemicellulosic oligosaccharides is almost a compulsory requirement to upgrade hemicellulosic sugar stream. Actually, there are not yet many effective microbial catalysts that can directly metabolize oligosaccharides to produce marketable products, exceptions being production of xylanases by fungi (*Aspergillus niger*[112]) or actinomycetes[167]. Posthydrolysis options for oligosaccharide hydrolysis can be reduced to acid[57,63,109,161,168-171], or enzymatic catalyzed processes[62,161,172-175]. This process has been applied to hydrolyzates obtained from all type of materials (softwoods[109,170], hardwoods[168] and herbaceous[57,63,161,169,171]) catalyzed by H_2SO_4 (conc. 0.5-6.5% w/w) at 100.5-135°C and reaction time up to 10 h[57,109,161,168,169].

Under fully optimized conditions, sugar recovery is around 100%[57,109,161,168,169,171], as compared to standard dilute acid hydrolysis (121ºC, 4% H_2SO_4 and 60 min). Actually, this can be considered major advantage of acid posthydrolysis procedure. Other advantages associated to this process are its high-speed and low catalyst cost. An increase in hydrolyzate fermentability due to a lower inhibitory effect has also been reported[170]. On the other hand, it can induce significant equipment corrosion and hence higher equipment costs. Media neutralization is an absolute need, thus increasing both alkali consumption and costs associated with salts (typically gypsum) removal.

Actually, as much of hemicellulose complex structure is still present in oligosaccharides[79,176], action of several enzyme activities are usually required for complete hydrolysis. These enzymes act synergistically, since endoxylanase activity is highly dependent on the presence of debranching enzymes and vice versa[62,165,174,177-179], as well as chain length and degree of substitution[180]. Toxic/inhibitors compounds potentially present in hydrolysates can significantly reduce enzyme activity [62,181-183]. Indeed, each enzyme performance will largely depend on the type and concentration of inhibitors. Phenolic compounds are particularly toxic, once they easily form crosslinkage with proteins, inactivating them[184]. Walch[62] also reported inhibitor effect of tannins on xylanase activity. On the other hand, there are no evidences of synergism or cumulative toxic effect amongst different inhibitors simultaneously present in hydrolyzates[183].

Main advantage of enzymatic posthydrolysis over acidic process is milder operation conditions (temperature and pH), which leads to reaction media free of further sugar degradation compounds that can limit microbial performance, together with potential economical advantages related to energy savings and equipment cost. Main bottleneck is slow kinetics that may induce considerable problems for large-scale operations, besides cost of enzyme preparations.

Integration of Biomass Pretreatments in Biorefinery Concept

Numerous hydrolysis methods or combinations are available having specific advantages and disadvantages and yielding very different reaction products. Table 3

Table 3—Hemicellulosic hydrolyzates composition (g.l^{-1}) obtained from different lignocellulosic raw materials by dilute acid hydrolysis

Material	Xyl	Glc	Ara	Gal	Man	Acetic	Furfural	HMF	Phenolics
Agricultural byproducts									
Sugarcane bagasse[185]	46.0	3.0	5.0	-	-	10.0	0.60	< 0.1	-
Sugarcane bagasse[186]	18.5	1.2	1.7	-	-	6.5	< 0.1	< 0.1	-
Sugarcane bagasse[187]	26.4	5.5	2.1	-	-	5.5	< 0.5	< 0.1	-
Sugarcane bagasse[164]	18.5	5.1	-	-	-	3.7	2.0	< 0.1	-
Corn cobs[188]	35.3	3.2	4.6	-	-	3.7	0.30	< 0.1	-
Corn stover[188]	22.5	2.0	3.6	-	-	2.3	0.30	< 0.1	-
Barley bran[188]	35.6	5.9	7.1	-	-	2.4	0.30	< 0.1	-
Rice straw[189]	16.2	6.0	2.2	-	-	0.63	-	-	-
Rice straw[189]	16.4	4.4	2.4	-	-	1.4	0.41	< 0.1	-
Hardwoods									
Oak[190]	43.5	9.0	0	3.3	2.9	10.9	0.30	0.9	-
Oak+yellow poplar[191]	44.8	2.4	-	-	-	9.2	0.91	0	-
Eucalyptus[192]	30.0	1.5	2.8	3.7	1.0	10	-	-	-
Eucalyptus[193]	17.0	2.8	1.9	-	-	2.3	0.50	-	-
Eucalyptus[127]	18.0	3.6	0.6	-	-	5.2	< 0.5	-	-
Eucalyptus[194]	19.2	2.5	0.4	-	-	5.0	-	-	-
Eucalyptus[188]	17.1	1.7	2.1	-	-	5.2	0.50	< 0.1	-
Aspen[195]	12.3	0.9	-	-	-	3.1	0.14	-	0.63[a]
Aspen[195]	23.7	1.7	-	-	-	6.1	0.28	-	1.28[a]
Aspen[196]	15.3	6.3	0.9	0.8	1.2	0.6	-	-	-
Birch[197]	15.4	24.5	-	-	2.5	8.3	2.2	7.3	-
Softwoods									
Spruce[197]	3.3	39.0	-	-	12.3	3.2	2.2	7.3	-
Spruce [b, 198]	7.8	20.0	-	4.2	18.1	3.0	0.8	2.0	2.4
Douglas fir[199]	15.6	42.0	4.8	10.3	32.6	7.3	2.0	4.1	-

Xyl, xylose; Glc, glucose; Ara, arabinose; Gal, galactose; Man, mannose; HMF, hydroxymethylfurfural, [a]*p*-hydroxybenzoic acid and vanilinne; [b]majority

Table 4—Relevant enzymatic activities for the enzymatic posthydrolysis of xylooligosaccharides[200]

Enzyme	EC	Hydrolyzed linkage	Substrate	Main product
Endoxylanase	3.2.1.8	Internal β-1,4	Main chain	Oligomers
Exoxylanase	n.c.	Terminal β-1,4 (reducing end)	Main chain	Xylose, xylobiose
β-Xylosidase	3.2.1.37	Terminal β-1,4 (non-reducing end)	Oligomers	Xylose
Arabinosidase	3.2.1.55		Side groups	Arabinose
Glucuronidase	3.2.1.139		Side groups	Methylglucuronic acids
Acetyl xylan esterase	3.1.1.72	Ester bond	Side groups	Acetic acid
Feruloyl esterase	3.1.1.73	Ester bond	Side groups	Ferulic acid

n.c., Not yet classified

shows different hemicellulosic hydrolyzates composition[127,164,185-199] after dilute acid hydrolysis of LCM. Table 4 presents most important enzyme activities[200] required for hydrolysis of xylooligosaccharides obtained from hardwoods and herbaceous type materials. Fig. 2 presents a cartoon of typical polymeric composition after BP as a function of reaction pH characteristic of each pretreatment. Generally, low pH pretreatments tend to solubilize hemicellulose and render it in oligomeric form, conversely
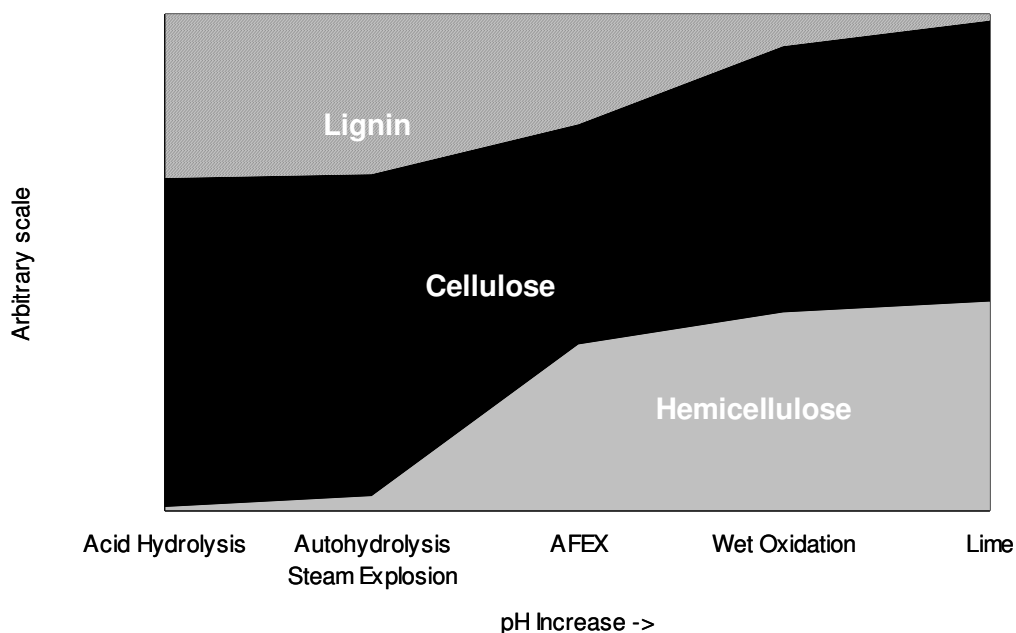
Fig. 2—Typical polymeric solids composition after biomass pretreatment as a function of reaction pH characteristic of each pretreatment
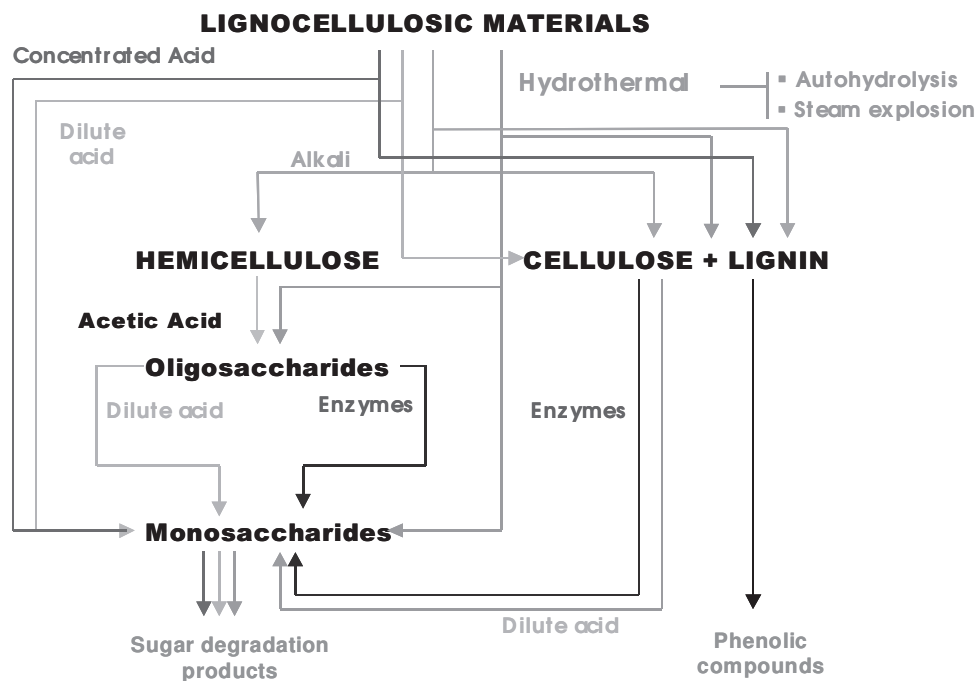
Fig. 3—Main hydrolysis processes options for biomass fractionation in a biorefinery context

to alkaline pretreatments that selectively solubilize lignin. In depth knowledge of main reactions and consequences of each pretreatment can help to better design most convenient strategy to treat a given material for a specific aim. Fig. 3 presents a simple schematic representation of most commonly used processes and their main effects on LCM.

Choice for BP technologies heavily influences cost and performance in subsequent hydrolysis and fermentation. Therefore, following criteria should be met: i) Maximize pentoses yield in non-degraded form; ii) Produce reactive (cellulosic) fibers; iii) Get hydrolysates with no significant inhibition of fermentation; iv) No extensive feedstock size reduction; v) Design reactors

of high solids loading and built from materials not expensive ones; and vi) Produce zero waste[1,2].

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