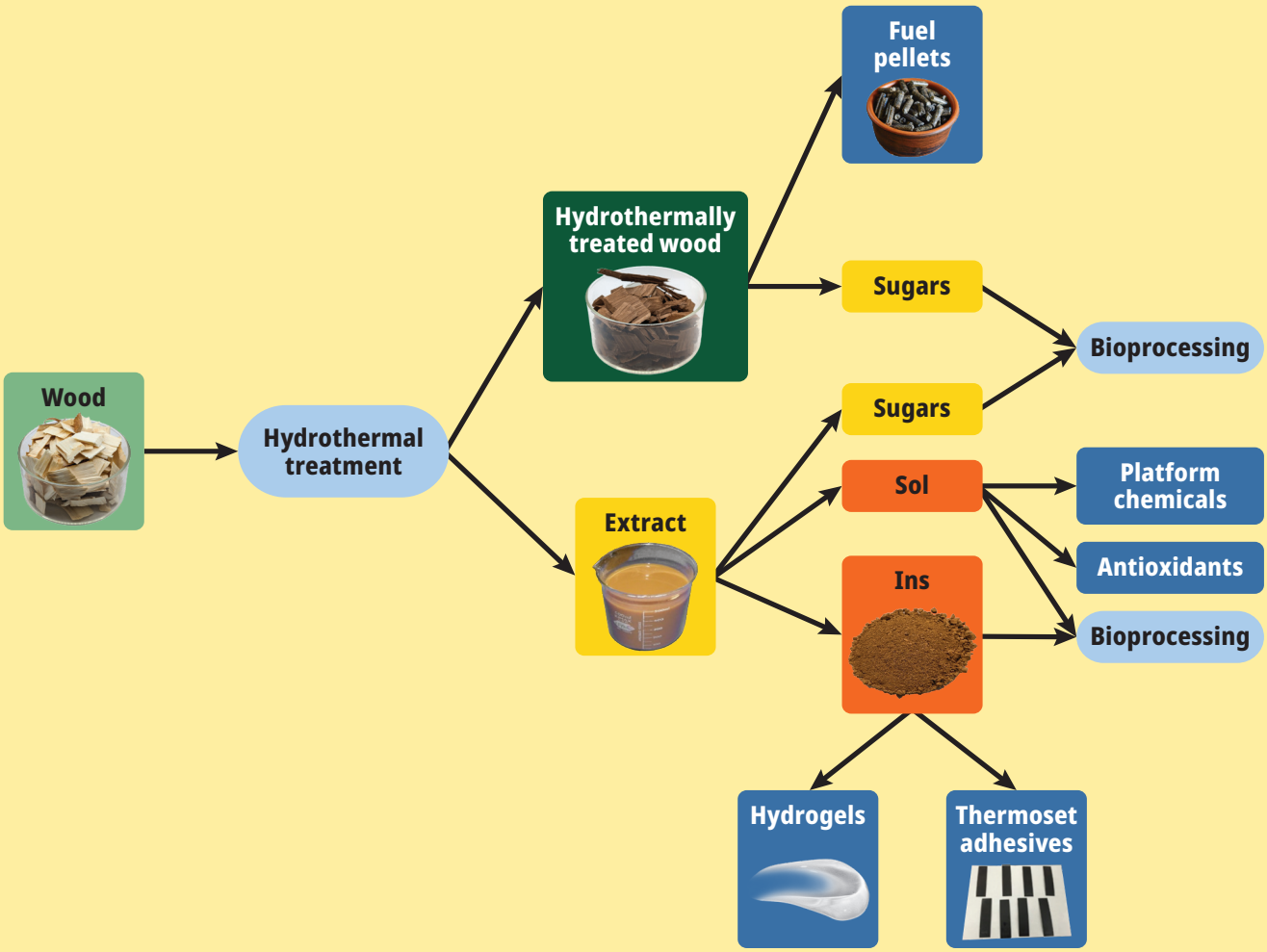
Forest Service
U.S. DEPARTMENT OF AGRICULTURE

Forest Products Laboratory | General Technical Report FPL–GTR–304 | September 2024

Hydrothermal Pretreatment of Woody Biomass and Potential Value-Added Applications of the Resulting Autohydrolysates

Derek Corbett
Aditi Nagardeolekar
Prajakta Dongre
Biljana Bujanovic



Abstract

Economic feasibility of wood-based biorefinery depends on the development of efficient, environmentally acceptable, robust, and low-cost pretreatment technologies for the production of high-value products. Hydrothermal pretreatment successfully meets these needs because it is environmentally friendly and cost-efficient. Not only does this pretreatment effectively reduce the recalcitrance of wood to further conversion, it also provides suitable byproduct streams for the production of a broad palette of value-added products. High-value coproducts have been recognized as essential to the success of the multiproduct wood-based biorefinery. In this review, the chemistry of hydrothermal pretreatment is discussed in the context of each of the major wood constituents. This is followed by an overview of the various value-added coproducts that can be produced from the "waste" streams of hydrothermal pretreatment including materials, nutraceuticals, pharmaceuticals, fine chemicals, and commodity chemicals. Finally, valuable products that can be extracted by hydrothermal treatment from underutilized bark and knotwood are discussed. With this report, our aim is to motivate researchers and industry professionals to pursue innovative approaches toward a diversified portfolio of wood biorefinery products, thus advancing the bioeconomy.

Keywords: Hydrothermal pretreatment; autohydrolysis; value-added products; woody biomass; lignin; hemicelluloses; extractives

September 2024

Corbett, D.; Nagardeolekar, A.; Dongre, P.; Bujanovic, B. 2024. Hydrothermal pretreatment of woody biomass and potential value-added applications of the resulting autohydrolysates. General Technical Report FPL-GTR-304. Madison, WI: U.S. Department of Agriculture, Forest Service, Forest Products Laboratory. 38 p.
https://doi.org/10.2737/FPL-GTR-304

USDA Forest Service, Forest Products Laboratory
One Gifford Pinchot Drive, Madison, WI, USA 53726
(608) 231-9200
SM.FS.MailroomFPL@usda.gov

This and many other Forest Service research publications are available for download through TreeSearch at https://research.fs.usda.gov/treesearch/.

The Forest Products Laboratory is maintained in cooperation with the University of Wisconsin.

Contents

Hydrothermal Pretreatment of Woody Biomass and Potential Value-Added Applications of the Resulting Autohydrolysates

Derek Corbett, Senior Process Scientist
Enviva Inc., Bethesda, Maryland, USA

Aditi Nagardeolekar, Postdoctoral Research Scientist
Prajakta Dongre[1], Research Assistant
State University of New York, College of Environmental Science and Forestry, Syracuse, New York, USA

Biljana Bujanovic, Project Leader and Associate Professor Emeritus
USDA Forest Service, Forest Products Laboratory, Madison, Wisconsin, USA
State University of New York, College of Environmental Science and Forestry, Syracuse, New York, USA

Introduction

Predictions as to the worldwide available quantity of lignocellulosic biomass (LCB) vary considerably. Estimates range from at least 4 billion to as much as 170 billion metric tons per year worldwide (Klass 1998, Rowell 2007). Estimates of total quantity of woody biomass available range from at least 1.75 to 75 billion metric tons per year worldwide (~43% of total LCB) (Klass 1998, Rowell 2007). In the United States, the forest-based biomass supply physically available in 2011 was estimated to be ~45 million metric dry tons per year depending on market economics and a feasible increase to as much as 225 million metric dry tons was predicted to be achievable with longer-term industry growth (Skog and Stanturf 2011). In contrast, the U.S. Department of Energy's (DOE) "Billion Ton Study" predicted a potential supply of 1.3 billion metric dry tons of forest-based woody biomass to be sustainably available by the mid-21st century (Skog and Stanturf 2011).

Nonwoody LCB types such as agricultural residues, grasses, and leaves, constitute additional potentially available biomass not included in the estimates above. Both woody and nonwoody LCB types have certain advantages. However, woody biomass is probably the best candidate for near-term implementation because it allows for year-round harvesting and supply (eliminating the need for long-term storage), it has a relatively higher density (decreasing transportation costs), it has a relatively higher lignin content (increasing combustion energy), and it has a relatively lower inorganics content (decreasing combustion ash and reducing physical attrition during processing). Furthermore, forest growth has environmental benefits compared with traditional agriculture (substantial carbon sink and less need for fertilization) (Zhu and others 2010). It is also of paramount importance that the production of fuels, materials, and chemicals in wood-based second generation biorefineries is not in conflict with food and feed production as in the case of first generation biorefineries based on crops such as corn kernel and sugarcane. Sustainable forestry practices and efficient supply chains are essential to unlocking this potential resource in a commercially viable and environmentally responsible way (Rowell 2007). For these reasons, this review will focus on wood as the cornerstone of lignocellulosic-based second generation biorefineries.

In addition to chemical and thermochemical pathways, biochemical pathways are viewed as promising for commercial biorefineries and, in some cases, are regarded as more environmentally compliant, sustainable, and cost-effective (Bhutto and others 2017). Generally, the biochemical pathway consists of feedstock preparation–comminution, pretreatment (PT), enzymatic hydrolysis (EH), fermentation, and product separation (Bhutto and others 2017). In this design, the use of polysaccharides is emphasized, whereas biofuels may also be produced from lignin in conversion through chemical (Shu and others 2020) and biological (Chen and Wan 2017) pathways. Moreover, to take advantage of lignin as the most abundant renewable aromatic polymer, new strategies of lignin valorization through lignin-first biomass fractionation are being suggested (Renders and others 2017). This is in contrast to

[1] Current affiliation: Research Associate, Department of Chemistry and Wisconsin Energy Institute, University of Wisconsin-Madison, Madison, Wisconsin, USA. Also served as a volunteer at USDA Forest Service, Forest Products Laboratory.

the use of lignin as a (low-value) fuel, which is currently the main use of lignin produced by the chemical pulp industry (mostly kraft lignin). This review, however, concentrates on potential opportunities to diversify the product portfolio of wood-based biorefineries that use cellulose to produce biofuels. In this context, select opportunities for using hemicelluloses and lignin, specifically their degradation products released from wood during hydrothermal pretreatment (HTP), are introduced. These can be separated from the spent liquor–autohydrolysate and used either as recovered (e.g., acetic acid) or after chemical (e.g., furfural from pentoses) or biological conversion (e.g., production of lactic acid by fermentation of monomeric sugars), offering a broad palette of potential value-added products (Fig. 1). Efforts toward valorization of the wood degradation products present in autohydrolysate aim to provide additional revenues to advance the implementation of biorefineries and contribute to the growing bioeconomy (Bhutto and others 2017, Bujanovic and others 2012, Devaney and Iles 2019, Ragauskas and others 2014). The reaction pathways leading to these degradation products during HTP of wood are summarized in the following sections.

Pretreatment

The goal of PT is to reduce the recalcitrance of wood by disrupting its tight composite structure to allow enzymes to access the polysaccharide–cellulose component for the release of sugars–glucose (Fig. 2). Extensive research has been performed to evaluate PTs designed for one or more of the following results: decrease the content of hemicelluloses and/or lignin, decrease cellulose crystallinity and degree of polymerization (DP), deacetylation, relocation of lignin and modification of lignin structure, and increase in both accessible surface area and porosity (Chandra and others 2007). Lignin–carbohydrate bonds (LC bonds), which play a critical role in networking wood polymers, are considered to contribute to wood recalcitrance. Nevertheless, the cleavage of LC bonds has rarely been included as a goal of PT, mainly because it is impossible to consider the cleavage of LC bonds independently from the removal of hemicelluloses and/or lignin without the use of highly specialized enzymes (Giummarella and others 2019, Martínez-Abad and others 2018).

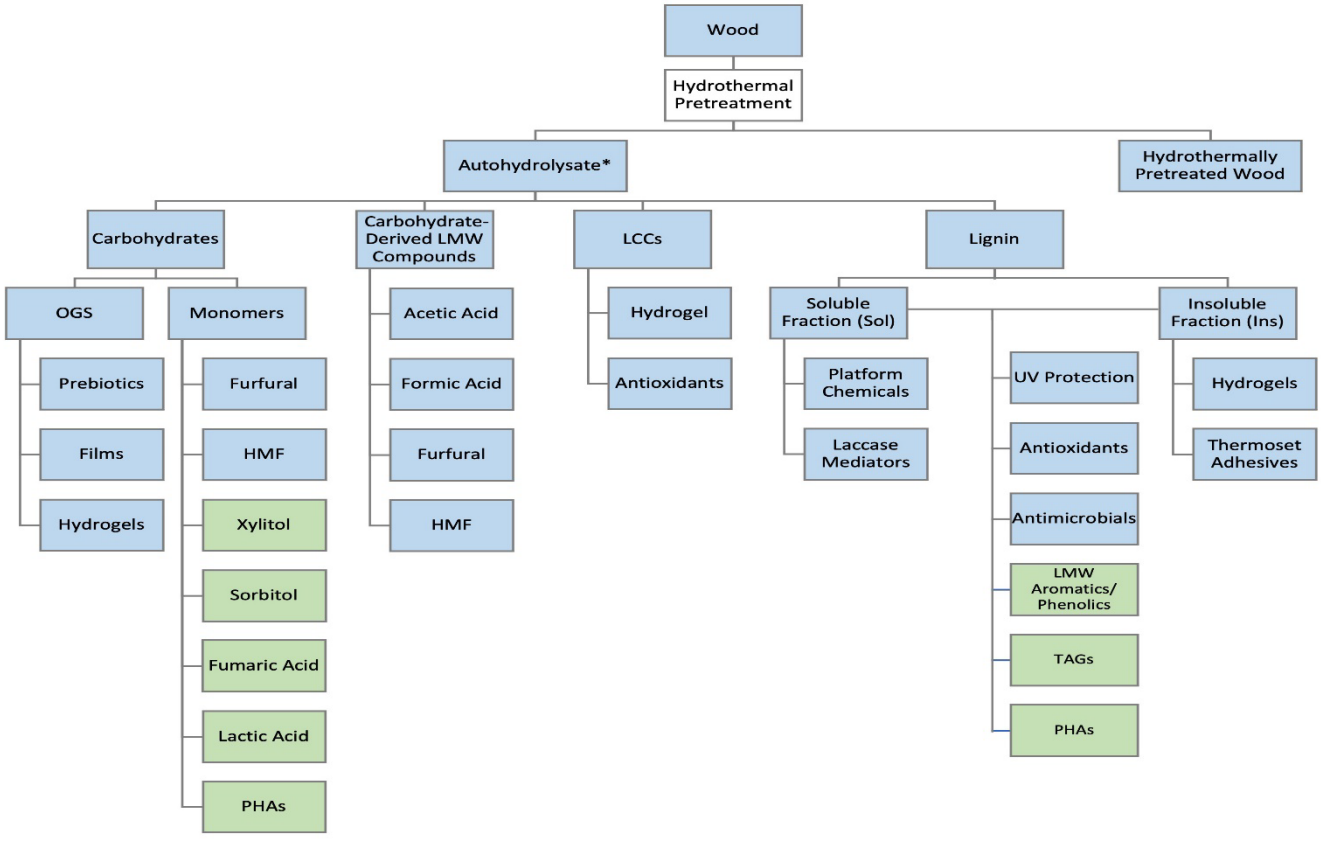


Figure 1—Potential value-added products from wood autohydrolysate. Products of biological conversion are shown in green. *For some applications, autohydrolysate may be used without treatment or separation (raw or crude). Note that this scheme is not inclusive of all potential nonfuel value-added products that have been suggested in the literature (HMF: hydroxymethylfurfural; LCC: lignin–carbohydrate complex; LMW: low molecular weight; OGS: oligosaccharides/short chain hemicelluloses; PHAs: polyhydroxyalkanoates; TAGs: triacylglycerides).

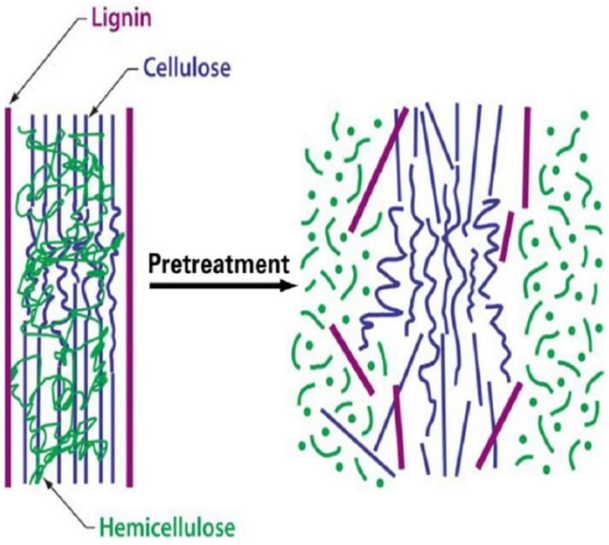


Figure 2—The goals of pretreatment: disrupt the native lignocellulosic structure, remove hemicelluloses and lignin, reduce cellulose degree of polymerization and crystallinity, and increase cellulose accessibility to enzymes (Bhatia and others (2012); Used under terms of license CC BY 2.0 (https://creativecommons.org/licenses/by/2.0/legalcode.en)).

Because sugar degradation products are notorious enzyme inhibitors and are not fermentable, PT should be conducted with minimal degradation of sugars. In this context, PT has a large impact on the success of downstream operations, including the efficiency of EH and fermentation yields. Also, PT should be cost-effective for both capital and operational requirements, with low energy demand, including low demand for comminution. Currently, PT is regarded as a major barrier to advancing second generation biorefineries because PT costs may account for anywhere from 11% to more than 40% of the total cost of biofuel production (Bhutto and others 2017, Su and others 2020).

Multiple PT technologies have been suggested, including physical, chemical, and biological methods. Hydrothermal, dilute-acid, and ammonia-based processes have already been implemented at industrial scale. Among these, autocatalyzed HTP has frequently been recommended as a green PT method; HTP using only water and heat is a clean and environmentally benign process (Beckham and others 2016, Bhutto and others 2017, Hu and Ragauskas 2012).

Through the series of reactions collectively termed autohydrolysis, HTP aims to remove hemicelluloses (mostly xylans) from biomass. Hardwoods (HWs) are more amenable to autohydrolysis than softwoods (SWs). This is attributed to the differences in the content and chemical nature of their main hemicelluloses and lignin; SWs contain (galacto)glucomannans (GGMs) and guaiacyl (G) lignin , which in the mild acid conditions of HTP are more stable and more prone to condensation, respectively, compared with the glucuronoxylans (GXs) and syringyl–guaiacyl (SG) lignin, which are predominant in HWs (Leschinsky and others 2008a, Pu and others 2013, Ye and others 2012). LC bonds have also been reported to be affected by HTP, although the extent of the impact is difficult to measure (Li and Gellerstedt 2008, Tunc and others 2010, Ye and others 2012). Although the removal of hemicelluloses–xylans has been shown to be more complete in acid PT than in HTP (99% vs. 75% removal of starting xylan in poplar (*Populus trichocarpa x deltoides*) (Samuel and others 2011)), HTP is favored because of some benefits. These benefits include using water only, avoiding the need for expensive alloys to resist corrosion in acid conditions (lowering cost), and reduced sugar degradation, which enables more efficient downstream processing, including EH and fermentation (Jönsson and Martín 2016). Only minimal dehydration of released monosaccharides has been observed during HTP conducted under relatively mild conditions (P-factors up to 600) (Ye and others 2012).

Hydrothermal Pretreatment

Autohydrolysis is initiated by deacetylation of hemicelluloses leading to a decrease in pH. Subsequently, acid-labile glycosidic bonds are cleaved (Fig. 3), leading to the degradation of hemicelluloses (especially xylans) and dissolution of the resulting oligosaccharides (OGS), monosaccharides, and their degradation products (furfural from pentoses and hydroxymethylfurfural (HMF) from hexoses). The extent of degradation of hemicelluloses depends on the severity of HTP (temperature and time of the process), although the removal of xylans usually exceeds 70% of the xylans originally present in HWs (Amidon and others 2008):

$$S_O = \log R_0, R_0 = \int_{t_0}^{t} e^{\left(\frac{T_r - T_{ref}}{w}\right)} dt \quad (1)$$

where t is the reaction time (min), T_r is the reaction temperature (°C), T_{ref} is the reference temperature (most often 100 °C), and w is an empirical constant (most often 14.75 °C) (Garrote and others 2004).

$$P = \int_{t_0}^{t} e^{\left(40.48 - \frac{15{,}106}{T}\right)} dt \quad (2)$$

where t is the reaction time (h) and T is the reaction temperature (K) (Wood and others 2020).

More severe conditions may lead to almost complete removal of xylans, for example, HTP of beech at 220 °C for 15 min (Nitsos and others 2013) and HTP of eucalyptus (*Eucalyptus urophylla*) at 240 °C for 30 min (Sun and others 2014) (Fig. 4). However, HTP at higher temperatures

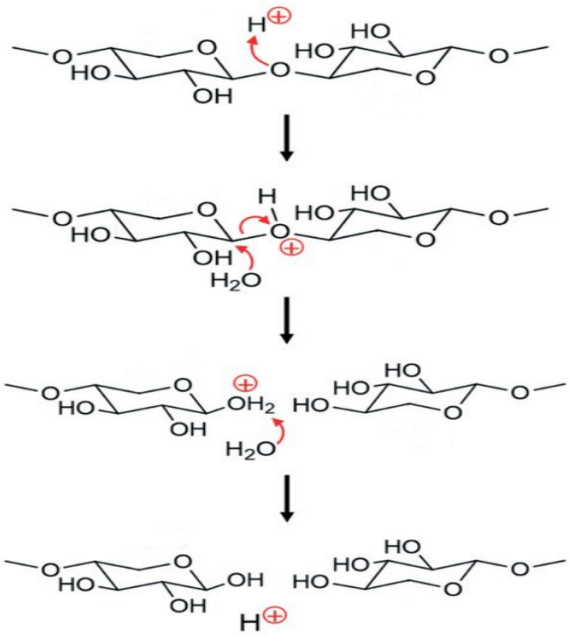


Figure 3—Hydronium ion catalyzed cleavage of the glycosidic bond between XylP units in xylan (Zhu and others 2017; Used with permission from Royal Society of Chemistry through Copyright Clearance Center, Inc.).

promotes degradation of sugars resulting in a higher concentration of furfural in the autohydrolysate (Fig. 5). For example, the furfural concentration in beech autohydrolysate was found to increase dramatically with an increase in temperature; almost a nine-fold increase from 190 to 220 °C after 15 min of HTP (Nitsos and others 2013).

Lignin is modified and dissolved to a much lesser extent than hemicelluloses during HTP. Removal of lignin is expressed as delignification degree (DD; lignin removed during HTP calculated as a percentage of the total lignin present in starting or native wood) and is typically less than 20% but may exceed 40% (Fig. 6) (Amidon and others 2008, Corbett and others 2017, Gong and Bujanovic 2014, Nagardeolekar and others 2020, Narron and others 2017, Nitsos and others 2013). Some acid-labile LC bonds are cleaved, and some may still be found in lignin recovered from autohydrolysate; e.g., 10.2 LC bonds/100 lignin aromatic rings in the case of HTP of red maple (*Acer rubrum*) (Narron and others 2017). During HTP, cellulose remains mostly unaffected (Li and Gellerstedt 2008, Nitsos and others 2013, Ye and others 2012), although it has been shown that cellulose undergoes decomposition at higher severities (Sun and others 2014).

The removal of a majority of hemicelluloses leads to an overall increase in the porosity of the hydrothermally treated wood. A steady increase in both porosity (expressed as average pore size) and surface area (BET surface area) of poplar wood chips with the progress of autohydrolysis has been demonstrated in recent work by Guo and others (2021).

This improves the accessibility of cellulose as demonstrated by the improvements in EH of wood after HTP (Romaní and others 2010, Tan and others 2020).

Evidently, the effects of HTP depend strongly on the employed conditions and, therefore, they are tunable based

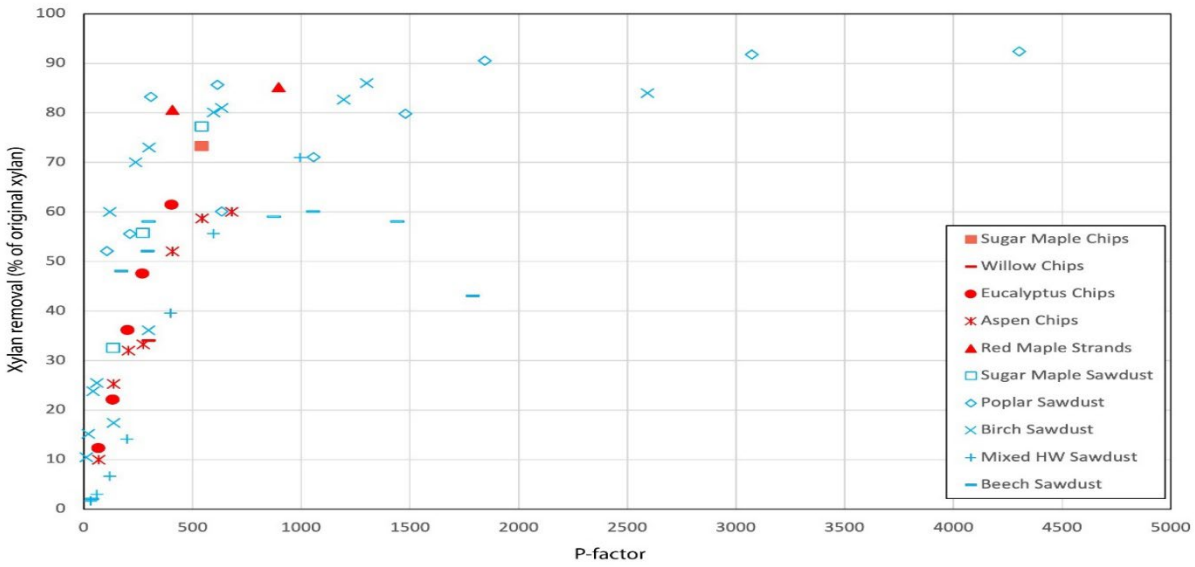


Figure 4—Removal of xylans during hydrothermal pretreatment of hardwoods. Xylan removal (%) = [(xylan in original wood (g) – xylan remaining in pretreated wood (g))/xylan in original wood (g)] × 100. Sources of data: sugar maple chips (Amidon and others 2008); willow chips (Nagardeolekar and others 2020); eucalyptus chips (Colodette and others 2011); aspen chips (Mittal and others 2009a); red maple strands (Jara 2010); sugar maple sawdust (Mittal and others 2009b); poplar sawdust (Kučerová and others 2016); birch sawdust (Kilpeläinen and others 2012, Wojtasz-Mucha and others 2021); mixed hardwood (HW) sawdust (Tunc and van Heiningen 2008); beech sawdust (Nitsos and others 2013).

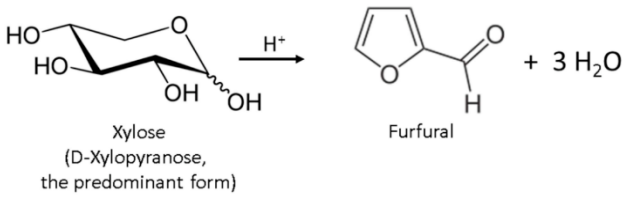


Figure 5—Dehydration of xylose to form furfural in acidic conditions. The detailed reaction pathway is not well agreed upon.

on desired outcomes. HTP may be performed under a wide range of conditions, including variations in particle size, water-to-wood ratio, temperature, time, and pressure. Conditions are most often tuned to maximize enzyme accessibility to cellulose and minimize the production of compounds inhibitory to EH. However, other factors may also be considered, such as maximizing the concentration of low DP xylooligosaccharides (XOS). HTP may be conducted in subcritical and supercritical conditions, i.e., below and beyond the critical point of water (373 °C and 22.1 MPa). To compare the effects of different HTP conditions, it is useful to express the treatment severity as a single number. The two most popular methods to express PT severity are the "severity factor" (log R_0; Eq. (1)) and the "P-factor" (Eq. (2)) (Abatzoglou and others 1992, Sixta 2006). These two factors take into account the treatment time and temperature, similar to the H-factor, which was developed to express the severity of kraft pulping and predict the content of lignin remaining in the pulp (Leschinsky and others 2008b, Sjöström 1993). The P-factor, originally developed for prehydrolysis in the pulp and paper industry, is used to express the severity of HTP and predict the content of xylans remaining in pretreated wood. It is calculated using an Arrhenius-type equation postulating hydrolysis of xylan, i.e., cleavage of the glycosidic bonds in the main xylan chain is the predominant reaction during HTP (Sixta 2006, Wood and others 2020). The combined severity factor (CSF), which includes the effect of pH, has also been proposed to predict the effects of HTP (Lee and Jeffries 2011, Lloyd and Wyman 2005):

$$\mathrm{CSF} = \log\left\{ t \times e^{\frac{T_H - T_R}{14.75}} \right\} - \mathrm{pH} \qquad (3)$$

where t is the reaction time (min), T_H is the reaction temperature (°C), T_R is the reference temperature (most often 100 °C), and pH is the acidity of the aqueous solution (Lee and Jeffries 2011).

HTP performed at temperatures between 140 and 180 °C for 30 to 180 min is often called hot water extraction (HWE) and results in the removal of 15% to 30% of wood

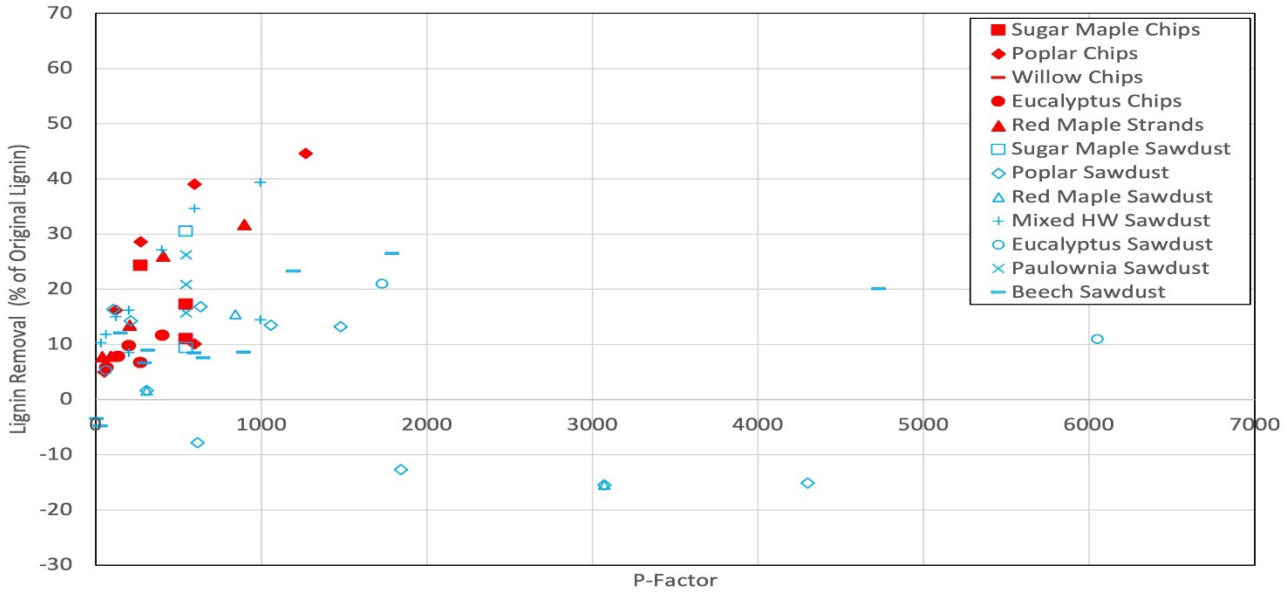


Figure 6—Delignification of hardwoods during hydrothermal pretreatment: Delignification degree vs. P-factor: Delignification degree refers to the amount of removed lignin expressed as a percentage of the amount of lignin present in native wood. Sources of data: sugar maple chips (Amidon and others 2008, Corbett and others 2017, Mittal and others 2009a); poplar chips (Wang and others 2016); willow chips (Nagardeolekar and others 2020); eucalyptus chips (Colodette and others 2011); red maple strands (Jara 2010), sugar maple sawdust (Gong and Bujanovic 2014); poplar sawdust (Kučerová and others 2016); red maple sawdust (Narron and others 2017); mixed hardwood (HW) sawdust (Tunc and others 2010, Tunc and van Heiningen 2008); eucalyptus sawdust (Gütsch and others 2012); paulownia sawdust (Gong and Bujanovic 2014); beech sawdust (Nitsos and others 2013).

constituents, hemicelluloses, lignin, hydrophilic extractives, and inorganics from HWs (Amidon and others 2008, Bujanovic and others 2012, Corbett and others 2017, Dongre and others 2015, Gong and Bujanovic 2014, Goundalkar and others 2010, Mante and others 2014, Nagardeolekar and others 2020, Wood and others 2020, Ye and others 2012).

Pressurized hot-water extraction (PHWE), also called subcritical water extraction (SWE), is a form of HPT that involves pressurization of the reaction vessel beyond the saturated vapor pressure of water (Kilpeläinen and others 2012, Kyyrö and others 2020, Leppänen and others 2011, Martínez-Abad and others 2018, Penttilä and others 2013, Rissanen and others 2014). PHWE allows for enhanced mass transfer while minimizing decomposition or condensation of reaction products designed for further processing (Martínez-Abad and others 2018). PHWE is conducted as accelerated solvent extraction (ASE) and is performed at high temperature and pressure (usually <200 °C combined with pressurized water, usually <100 bar) (Teo and others 2010). PHWE is more often performed on SWs because HTP performed on SWs has been shown to be less effective in reducing recalcitrance than when it is performed on HWs.

Removal of Hemicelluloses

The reactions of hemicelluloses, particularly of HW glucoronoxylans (GXs), are critical to effective HTP. Initially, hydronium ions (H^+ originating from water; pK_w of water decreases with an increase in temperature) catalyze the cleavage of acid-labile bonds present in GXs. This leads to partial deacetylation to form acetic acid ($pK_a = 4.76$), which is primarily responsible for the initial reduction in pH. As HTP progresses, the pH is further reduced by the release of other acidic moieties, including aromatic acids such as vanillic acid ($pK_a = 4.08$), syringic acid ($pK_a = 3.86$), and ferulic acid ($pK_a = 4.27$), and the dissociation of acidic side chains of GXs (4-O-methyl-glucuronic acid; MeGlcA; $pK_a \sim 2.8$) and pectins (α-D-galacturonic acid; $pK_a = 3.5$). Depending on the conditions, ~60% to 75% of the acetyl groups present in native HWs are cleaved (Mittal and others 2009a). Decomposition of monosaccharides has also been reported to contribute to pH reduction during HTP through the formation of formic acid ($pK_a = 3.75$). The final pH depends on the severity of treatment and the specific wood being treated but typically reaches 3.8 to 3.2 (Ye and others 2012).

With the decrease in pH, the β-(1→4)-glycosidic bonds in the xylan backbone are cleaved. Bonds cleave internally (in contrast to peeling-type reactions) through proton-catalyzed insertion of a water molecule (Fig. 3) (Ma and others 2015, Zhu and others 2017). This results in a substantial reduction in DP and molecular weight (MW) and dissolution of a polydisperse mixture of OGS (predominately XOS in HWs) accompanied by the release of XylP monomers (Xylan → XOS → XylP). The degree of branching and the pattern of acetylation/glucuronation has been confirmed to affect the hydrolyzability of GXs during HTP, with more linear (less substituted) xylan being more resistant to extraction (Tunc and van Heiningen 2008). In addition, the removal of xylans is strongly dependent on their association with lignin and cellulose (Ma and others 2015).

The removal of hemicelluloses increases with severity, and substantial quantities of xylans may be removed during HTP of HWs (featuring a high content of acetylated GXs; 15% to 30% oven-dried (OD) wood, 7Ac/10Xylp) (Sjöström 1993) (Fig. 4). It should be noted that the increase in severity promotes degradation of XOS leading to decrease in the recovery of XOS (Mittal and others 2009a). Even under the relatively mild conditions of HTP, degradation of XOS to xylose and further to furfural occurs (P-factors > 600) (Fig. 5). Likewise, glucomannans may be converted to HMF. These carbohydrate degradation products are well-known inhibitors of EH (Palmqvist and Hahn-Hägerdal 2000a,b). A decrease in the content of cellulose may also be observed (Nitsos and others 2013, Sun and others 2014). Therefore, HTP conditions must be tuned properly to minimize degradation reactions while maximizing the fermentable sugars and cellulose accessibility to further treatments (Nitsos and others 2013, Sun and others 2014, Wood and others 2020, Ye and others 2012).

The PHWE of SWs leads to efficient removal of GGMs. For example, in studies conducted on spruce groundwood (170–180 °C, 1 h), removal of >80% of GGMs was observed. These studies demonstrated that at severe conditions (180 °C, 100 min), arabinoglucuronoxylans (AGXs) undergo hydrolysis even to a higher extent than GGMs (Song and others 2008). However, at less severe conditions (PHWE, 163 °C, 60 min), AGXs were removed to a lesser extent. According to this study, the relative insolubility of xylans may be attributed to the lignin–xylan bonds, which remain stable at these conditions (Westerberg and others 2012).

Effects on Cellulose

In general, cellulose remains mostly intact during HTP at relevant conditions. However, at higher severities, cellulose losses are observed. For example, HTP for 30 min at 240 °C (P-factor 31,227) resulted in the loss of 58.7% of starting cellulose (Sun and others 2014). It has been proposed that under less severe conditions, the tightly packed nature of cellulose in the cell wall protects cellulose from degradation. In addition, the glycosidic bonds in cellulose cleave ~1,500 times slower than the glycosidic bonds in the xylan main chain (Tunc and van Heiningen 2008). Therefore, it has been proposed that glucose observed in hydrolysate liquor mainly originates from the hydrolysis of hexosans/(G)GMs (Amidon and others 2008).

Hydrothermal treatment of microfibrillated cellulose was studied at temperatures up to 150 °C. Only slight degradation occurred, if any. Rheological properties remained stable even after 24 h of treatment. However, at more severe conditions (180 °C), a lower pH was observed and a decrease in rheological properties and an increase in the production of degradation compounds were reported (Hiltunen and others 2018). Cellulose-derived compounds removed during HTP have received limited attention in the context of high-value applications because HTP is tailored to maintain cellulose yields and avoid the production of degradation compounds to maximize yields during EH (Chandra and others 2007).

Delignification

Although HTP is designed to remove hemicelluloses, it simultaneously leads to the removal of a portion of the lignin from lignocellulosics–wood (expressed as DD). The amount of lignin that colloidally dissolves under autohydrolytic conditions is highly species dependent and varies based on the employed conditions, including the severity of HTP, particle size, and water-to-biomass ratio (Fig. 6). Two distinct fractions of dissolved lignin may be recovered from the hydrolysate after HTP: a soluble (Sol) and an insoluble (Ins) fraction (Leschinsky and others 2008b, 2009). The Ins fraction, composed of lignin degradation products (LDPs) of relatively higher molecular weight (HMW), readily precipitates after cooling to room temperature or below and can be removed via decantation, centrifugation, and/or filtration. The Sol fraction remaining in the solution is composed of LDPs, hydrophilic lower molecular weight (LMW) lignin, and lignin monomers. Nevertheless, the content of lignin in the extracted wood increases because of the much higher removal of hemicelluloses.

The two lignin fractions that are quantified at the end of a two-step acid hydrolysis of extracted biomass during lignin content determination, known as Klason (acid-insoluble lignin) and acid-soluble lignin (ASL) (Hatfield and Fukushima 2005), are removed to different extents during HTP. For example, HTP of eucalyptus (*Eucalyptus globulus*) at 200 °C for 20 min led to the removal of ~80% of the starting ASL (a four-fold increase from ~20% at 150 °C for 30 min), whereas the difference in Klason lignin removal was much lower (ranged from ~2% at 150 °C for 30 min to ~9% at 200 °C for 20 min) (Gütsch and others 2012). Accordingly, the main portion of lignin removed during HTP appears to be lignin originally present in wood as ASL. Total DD increased from ~5.3% at 150 °C for 30 min to ~21% at 200 °C for 20 min after which it decreased to ~11% (200 °C, 70 min). This decrease in DD is consistent with lignin condensation reactions taking place through the formation of carbon–carbon bonds and surpassing lignin degradation reactions at high temperatures and high severities (Garrote and others 2004; Leschinsky and others 2009; Li and Gellerstedt 2008; Wang and others 2016, 2020a). It also underscores the importance of maintaining low temperatures during HTP. It has been shown that HTP of hybrid poplar resulted in a DD of 13.2% at 180 °C for 70 min (P-factor 1,478) compared with HTP performed at similar severity but at a higher temperature (195 °C for 30 min; P-factor 1,843), which resulted in a “gain” in lignin amount (negative DD of 12.7%; Fig. 6). The negative effects of high severity HTP also include a decrease in glucose content and degree of polymerization of cellulose and an increase in cellulose crystallinity (Kučerová and others 2016).

With regard to the primary goal of HTP, removal of hemicelluloses, it should be noted that with the progress of autohydrolysis, the selectivity of xylan removal has improved:

$$\text{selectivity: } \frac{\text{hemicelluloses removal (\% of original hemicelluloses)}}{\text{DD (\% of original lignin)}}$$

For example, 100 min of HTP of mixed southern HWs at 150 °C and 170 °C resulted in 8.6% and 14.5% delignification, respectively, whereas the removal of xylans reached 12.3% and 67.6% in the respective experiments, i.e., a 20 °C increase in temperature resulted in ~3.3-fold increase in selectivity (Tunc and others 2010). Because of the relatively selective removal of hemicelluloses, the content of lignin in the HTP wood increases and leads to an overall increase in the energy of combustion. For example, HTP of eucalyptus (170 °C, 60 min) resulted in an increase of higher heating value (HHV) from 17.32 to 18.34 MJ/kg (Δ5.9%) (Ge and others 2020) and in the case of HTP of willow with bark (160 °C, 2 h), the HHV increased from 19.47 to 19.94 MJ/kg (Δ 2.4%) (Nagardeolekar and others 2020). This increase aids in the use of HTP lignocellulosics–wood for the production of fuel pellets (Eisenbies and others 2019).

Apparently, PHWE results in more pronounced delignification than HTP. In the case of HWs, PHWE of poplar performed at 200 °C for 5 min resulted in a DD of 38% (Tian and others 2021). In contrast, HTP of eucalyptus at 200 °C for 20 min led to a lower DD of ~21% (Gütsch and others 2012). Likewise, lignin in SWs is more degraded during PHWE than during HTP; PHWE of spruce at 180 °C for 100 min resulted in 30% delignification (Song and others 2008), whereas HTP of pine performed at 198 °C for 60 min ended up at ~17.5% delignification (Santos and others 2018).

The lignin reactions taking place in mild acid conditions during HTP are proposed to follow two competing reaction mechanisms leading to lignin depolymerization; acidolytic or homolytic cleavage of the β-O-4 bonds (Fig. 7, Routes 1 and 3, respectively) and lignin–lignin condensation–repolymerization through $C_{2/6}$–C_{α} cross-linking (Fig. 7,

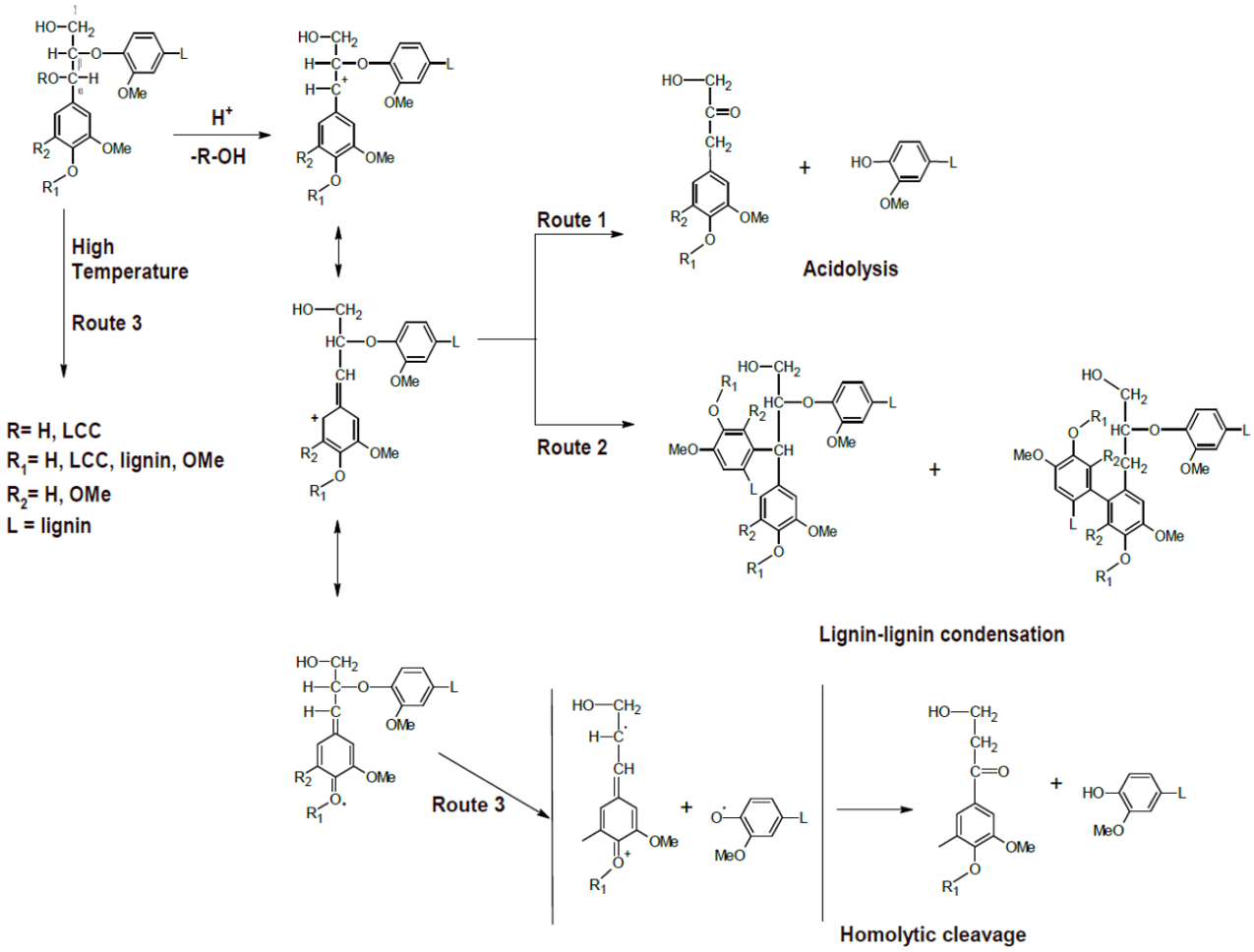


Figure 7—Proposed degradation of the β-O-4 bond between lignin C_9 units in the mildly acidic conditions of autohydrolysis.

Route 2). The chain of lignin reactions is initiated by the formation of resonance-stabilized benzyl carbocation after proton-induced elimination of water or alcohol (Fig. 7). The relative importance of these reactions depends on the conditions of HTP and the structure of lignin in wood. Lignin repolymerization, noticed mostly at higher temperatures (DD < 0 in Fig. 6), may be reduced with the addition of strong nucleophiles, such as 2-naphthol (Li and Gellerstedt 2008, Lora and Wayman 1980), *p*-hydroxybenzoic acid (*p*-HBA) (Chua and Wayman 1979), formaldehyde (Shuai and others 2016), and dimethylphloroglucinol (Pielhop and others 2016), to quench benzyl carbocations. In this regard, HTP experiments using the SWs spruce (*Picea abies*) and pine (*Pinus sylvestris*) in presence of 2-naphthol revealed a significant increase in digestibility, demonstrating that lignin repolymerization plays a major role in reducing cellulose accessibility, i.e., substantially contributes to recalcitrance (Pielhop and others 2017). Accordingly, stronger inhibition of EH was demonstrated from lignin derived from more severely pretreated HWs (Ko and others 2015a). Additionally, lignin derived from more severely pretreated HWs was shown to have higher glass transition temperature (T_g), implying that more advanced condensation reactions took place during HTP (Ko and others 2015a).

Confocal Raman microscopy analysis is a nondestructive method for qualitative monitoring and visualization of histochemical changes in biomass tissues at a subcellular level (Wang and others 2017). Compared with untreated samples of eucalyptus, HTP samples showed a gradual decrease in lignin content in all regions, although the delignification rate decreased from the secondary wall (S-wall) layers (highest lignin dissolution) to the cell corner (CC) areas (lowest lignin dissolution) (lignin content decreases in the following order: CC > compound middle lamella (CML) > S-wall). This effect may be attributed to the higher content of syringyl (S) units inherently present in lignin in the S-wall layers resulting in a more reactive lignin (Sjöström 1993). Nevertheless, in high-severity HTP (210 °C), the lignin concentration decreased significantly in both the S-wall and CC regions. The results have also shown that HTP of HWs leads to a more effective removal of lignin enriched in S units compared with original lignin in the wood (Bujanovic and others 2012, Corbett and others

2017, Goundalkar 2014). Accordingly, with an increase in severity of HTP, a decrease in S/G ratio has been revealed for lignin remaining in the wood (Wang and others 2020a).

The disruption of the cell walls with increased HTP severity has also been observed by scanning electron microscopy (SEM). The SEM images revealed the appearance of spherical droplets on the surface of the cell wall at higher temperatures. It was confirmed that the droplets were composed mainly of lignin. It has been suggested that the temperature of HTP exceeds the T_g of lignin allowing lignin moieties to migrate and then coalesce and redeposit as droplets on the surface (Wang and others 2017). However, these droplets may also originate from acid-catalyzed condensation reactions of dehydrated carbohydrates, which yield polyphenolic compounds resembling lignin (pseudo-lignin) (Shinde and others 2018).

In recent studies of autohydrolysis of poplar, the migration of lignin from internal areas and the formation of droplet clusters on the surface of the chips and also at the pit membranes of fibers have been observed (Guo and others 2021). The hydrophobicity of poplar chips was evaluated by measurements of external and internal surface contact angles (Guo and others 2021). With an increase in HTP severity, a steady increase in the external surface contact angle and a decrease in the internal surface angle were revealed, corroborating the redistribution of lignin, which is migrating from the cell wall to the surface (Guo and others 2021). In contrast, a study of three grass residues (Family Poaceae; *Miscanthus*, wheat straw, and corn stover) reported a lower external surface contact angle after HTP (Djajadi and others 2017). In general, surface hydrophobicity – wettability – has been recognized as an effective means to predict the success of EH (Ruiz and others 2020) with enzymatic digestibility typically correlated negatively with contact angle (higher digestibility at reduced hydrophobicity in accordance with lower overall lignin content) (Djajadi and others 2017).

Effects on Lignin–Carbohydrate Complex

LC bonds, which are essentially involved in creating the composite entangled structure of wood, are also proposed to contribute to the native recalcitrance of wood (Chen and others 2010). They lead to longer treatments and/or higher temperature requirements for the removal of lignin and hemicelluloses (Chen and others 2010). Accordingly, in HTP, as noticed using mixed southern HWs, lignin-free and lignin-bound xylan chains were removed at different rates; the former were removed relatively readily during the initial stages of autohydrolysis, whereas the latter (i.e., xylans incorporated into the lignin–carbohydrate complex (LCC)) were removed at a slower rate in advanced stages of autohydrolysis. Accordingly, the cleavage of xylan-bound lignin to produce soluble LCC xylan is considered the rate-limiting step (Chen and others 2010).

Differences in relative stability between the three types of LC bonds (benzyl ether (BE), γ-ester (γE), and phenyl glycoside (PG)) have been demonstrated by their extractability during HTP (Giummarella and others 2016a, 2019; Martínez-Abad and others 2018). Hot-water extracts of birch (*Betula pendula*) obtained at 160 °C for 2 h contained PG and BE linkages, whereas spruce extracts obtained at the same conditions contained only the PG linkages (Giummarella and Lawoko 2017). Further, for both species, BE and γE LC bonds were found in higher amounts in the HWE wood than in native wood, indicating their relative resistance toward extraction and/or autohydrolytic cleavage. The relative stability of γE to extraction is hypothesized to stem from their increased hydrophobicity compared with that of C_γ-hydroxymethyl groups of lignin, which decreases their water solubility.

In a separate study, PHWE was carried out using birch (170 °C, 100 bar) (Martínez-Abad and others 2018). Similar to the findings of HTP mentioned earlier (Giummarella and Lawoko 2017), PG linkages were found in the extracts obtained at an early phase of autohydrolysis. However, contrary to the previous findings, γE linkages also appeared in these extracts, whereas BE linkages were found in the extracts obtained in advanced stages of autohydrolysis. The relative ease of removal of PG linkages was also observed in fractionation studies conducted on warm water extracts (80 °C, 4 h) of ball-milled wood of spruce using a hydrophobic resin, where the retentate adsorbed on the resin was found to contain PG and BE LC bonds (Giummarella and others 2016b). Conversely, in a study of HTP of maple wood (180 °C, 40 min), all types of LC bonds were found in the adsorbate obtained after fractionation of autohydrolysates using hydrophobic resin; ~10 LC bonds (0.6 PG, 2 (C-1) BE, and 7.6 γE) per 100 aromatic rings were reported (Narron and others 2017). In a similar study, autohydrolysates of spruce (120 and 160 °C for 20 and 60 min each) were fractionated using hydrophobic resin. The resultant retentates were found to contain three types of LC linkages with ester linkages being the most prominent (Zhang and others 2019).

These reports established LCCs as a hurdle in the separation of lignin and carbohydrates from the hydrolysates, which complicates attempts at the valorization of the individual components after extraction (Corbett and others 2019, Narron and others 2017). Additionally, the difference in the extractability of the different types of LC bonds highlights the complexity of the lignocellulosic matrix and underlines the role of LCCs in mediating biomass recalcitrance. This can also lead to possible strategies for optimizing the temperature and duration of HTP to effectively tailor the composition of the residue and hydrolysate to suit the needs of particular applications.

Removal of Inorganics — De-ashing

Wood contains a small amount of inorganic material (~1%), typically measured as the ash content after combustion (Sjöström 1993). Removal of inorganic compounds is usually performed by water washing–acid leaching in mild acid wherein water-soluble metal compounds are efficiently and completely removed. HTP is also relatively effective in removing ash, to an extent that is dependent on the species, particle size, and the severity of treatment. Ash removal from ground poplar during HTP increased from 33% to 85% (based on the original mineral matter) with an increase in severity (log R_0) from 1.3 to 4.8 (0.5 mm; *P. deltoides*) (Bouchard and others 1990, 1991). During HTP of poplar chips, ash removal increased from ~22% at 150 °C for 60 min to ~47.5% at 170 °C for 60 min (Guo and others 2021). During HWE at 160 °C for 2 h, ~45% of the ash originally present in willow with bark (commercial cultivars) was removed (Nagardeolekar and others 2020).

The de-ashing effects of HTP have beneficial impacts on further processing of the residual wood. Notably, a decrease in inorganic matter alleviates issues that are common during pyrolysis of biomass rich in alkali (K) and alkaline earth metals (Ca, Mg) (may be quantified by inductively coupled plasma optical emission spectroscopy (ICP-OES)). These metals influence the thermal decomposition of biomass and affect the composition of produced bio-oils. In studies of catalytic fast pyrolysis of sugar maple (*Acer saccharum*), HWE (160 °C, 2 h) reduced char–coke formation and increased yield of benzene, toluene, and xylene (46%, 35%, and 26%, respectively) in the pyrolysis oils (Mante and others 2014). Additionally, ash removal during HTP can be expected to have beneficial impacts for the wood pelletizing industry because of reduced degradation of process metal, higher calorific value, and decreased fouling of furnaces at end-use (Eisenbies and others 2019).

Challenges with Implementing Hydrothermal Pretreatment

Although the advantages of HTP relative to other PT technologies are evident, there are limitations and challenges that must be considered and overcome. First and foremost, water and energy consumption must be reduced. High water demand could be mitigated by maximizing solids loading (>25% w/w (del Río and others 2022)) as well as various water recovery methods such as filtration. Continuous counter-current operation with a heat recovery step could mitigate high energy demand. Currently, continuous operation HTP is an unproven technology at commercially relevant scales, but the technology that enables continuous kraft pulping in the paper industry is expected to be effective for HTP. Alternatively, continuous HTP technologies that use screw conveyance reactors have been trialed at a pilot scale (Corbett and others 2019). Engineering obstacles related to mass and energy transfer must be overcome.

As has been discussed, HTP is minimally effective when applied to SWs because of the lack of acetylated xylans, a higher stability of GGMs, and the condensation tendency of G-lignin. As demonstrated in earlier studies, PHWE or SWE may be effective alternatives to the addition of chemicals for pretreatment of SWs (Kilpeläinen and others 2012, Kyyrö and others 2020, Leppänen and others 2011, Martínez-Abad and others 2018, Penttilä and others 2013, Rissanen and others 2014).

The separation of dissolved constituents after HTP is an additional challenge that must be overcome. A variety of strategies have been proposed, the nature of which depends on the desired end products. Challenges with separation are not unique to the HTP approach. The highly heterogeneous and interconnected nature of woody biomass inherently complicates efforts to utilize more than just one of its individual constituents in high-value applications (in contrast to pulping chemistry, for example, in which the fate of lignin is not a priority). Separation strategies will be touched on throughout the rest of this report in the context of the specific products or categories of chemicals being discussed.

An important consideration is that although the conditions of HTP can be "tuned" for various applications, this also means that the optimal conditions for different target applications are not the same. For example, the optimal set of HTP conditions for XOS production is not the same as the set of conditions that maximizes cellulose hydrolyzability. To optimize multiple end-uses, a combination of pretreatment technologies could be considered; e.g., mild HTP to promote XOS production followed by a delignification technology to maximize cellulose conversion to glucose (del Río and others 2022).

These challenges have hindered efforts to commercialize HTP, particularly when woody biomass is being used as a feedstock. To date, only two purportedly commercial-scale implementations of HTP have been undertaken (Inbicon (Fredericia, Denmark) and Clariant (Muttenz, Switzerland)) and both were used with wheat straw not woody biomass (Martin and others 2022). Nonetheless, HTP remains a popular strategy touted by both researchers and entrepreneurs because of its relatively low capital costs, benign environmental profile, and potential to produce a diverse range of value-added coproducts.

Potential Value-Added Applications — Carbohydrates

Materials

The carbohydrates present in wood autohydrolysates are derived almost exclusively from noncellulosic polysaccharides. Although they might be substantially

modified from their native form, many structural and functional features beneficial for value-added material applications remain intact. Depending on the starting biomass and the conditions of the HTP, these may include some degree of branching, abundant hydroxyl groups, high hydrophilicity, biodegradability, and nontoxicity (Maleki and others 2014). The large number of hydroxyl groups, in particular, provides an excellent opportunity for various chemical manipulations, including functionalization (Hansen and Plackett 2008) and cross-linking reactions (Maleki and others 2014).

Traditional preparation of hemicelluloses for material applications involves highly specific extraction techniques, e.g., alkaline, hydrogen peroxide, or organic solvent extraction and/or purification to produce a relatively homogeneous hemicellulosic mixture (Geng 2019). In contrast, hemicelluloses recovered from wood autohydrolysates are produced using only water, but they are typically less clean. In this regard, there are associated challenges with their use in material applications that must be overcome. There is substantial evidence that close interaction with lignin, including LC bonds, influences extractability of hemicelluloses even by conventional means, with both lignin content and lignin type contributing to this effect (Geng 2019). In the case of hemicelluloses obtained from wood autohydrolysates, the presence of at least some lignin is a near certainty. A notable portion of hemicelluloses in wood autohydrolysates remains covalently attached to lignin fragments via LC bonds, indicating the presence of extracted portions of LCC (Corbett and others 2019, Maleki and others 2014, Narron and others 2017). Although chromatographic, resin, or enzyme-based purification of these hemicelluloses is possible, such upgrading strategies reduce economic feasibility (Al-Rudainy and others 2019, Maleki and others 2014). It has been reported that lignin remaining in hemicelluloses negatively affects their use in various materials, including hydrogels, in which lignin can result in darkening and decreased swellability and reactivity (Al-Rudainy and others 2019). However, several studies have demonstrated that swellable hydrogels, barrier coatings, and other value-added materials can be produced from lignin-containing "crude" hemicelluloses (Hartman and others 2006, Ibn Yaich and others 2012, Maleki and others 2014, Saadatmand and others 2012, Zhu Ryberg and others 2011).

Several researchers have studied the utilization of noncellulosic polysaccharides obtained from wood hydrolysate produced via HTP of both SWs and HWs (Hartman and others 2006, Ibn Yaich and others 2012, Maleki and others 2014, Saadatmand and others 2012, Zhu Ryberg and others 2011). In one such study, hemicellulose–carboxymethyl cellulose (CMC) films were produced to serve as an oxygen barrier in packaging applications. HW autohydrolysates were upgraded via ultrafiltration, diafiltration, or precipitation to produce three different xylan-rich hemicellulose fractions. The films produced using the crudest hemicellulose fraction displayed better oxygen barrier properties than those produced from highly purified hemicelluloses, although the films produced from the more purified hemicelluloses displayed better strength properties. The improved barrier properties were attributed to strong component affinities between the CMC and hemicelluloses in the film, leading to more immobilized chain segments and a dense disordered structure (Ibn Yaich and others 2012). In addition to reducing lignin content, upgrading led to a reduction in the degree of substitution and extent of branching. As such, the role of lignin was unclear.

The production of hydrogels from acetylated GGMs recovered from two different spruce autohydrolysates (hydrolysis before sulfite pulping and steam explosion for fiberboard production) via ultrafiltration has also been reported (molecular weight cut-off (MWCO) of 1 kDa) (Maleki and others 2014). Prior to hydrogel formation, allyl modification was performed with acrylic acid and a radical initiator in DMSO. Acrylic acid accounted for between 40% and 60% of the dry weight of the resulting hydrogels. Hydrogels with various swellabilities (equilibrium swelling ratio, Q_{eq}, from 50 to 270 g water/g dry hydrogel) were produced with potential applications including absorbing materials and drying or water-retaining agents (Maleki and others 2014). Hydrogels were reported to have a light brown color, although the impact of lignin on the hydrogel properties was not discussed.

The effect of lignin on the properties of hemicellulose-based films and hydrogels has been directly studied, although these studies did not use hemicelluloses derived from wood autohydrolysates (Al-Rudainy and others 2019, Goksu and others 2008). Goksu and others (2008) successfully formed films from xylan–lignin mixtures extracted from cotton stalks in an alkaline solution. The films were produced with no cross-link or plasticizer additive. It was reported that coherent films could be formed only if lignin was still present in the film-forming solution (Goksu and others 2008). Al-Rudainy and others (2019) produced lignin-containing hydrogels from GGMs and lignin isolated from SW spent sulfite liquor. The increased presence of lignin was found to decrease mechanical strength and stiffness and increase hydrogel swellability probably because of reduced cross-linking as a result of consumption of the cross-linking agent (epichlorohydrin) by lignin (Al-Rudainy and others 2019).

Platform Chemicals

Carbohydrates present in autohydrolysates have also been explored for conversion into platform chemicals to produce high-value nonfuel products through chemical and biological conversion. Furfural and HMF, which are furan-based derivatives of pentoses and hexoses, respectively, are

regularly included in the list of top chemicals from biorefinery carbohydrates (Bozell and Petersen 2010, Werpy and others 2004). Although furfural and HMF can be produced during HTP, because they are products of acid-catalyzed dehydration of pentoses–hexoses (Fig. 5), it has been demonstrated that this conversion is minimal at P-factors <600 (Tunc and van Heiningen 2008, 2009). Chemical conversion of dissolved pentoses–xylose into furfural (Fig. 1) is one of the most common valorization strategies because of the wide range of chemicals into which furfural can be further upgraded including dihydropyran, tetrahydrofuran (THF), 2-methylfuran, furfuryl alcohol, and furoic acid (Amidon and others 2008, Barbosa and others 2014, Gairola and Smirnova 2012, Kabbour and Luque 2020, Mandalika and Runge 2012, Mariscal and others 2016, Werpy and others 2004, Wood 2016, Xing and others 2011). Furfural and its derivatives have found use in applications as far ranging as insecticides and pharmaceuticals as well as being a core moiety in various antihypertensive (ancarolol, beta-blocker), antidepressant, anxiolytic, and anti-inflammatory drugs (Espro and others 2021).

Various thermocatalytic systems have been proposed to improve the relatively poor yields and high energy requirements of traditional furfural production technologies. Most furfural production technologies require strong mineral acids and/or solid catalysts, but more complex systems have recently been proposed involving biphasic reactors, ionic liquids, and soluble organic acid catalysts (Wood 2016). The inherent reactivity of furfural poses a critical challenge to high-yield furfural production; furfural is readily lost to side reactions such as degradation and condensation with itself and xylose. Most strategies to convert xylose to furfural suffer from either poor selectivity, poor conversion, or both. However, both selectivities and conversions of xylose to furfural higher than 98% have been reported (Lessard and others 2010). To achieve these results, a biphasic plug flow reactor system with a specially modified solid zeolite catalyst was used. Nevertheless, this technology would benefit from further efforts toward improving catalyst regeneration (Lessard and others 2010).

Most studies use a pure xylose solution for the investigation and optimization of solvent-catalyst-reactor systems. The production of furfural from xylose-rich autohydrolysates poses further challenges as side reactions with dissolved constituents (LDPs and (poly)phenolics) become important (Liu and others 2018). Nonetheless, several studies have reported on the relatively successful production of furfural from autohydrolysates either as a second stage or directly from biomass in a "one-pot" strategy (Liu and others 2018, Baktash and others 2015). Liu and others (2018) studied batch, biphasic, and vapor release reaction systems that relied only on the soluble acids present in mixed southern HW autohydrolysates. Acetic acid and formic acid were present in the starting liquor at 3 to 7 g/L and 0.5 to 0.6 g/L, respectively, depending on whether autohydrolysates were autoclaved before use. The highest molar yield of furfural (73%) was achieved with the vapor release reaction system. Furfural was collected as a highly dilute aqueous solution (Liu and others 2018). Baktash and others (2015) reported 57% furfural yield from kraft prehydrolysis liquor using sulfuric acid as a catalyst. Treatment of the raw liquor with activated carbon was found to be necessary to remove dissolved lignin.

Acetic acid is inherently present in autohydrolysates produced from wood, particularly because of deacetylation of acetylated hemicelluloses. Notably, HWs contain highly acetylated xylans (~7 acetyl residues per 10 XylP units) (Sjöström 1993). Agricultural residues such as straw, stover, and fruit pits also contain acetylated xylans (Chen and others 2013, Corbett and others 2015, Yelle and others 2013). Acetic acid is a well-known inhibitor of EH and fermentation and should be removed from autohydrolysates before further processing (Ye and others 2012). After removal and concentration, acetic acid becomes a significant potential revenue source for a multiproduct biorefinery (Amidon and others 2011).

Membrane filtration is the most promising technology for the separation of acetic acid from autohydrolysates because of its relatively low cost, simplicity, and noninvasiveness (Amidon and others 2008, Liu and others 2018). Membranes in the MWCO range of reverse osmosis and nanofiltration are the most applicable for separating monomeric hexoses, pentoses, and acetic acid, which have molar masses of 180.16, 150.13, and 60.05 g/mol, respectively (Amidon and others 2008). Strategic choice of membrane MWCO could also conceivably allow for the selective removal of HMW constituents, such as lignin fragments, that could be detrimental to further processing.

Food and Nutrition

By far, the most plentiful dissolved constituents in autohydrolysates are mono- and oligosaccharides derived from hemicelluloses. Dissolved noncellulosic polysaccharides can account for 60% to 70% of the total solids in autohydrolysates (Vegas and others 2006). Near complete removal of hemicelluloses from solid biomass can be achieved. For example, Fig. 4 shows the removal of xylans vs. severity (P-factor). PT severity also determines the extent to which hemicelluloses are hydrolyzed, completely to monomers or partially to OGS (Fig. 1) (Vázquez and others 2005). These dissolved carbohydrates, including xylose, mannose, galactose, glucose, and their OGS have prospective applications as functional additives to food.

Prebiotics

Prebiotics are a class of carbohydrates that promote the growth and maintenance of beneficial bacteria in the gut.

Prebiotics have been shown to improve human health in a variety of ways, including facilitating digestion, improving immune function, improving mineral adsorption, and reducing blood glucose levels, cholesterol levels, and procarcinogenic enzymes in the gastrointestinal tract. A carbohydrate is an effective prebiotic if it is resistant to hydrolysis by human digestive enzymes but it can also be readily consumed by bacteria in the gut (Hong and others 2019, Mussatto and Mancilha 2007). OGSs have been shown to act as effective prebiotics (Hong and others 2019, Mussatto and Mancilha 2007).

The OGSs are short-chain saccharides containing 3 to ~10 monosaccharide units (although saccharides with up to 19 monosaccharide units have sometimes been classified as OGS) (Mussatto and Mancilha 2007). Disaccharides have been demonstrated to show similar characteristics to longer chain OGSs and therefore are often included as OGS (Crittenden and Playne 1996). The OGSs considered for prebiotic applications include galactooligosaccharides (GOS), fructooligosaccharides (FOS), XOS, inulin, lactulose, and polydextrose. The OGSs that can be produced from wood, including GOS (from pectins, mainly homogalacturonans (Teleman 2009)) and XOS, have been gaining popularity as potential value-added products from autohydrolysates (Fig. 1). The XOSs are particularly attractive because their sweet taste allows for applications as artificial sweeteners (Samanta and others 2015). In addition to potential applications in human health supplements, OGSs such as XOS have applications as animal feed additives (Samanta and others 2015).

The nature of the OGS dissolved during HTP depends on the hemicelluloses present in the starting biomass. Additionally, OGS solubilization and DP distribution can be tuned based on the severity of treatment (Jang and others 2021, Mittal and others 2009a, Parajó and others 2004). Up to 75% of the starting xylan in sugar maple was dissolved during HTP at 175 °C for 2 h (P-factor ~1,750), whereas a maximum XOS yield of 51% was achieved at less severe conditions (125 °C, 4.5 h, P-factor ~57) (Mittal and others 2009a). The OGSs accounting for >60% of the starting xylan in *E. globulus* were identified in autohydrolysates produced at varying conditions from 145 to 190 °C. The XOS concentration in the autohydrolysate was highly dependent on the reaction time and temperature and was maximized after ~1 h at 145 °C and only after ~5 min at 190 °C (Garrote and others 1999). In studies of the tropical HW *Eucalyptus pellita*, 77.6% of original xylan was removed in HTP at 170 °C for 50 min. The XOS accounted for 76.5% of the total sugars in the autohydrolysate (Jang and others 2021). The nonspecific hydrolysis and solubilization taking place during HTP resulted in a heterogeneous mixture of OGS (often still substituted with side-chains such as MeGlcA) and monomeric sugars in addition to noncarbohydrate contaminants, such as lignin, organic acids (acetic acid, formic acid, glucuronic acid), and various degradation products (notably furfural and HMF) (Fig. 5).

The DP of OGS is an important factor determining prebiotic activity, along with the degree of branching, linkage type, water solubility, and purity (Biedrzycka and Bielecka 2004, Mussatto and Mancilha 2007, Singh and others 2015). It has also been shown to influence downstream processing strategies. Although lower DP is preferred because of enhanced prebiotic activity, complete hydrolysis to monosaccharides must be avoided (Corbett and others 2019). Both monosaccharides and noncarbohydrate solids are considered impurities. Enzymatic treatment of raw autohydrolysates has been conducted to increase the concentration of low-DP OGS available for prebiotic production. Traditional xylanase cocktails contain both endo- and exo-xylanases and are designed to promote EH of the cellulose in pretreated biomass. However, commercial endo-xylanases are available and have been shown to effectively reduce DP of xylans in autohydrolysate without producing xylose (Biedrzycka and Bielecka 2004, Garrote and others 1999, Martínez-Abad and others 2018).

Removal of noncarbohydrate impurities from wood autohydrolysates poses additional challenges. A number of strategies have been investigated for purification of XOS, including solvent extraction, solvent precipitation, ion-exchange, flocculation, adsorption, ultrafiltration, freeze drying, and hydrophobic resin treatment (Vegas and others 2008). Often a combination of strategies is used. Recently, XOS prebiotics were produced from *E. nitens* via HTP at 195 °C, using a combination of filtration (MWCO 1 kDa, to remove LMW components) and anion exchange resin (AmberLite IRA96, DuPont, Inc., Wilmington, Delaware, USA). Processing successfully increased the substituted XOS content from 57% to 83% (percentage of nonvolatile components in solution). However, the overall yield of XOS after processing was only 38% (based on hemicelluloses in the starting wood), implying significant losses during production and/or processing (Míguez and others 2021). Hydrophobic resin (AmberLite XAD 16N, DuPont, Inc.) was used to process sweetgum autohydrolysate (180 °C, 40 min) to produce a purified XOS product. The authors reported an overall XOS yield of 48% (based on starting xylan) with only 7% of the XOS in the raw autohydrolysate lost during resin treatment (Huang and others 2016).

Isolation–purification of XOS from autohydrolysate is impeded by association of hemicelluloses (and solubilized OGS) with lignin. In recent work, it was reported that ~30% of the XOS dissolved in *Miscanthus* autohydrolysate was measurable by analytical chromatography only after complete EH to xylose (Corbett and others 2019). This result implies that this portion of XOS may feature association with lignin or has side-chain decorations that may impede purification efforts (Corbett and others 2019).

During purification using hydrophobic resin (XAD 16N), 100% of the dissolved "tied" XOS (branched or associated with lignin) was lost on the resin surface even after extensive water washing (additionally, 80% of XOS with a DP >6 was lost despite that carbohydrates are typically considered to be hydrophilic in nature). This finding further suggests that strong association between OGS and lignin fragments must be addressed during purification efforts. The significant loss of XOS during hydrophobic resin treatment that was observed in Corbett and others (2019) contradicts the reports by Huang and others (2016).

Other Food Applications

Xylitol and sorbitol are carbohydrate derivatives (C5 and C6 polyols, respectively) that have been growing in popularity as low-calorie food sweeteners (Bozell and Petersen 2010, Espro and others 2021, Mäki-Arvela and others 2011) (Fig. 1). Xylitol and sorbitol are produced by hydrogenation (Raney nickel catalyst, W.R. Grace and Company, Columbia, Maryland, USA) or biological transformation of xylose, using the yeast *Candida guilliermondii*, or glucose, using a gram-negative bacterium, *Zymomonas mobilis*, respectively (Jofre and others 2022).

Xylitol and sorbitol were included in the top 12 value-added products from biomass in the original DOE report in 2004 (Werpy and others 2004) and were confirmed in a reinstated list by Bozell and Petersen (2010). Global xylitol production is estimated to exceed 260 kt by 2027, with a compound annual growth rate (CAGR) of 5.5% (Jofre and others 2022). It is considered to be one of the sweetest polyols, with a very low calorific value and glycemic index, and as such, xylitol is used as a sugar substitute and food additive (Espro and others 2021). DuPont produces xylitol of 99% purity under the brand name Xivia by catalytic reduction of xylose derived from wood-based xylans (Espro and others 2021). Sorbitol is a sweetener, humectant, pharmaceutical excipient, and emulsifier for food, pharmaceuticals, and cosmetics. It is especially beneficial in the production of dietetic and diabetic food and beverages. Sorbitol is also the starting substrate for the microbial production of vitamin C (Espro and others 2021). Global sorbitol production is estimated to exceed 2,000 kt by 2027, with a CAGR of 3.7% (Jofre and others 2022).

Fermentation

Fermentation of the carbohydrate constituents dissolved in autohydrolysate is a thoroughly examined subject. Although fuels such as ethanol and butanol are the most cited fermentation products, a variety of nonfuel products can be produced from autohydrolysates via fermentation, including LMW products such as lactic and fumaric acid and polymers such as polyhydroxyalkanoates (PHAs) (Fig. 1).

Lactic acid (LA), which was included in the top 30 potential building-block chemicals from biomass in 2004 (Werpy and others 2004) and the top 10 chemicals from biorefinery carbohydrates in 2010 (Bozell and Petersen 2010), is an important platform chemical for several commercially relevant products, such as preservatives, acidulants, flavoring agents, lactate esters as new green solvents, and other fine and commodity chemicals, and a building block for biodegradable polymers (polylactic acid (PLA)) (Bozell and Petersen 2010, Pontes and others 2021, Walton and others 2010). LA has also been spun by various spinning techniques to produce biodegradable fibers with a broad range of potential applications (Bozell and Petersen 2010). LA is a chiral molecule; however, optical purity is necessary for the production of high-quality PLA thermoplastics. LA can be produced synthetically or through fermentation of monomeric sugars by a variety of lactic acid bacteria (LAB), e.g., *Lactobacillus delbrueckii*, some of which naturally produce optically pure LA (Pontes and others 2021), and yeasts; e.g., fermentation of xylose to LA by the yeast *Scheffersomyces stipitis* (formerly *Pichia stipites*) (Bozell and Petersen 2010).

Both pentose and hexose sugars present in wood autohydrolysate have been successfully converted to LA in laboratory studies. The monomeric sugars from mixed hardwood autohydrolysate were converted to LA at 94% yield by *Bacillus coagulans* MXL-9 (following complete hydrolysis in sulfuric acid and neutralization) (Walton and others 2010). Similarly, LA was produced at high yield from the sugars in Siberian larch (*Larix sibirica* Lebed.) autohydrolysate by the same organism (Hörhammer and others 2011). *B. coagulans* is a moderately thermophilic LAB that is capable of surviving at pH as low as 4. The conditions of optimal temperature and pH are similar to those suggested for hydrolytic enzymes, and it is thus well-suited for simultaneous saccharification and fermentation (SSF). Most strains of *B. coagulans* can utilize xylose whereas all can utilize glucose (Walton and others 2010). More recently, LA production by *Lacticaseibacillus rhamnosus* was studied using a mixture of wood species (mostly eucalyptus and pine) following HTP (Pontes and others 2021). Both separate and SSF were tested. SSF had higher volumetric productivity, but the yield was unchanged. In this scheme, both the extract and residual solids are converted during saccharification and fermentation.

Vila and others (2008) studied the production of LA from eucalyptus autohydrolysate. The autohydrolysate was subjected to secondary hydrolysis with sulfuric acid prior to fermentation of the resulting monomers by *Lactobacillus pentosus* to produce LA, which was then converted to PLA. The authors combined the pretreated eucalyptus with the produced PLA to yield a composite material that was 57% stiffer than PLA alone (Vila and others 2008).

Fumaric acid is traditionally used in the food industry as an acidulent for food and beverages but has other applications in the chemical, pharmaceutical, and cosmetic industries

(Rodríguez-López and others 2012). *E. globulus* chips were subjected to autohydrolysis followed by EH and then subjected to fermentation by *Rhizopus arrhizus* DSM 5772 to produce fumaric acid. Membrane filtration (MWCO 1 kDa) and anion exchange processing (AmberLite IRA96) along with the addition of commercial glucose (15% by mass of total resulting glucose) were found to be essential for achieving reasonable yield to fumaric acid (0.44 g/g). Attempts to ferment raw hydrolysate were relatively unsuccessful (Rodríguez-López and others 2012).

PHAs are biodegradable polyesters that exhibit properties similar to petroleum-derived thermoplastics. They can be produced by a variety of micro-organisms under the correct nutritional conditions (Pan and others 2012). The most studied PHA is the homopolymer polyhydroxybutyrate (PHB), but other PHA homopolymers and copolymers have been studied as well (e.g., polyhydroxyvalerate (PHV)). The PHA produced depends on the specific organism and nutrients present; many PHA-producing organisms can produce a variety of PHAs. Although PHAs hold promise as a biodegradable replacement for many synthetic materials, the costs associated with production impede their broader commercialization. The carbon substrate reportedly accounts for 40% to 50% of the total costs of conventional production (Dai and McDonald 2014, Pan and others 2012). Efforts to produce PHAs from autohydrolysate have been motivated by a need for a lower-cost carbon source (Pan and others 2012).

Autohydrolysate produced from hybrid poplar was used as a carbon source in a mixed microbial (activated sludge) fed-batch fermentation to produce PHB. The yield of PHB was lower than those produced using pure cultures potentially because of the presence of inhibitory compounds (Dai and McDonald 2014). In a different study, the impact on PHA production of inhibitors present in sugar maple autohydrolysates (160 °C, 2 h) that had been further treated by membrane filtration and acid hydrolysis (with 2% sulfuric acid) was examined (Pan and others 2012). Of the multiple detoxification strategies performed, overliming combined with low-temperature sterilization demonstrated the highest removal of phenolics (65% removal). However, with respect to PHB production, only a small difference in xylose consumption was observed between the control and the detoxified substrates (Pan and others 2012). In a different approach, recombinant *Escherichia coli* was engineered to produce an endoxylanase to enable the consumption of xylan directly (Salamanca-Cardona and others 2014). Results indicated that the addition of a single sugar (xylose or arabinose) to the culture medium was necessary and effective at promoting PHA production (an 18-fold increase compared with control) by allowing the accumulation of biomass-derived sugars before their uptake by microbes (Salamanca-Cardona and others 2014).

Production of a PHA with the thermoplastic properties necessary for practical use remains a challenge. PHB, particularly, is rather brittle ($T_g \approx 2$ °C, $T_m \approx 170$ °C) (Dai and McDonald 2014, Pan and others 2012, Salamanca-Cardona and others 2014). However, production of PHB-co-PHV copolymer may provide an avenue for improving the thermoplastic properties (Pan and others 2012).

Potential Value-Added Applications — Lignin

Recovery of Insoluble and Soluble Lignin

Autohydrolysates resulting from HTP contain lignin, LDPs, and various hydrophilic extractives ((poly)phenolic compounds). They appear in two phases, colloidal and soluble (Yasarla and Ramarao 2012). Colloidal lignin precipitates readily when the temperature and/or pH of wood hydrolysate is decreased. This scaling–incrustation tendency causes issues in the processing of hydrolysates (Gütsch and Sixta 2012, Leschinsky and others 2008a). The precipitate contains lignin of relatively HMW Ins fraction, whereas (poly)phenolics of LMW remain soluble (Sol fraction) (Fig. 1) (Bujanovic and others 2012; Gütsch and others 2012; Leschinsky and others 2008a, 2009). The total amount of (poly)phenolics depends on the properties of wood and HTP conditions. In HWE experiments conducted using sugar maple (P-factor 540, 160 °C), the Ins and Sol fractions accounted for ~1.8% and ~1.5% based on native wood, respectively, which combined made ~15% of the total amount of dissolved material (Bujanovic and others 2012). Similar studies on eucalyptus (P-factor 600, 170 °C) reported yields of 1.4% and 1.3% for the Ins and Sol fractions, respectively (based on native wood) (Leschinsky and others 2008a). This material presents a broad range of potential applications and represents an important avenue by which to improve the economics of biorefineries (Bujanovic and others 2012). Moreover, HMW lignin and LMW (poly)phenolics interfere with EH and fermentation and should be removed from autohydrolysate. HMW lignin poses a physical barrier restricting the access of enzymes to cellulose, and nonproductive adsorption of enzymes also occurs (SW lignin > HW lignin). Soluble LMW phenolics are toxic to cellulases and cause the disruption of microbial membrane integrity even when present at low concentrations. LMW (poly)phenolics have been widely recognized as more toxic to micro-organisms than HMW lignin–(poly)phenolics in the fermentation of both hexoses and pentoses by yeasts and bacteria. A complex structure–activity relationship and the synergistic effects of (poly)phenolics in inhibiting growth of various micro-organisms have been the focus of numerous efforts to improve the efficiency of fermentation processes (Klinke and others 2004, Ko and others 2015b, Palmqvist and Hahn-Hägerdal 2000a).

Efficient separation of (poly)phenolics is vital for the success of downstream biorefinery operations, and various separation techniques have been proposed. In bench-scale HTP experiments, the Ins fraction has been recovered by centrifugation of the crude hydrolysate (Gütsch and others 2012, Leschinsky and others 2009) or after acidification (of the hydrolysate). In HWE experiments (160 °C, 2 h), the Ins fraction was recovered from the hot-water extract after acidification to pH 2 (Corbett and others 2017). The Ins fractions recovered by centrifugation of either crude or acidified hydrolysate contained lignin as the dominant component (Leschinsky and others 2009); e.g., the Ins fraction recovered from sugar maple contained 83% lignin (Corbett and others 2017).

In pilot-scale HWE experiments performed in a 1.84-m^3 (65-ft^3) digester using willow (*Salix* spp., blend of commercial cultivars with bark) or sugar maple, acidification of hydrolysates followed by decantation (willow) or centrifugation (sugar maple) was used to recover the Ins fractions of up to 84% and 88% lignin purity, respectively (Dongre and others 2015, Elniski and others 2023, Nagardeolekar and others 2020). Ins fractions containing relatively high lignin content were also recovered by membrane separation of autohydrolysates in laboratory conditions (Bujanovic and others 2012, Goundalkar and others 2010, Tian and others 2021). For example, the Ins fraction produced by ultrafiltration (MWCO 1 kDa) of acidified sugar maple hot-water extract contained 87% lignin with xylose being the most abundant sugar among associated carbohydrates (Bujanovic and others 2012).

Although successfully used in small-scale investigations, membrane separation is known to cause issues in commercial-scale applications because of frequent fouling. However, the fouling tendency of membranes may be reduced by various pretreatments of autohydrolysate, such as centrifugation, pH adjustment, and oxidation, including the gas-phase pulsed corona discharge oxidation and laccase-catalyzed treatment of autohydrolysates (Mänttäri and others 2015). It is less problematic for membranes characterized by a lower contact angle (i.e., more hydrophilic membranes), which in turn are inferior in selectivity (retain more carbohydrates) (Koivula and others 2011, Mänttäri and others 2015, Virtanen and others 2020).

Adsorption on ion-exchange resins (Nilvebrant and others 2001), AmberLite XAD resins (Lehto and Alén 2012, Narron and others 2017, Nitzsche and others 2019, Schwartz and Lawoko 2010, Vithanage and others 2016, Zhang and others 2019), minerals such as siliceous materials, clay, and natural zeolites (Nitzsche and others 2019, Ranjan and others 2009, Soto and others 2011), and activated carbon (Kim and others 2013, Tian and others 2021) have been used to separate lignin and LDPs from carbohydrates in the spent liquor produced by HTP. One important advantage of activated carbon is its higher effective surface area compared with resins and its diverse feedstock base, which includes coal and renewable alternatives such as agriculture residues and lignin (Soto and others 2011). However, the adsorbed compounds have been found to desorb more readily from XAD resins than from activated carbon (Zhang and others 2019). In addition, in the adsorption of (poly)phenolics, activated carbon is considered less selective than XAD resins because of the simultaneous adsorption of carbohydrates. However, XAD 16N was also shown to adsorb a relatively high amount of XOS, accounting for more than 40% of the mass of adsorbate (Corbett and others 2019, Narron and others 2017). The high-temperature adsorption onto activated charcoal (HiTAC) process has been recommended for use promptly after HTP at the hydrolysate temperature to prevent lignin scaling and incrustation of the equipment (Gütsch and Sixta 2012). With respect to the use of adsorption methods for the recovery of (poly)phenolics from autohydrolysates, identification of adsorbents characterized by good adsorption and desorption properties remains the primary challenge.

Liquid–liquid extraction with apolar solvents such as ethyl acetate, ether, and methyl *tert*-butyl ether has also been used to remove phenolics and lignin–LDPs in one step (Bouchard and others 1991, Conde and others 2009, Garrote and others 2003, Goldschmid 1954, Gonzalez and others 2004, Lehto and Alén 2012, Mänttäri and others 2015, Tran and Chambers 1986, Vázquez and others 2005) or to separate the Sol fraction in sequence with the removal of the Ins fraction (Goundalkar and others 2010, Leschinsky and others 2009). This method has been used for the separation of (poly)phenolics from spent liquors produced in various biorefinery and pulping operations including steam-explosion hydrolysates of olive (*Olea europea*) tree branches (Castro and others 2008), mild-acid hydrolysates of oakwood before fermentation to 2,3-butanediol (Frazer and McCaskey 1989), and spent liquors produced in chemical delignification (Suzuki and others 2020). It has also been conducted in conjunction with an appropriate method for the removal of HMW lignin–LDPs such as centrifugation or acidification followed by decantation–centrifugation (Corbett and others 2017, Dongre and others 2015, Leschinsky and others 2009, Nagardeolekar and others 2020) or microfiltration–ultrafiltration (Bujanovic and others 2012, Goundalkar and others 2010, Tian and others 2021) because low recovery yields (Kim and others 2013) may occur if the method is conducted individually. The ethyl acetate extraction of crude hydrolysate was conducted with no loss of carbohydrates and the removal of half of the dissolved lignin in the case of birch HTP (Lehto and Alén 2012). Up to 65% of the lignin-related phenolics present in autohydrolysate of poplar (*P. deltoids*) was removed after acidification to pH 2, whereas the fraction remaining in the aqueous phase was believed to be either too hydrophilic or chemically bonded to carbohydrates–LCC (Bouchard and others 1991).

Potential Uses of Insoluble Fraction of Lignin

At a P-factor of ~540, it is expected that the total amount of Ins and Sol fractions will amount to ~3.5% of OD wood or 15% of the total material dissolved during HTP. Hence, at a biorefinery capacity of 1,000 tons of wood per day, the annual production of these two fractions may equal 10,000 tons. This is a significant amount of lignin-rich material with great potential to increase the economic returns of HW-based biorefineries. With a modest selling price of $0.66 per kilogram, this would be more than $6 million in potential revenue per year (Ye and others 2012). Recent studies have explored the use of willow Ins as an additive in willow pellets and documented numerous benefits, including significant increases in the energy content and durability and reduction in the emission of carbon monoxide as a health and safety concern in the storage of wood pellets in large quantities (Elniski and others 2023). However, to render the biorefinery focused on carbohydrate-based products more economically attractive, using lignin for higher-value products is imperative. This warrants a departure from the traditional view of considering lignin as an energy source or waste.

As one of the three pillars of wood biorefinery (PT, EH, and fermentation), PT must be conducted to facilitate EH. Because the quality of lignin is not the focus of the PT processes, it is not controlled. Therefore, in addition to the native structural diversity of lignin, the transformations induced by the processing conditions during lignin removal and recovery result in substantial variations in its structure and properties, further complicating its use for high-value products. Next, we discuss the application of the Ins fraction in thermoset and hydrogel applications (Fig. 1). The properties of the Ins faction recovered from the autohydrolysate (HTP, 160 °C, 2 h) of hardwoods are listed in Tables 1 to 4.

Table 1—Ins fraction chemical composition [lignin content (acid insoluble lignin (AISL) and acid soluble lignin (ASL)) and ash content of the Ins fraction recovered from hydrothermal pretreatment (160 °C, 2 h) based on oven-dried mass]

Lignin sample ID[a]	Lignin (%)			Ash at 900 °C (%)
	AISL	ASL	Total	
SM[b]	86.4	1.9	88.3 ± 1.48	0.29 ± 0.07
SM[c]	80.0	3.0	83.0 ± 1	—
W[b]	80.2	4.5	84.7 ± 0.8	0.20 ± 0.02
NHW[b]	78.2	4.7	82.9 ± 0.21	2.26 ± 0.03
KL[b]	51.6	19.9	71.5 ± 2.36	0.40 ± 0.01

[a]SM, sugar maple; W, willow; NHW, mixed northern hardwoods; KL, kraft lignin (given for reference).
[b]Dongre 2018.
[c]Corbett and others 2017.

Table 2—Carbohydrate composition of Ins fraction (recovered from hydrothermal pretreatment (160 °C, 2 h) based on oven-dried mass)

Lignin sample ID[a]	Carbohydrates (%)					
	Arabinan	Galactan	Glucan	Xylan	Mannan	Total
SM[b]	—	—	—	4.0	—	—
W[c]	ND[d]	ND	1.19	1.19	0.60	2.99
KL[c]	ND	ND	0.75	ND	ND	0.75

[a]SM, sugar maple; W, willow; KL, kraft lignin (given for reference).
[b]Corbett and others (2017).
[c]Method according to Corbett and others (2017).
[d]ND, not detected.

Table 3—Free phenolic hydroxyl groups (PhOH) content and syringyl–guaiacyl (S/G) ratios of the Ins fraction (Recovered from hydrothermal pretreatment (HTP) (160 °C and 2 h). Reference values of PhOH and S/G ratios from literature are also provided.)

Lignin sample ID[a]	S/G ratio		PhOH (mmol/g lignin)	
	HTP	Literature	HTP	Literature
SM	2.60[b]	2.66[d]	1.95 ± 0.06[c], 3.00[b]	0.4[d]
W	1.25[c]	1.70[e]	1.97 ± 0.02[c]	—
NHW	0.52[c]	0.78–2.12[f]	2.23 ± 0.01[c]	0.5–0.75[h]
KL	—	—	1.10 ± 0.01[g]	—

[a]SM, sugar maple; W, willow; NHW, mixed northern hardwoods; KL, kraft lignin (given for reference).
[b]Corbett and others (2017).
[c]Dongre (2018).
[d]Goundalkar (2014).
[e]Agarwal and others (2015).
[f]Milled wood lignin (Santos and others 2012, Obst and Landucci 1986).
[g]Corbett (2016).
[h]Sjöström (1993).

Table 4—Thermal properties and molecular weight distribution (number average (M_n), weight average (M_w), and polydispersity (PD)) of the Ins fraction recovered from hydrothermal pretreatment (160 °C and 2 h)

Lignin sample ID[a]	Glass transition temperature (T_g, °C)	Thermal degradation temperature (T_d, °C)	Molecular weight distribution			Reference
			M_n	M_w	PD	
W	159	358	2,186	5,962	2.7	Dongre and Bujanovic (2019)
SM	143	356	—	—	—	Dongre (2018)
	—	—	1,825	3,058	1.7	Dongre and others (2015)
	—	—	1,600	4,960	3.1	Bujanovic and others (2012)

[a]W, willow; SM, sugar maple.

Lignin-Based Thermosets

Lignin-based resins have been extensively studied in the literature. The polyphenolic nature of lignin renders it a renewable substitute for phenol in phenolic resins. Phenol-formaldehyde (PF) is a ubiquitous polymer that exhibits unparalleled mechanical strength, thermal stability, and chemical resistance in diverse applications including composites, laminates, coatings, molding compounds, friction materials, and abrasives (Pilato 2013).

Lignin recovered from HTP (160 °C, 2 h) of sugar maple or willow with bark was analyzed and used to synthesize phenol- and formaldehyde-free ecofriendly lignin–furfural resins (Dongre and Bujanovic 2019, Dongre and others 2015). This lignin contained xylose-derived carbohydrates (Table 2), which can convert to furfural in acid conditions, i.e., furfural may be produced in situ. Furfural is a good cross-linking agent for lignin because it can serve as a nontoxic substitute for formaldehyde, which is carcinogenic (Fig. 8; Bu and others 2011, Samuel and others 2013, Yang and others 2015). Glass fiber filters were impregnated with the synthesized resins and subjected to mechanical testing yielding a tensile index and Young's modulus comparable with those of commercial PF resins. The resins were further tested on kraft paper for a potential high-pressure laminate application (Dongre and Bujanovic 2019). The free phenolic hydroxyl groups (PhOH) and S/G ratio of lignin appeared to govern the mechanical properties. It was also observed that resins produced from wood lignin with molecular weights within a narrow range ($M_n \approx$ 6,000 to 9,000 Da) demonstrated good mechanical properties.

Molecular weights of resins outside this range adversely affected the tensile strength, indicating that an appropriate extent of mobility is favorable for good tensile strength and Young's modulus, i.e., excessive or limited mobility compromises the tensile properties (Dongre 2018). However, this observation is based on a limited amount of data and further research is required. Lignin–PF resins prepared from HTP-derived poplar lignin exhibited poorer bonding strength, lower formaldehyde emissions, and higher curing temperature (Yang and others 2015).

Lignin-Based Hydrogels

Hydrogels are three-dimensional networks of chemically or physically cross-linked polymers, which can interact and swell in aqueous solutions without dissolving in them. They can absorb up to thousands of times their dry weight when in contact with aqueous solutions (Hoffman 2012, Thakur and Thakur 2015). Hydrogels are a relatively recent discovery (Otto and Drahoslav 1965, Wichterle and Lím 1960) and have been an area of interest because of their versatility of application and tunable properties. Although petroleum-based polymers such as poly(acrylamide) and poly(acrylic acid) have traditionally been used for hydrogel

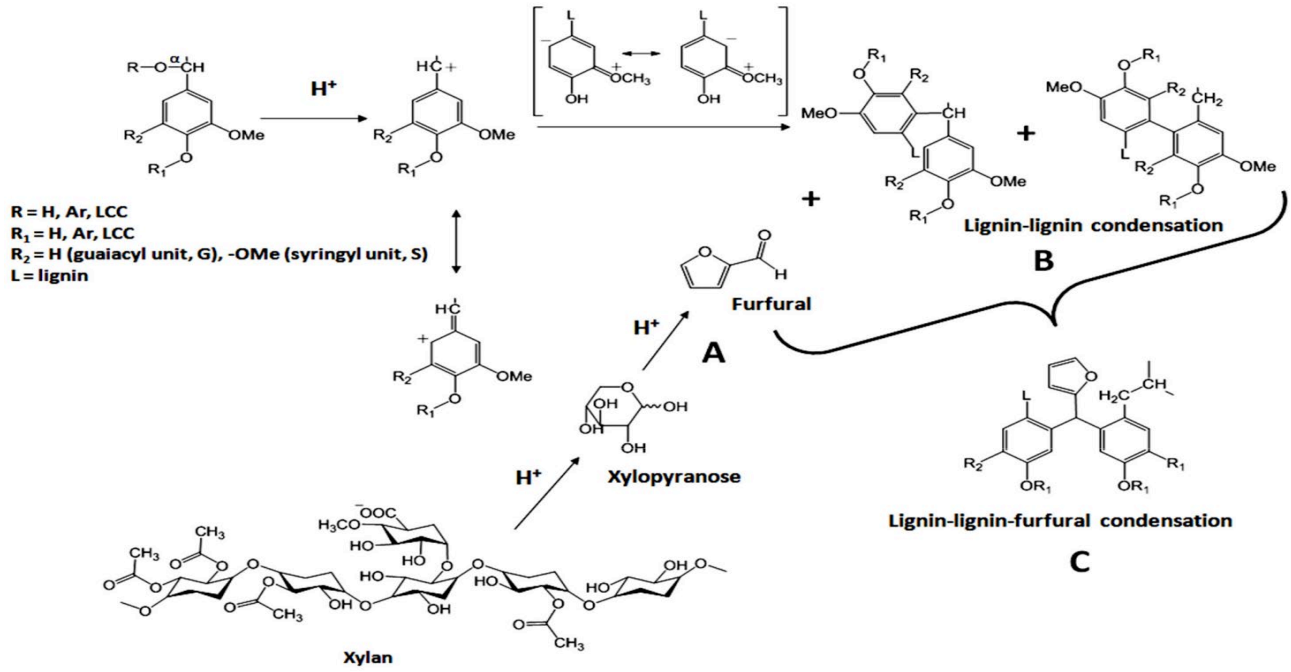


Figure 8—Proposed mechanism of lignin–lignin and lignin–furfural condensation products in acid conditions (in situ, xylan-containing lignin) (Dongre and others (2015); Used under terms of license CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/legalcode)).

synthesis, recent research has focused on using biopolymers as sustainable alternatives for synthetic polymers to produce greener hydrogels. As the most abundant aromatic biopolymer, lignin has been a popular choice for this purpose because of its unique properties (Figueiredo and others 2018, Thakur and Thakur 2015). Apart from being nontoxic, biocompatible, and biodegradable, the antioxidant (AO) and antimicrobial (AM) properties of lignin and its heterogeneous structure can offer added advantage for resisting biological attacks and extending the shelf life of resultant hydrogels (Gil-Chávez and others 2019, Witzler and others 2018). The relative hydrophobicity of lignin may also contribute to an increased shelf life compared with hydrogels produced from other common biopolymers such as cellulose (Thakur and Thakur 2015).

In one study, willow with bark was subjected to HTP (160 °C, 2 h) and the Ins fraction (W lignin) was recovered from the autohydrolysate by mild acid precipitation (Tables 1 to 4) (Nagardeolekar and others 2020). The recovered W lignin was found to be useful as a partial replacement of synthetic polymer in lignin–kaolin–acrylamide hydrogels (10.7% w/w lignin) and as the main biopolymer in lignin–poly(ethylene)glycol diglycidyl ether (PEGDGE) hydrogels (66.7% w/w lignin) (Figs. 9 to 11).

The hydrogels were found to be applicable as adsorbents of cationic pollutants in wastewater treatment and as absorbents of aqueous and nonaqueous solutions in sustained delivery applications, respectively. The PhOH groups on the W lignin served as the main polymerization sites to form the cross-linked networks in both hydrogels. These hydrogels were found to be more effective adsorbents of cations (72% of the original amount of cations removed) than those containing SW kraft lignin and hydrogels without lignin (56% and 31% of the original amount of cations removed, respectively). Further, the hydrogels containing the W lignin were found to have slightly higher swelling capacities (534%–743% of initial dry weight of hydrogel) than those containing SW kraft lignin (461%–529% of initial dry weight of hydrogel). The better sorption profile of the W lignin compared with kraft lignin was hypothesized to stem from its less condensed S/G nature (S/G = 1.25 (Dongre and others 2015)) and lower PhOH content (2.28 mmol/g (Nagardeolekar and others 2020)) compared with the more condensed G-type SW kraft lignin and its higher PhOH content (4.93 mmol/g (Nagardeolekar and others 2020)). As a result, the hydrogel network formed by kraft lignin was thought to be highly cross-linked and dense, hence less permeable to solutions than the hydrogel network formed by the W lignin. A recent study has also shown that lignin PEGDGE hydrogels synthesized from sugar maple and willow lignin Ins fractions outperformed kraft lignin PEGDGE hydrogel as carriers of chaga (*Inonotus obliquus*) mushroom extract–silver nanoparticles as a potent antioxidizing/radical quenching agent (Nagardeolekar and others 2024).

The overall beneficial effects of incorporating lignin into the hydrogel matrix were also observed in a separate study of steam explosion lignin isolated from aspen wood (Ciolacu and others 2012). In that study, lignin was used as one of the polymeric components to synthesize lignin-based hydrogels as in vitro controlled-release systems for bioactive polyphenols. Lignin was cross-linked with cellulose in an alkaline medium to synthesize a bio-based hydrogel network. Epichlorohydrin was used as the cross-linker with the hydroxyl groups on lignin and cellulose acting as the cross-linking sites to form the hydrogel matrix. The lignin to

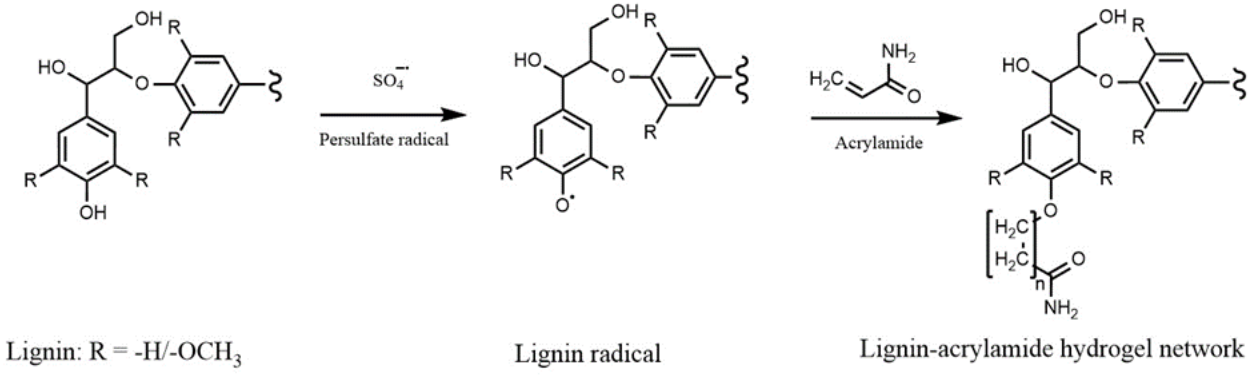


Figure 9—Proposed radical-initiated reaction for grafting of acrylamide on lignin; kaolin particles disperse into the resulting polymeric matrix.

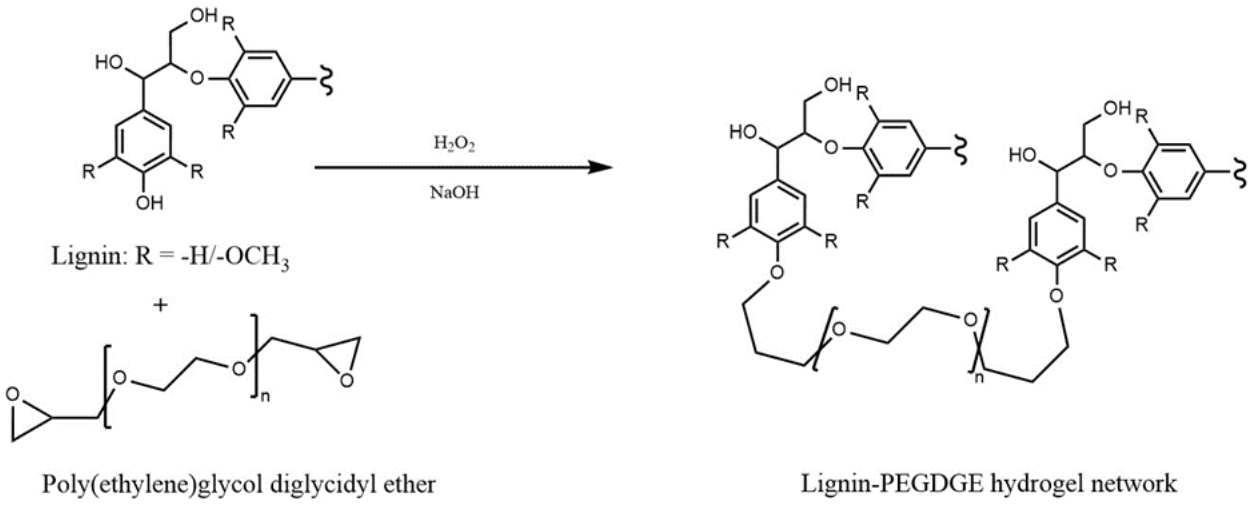


Figure 10—Proposed reaction for synthesis of lignin-poly(ethylene)glycol diglycidyl ether (PEGDGE) hydrogels.

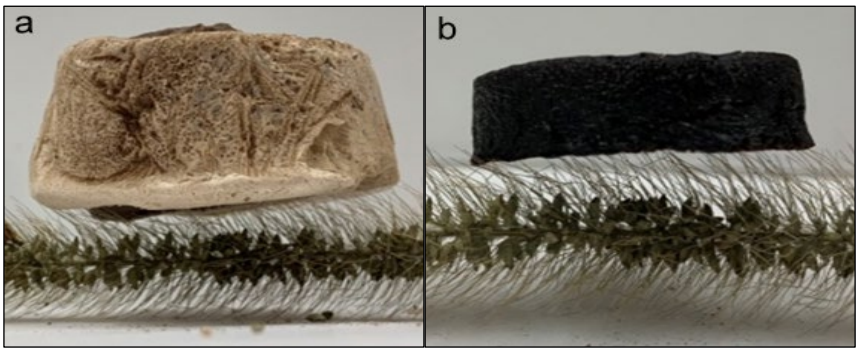


Figure 11—Lightweight and low-density (ρ ≤ 0.21 g/cm^3) hydrogels after lyophilization balanced on a spike of bristlegrass: (a) willow lignin-acrylamide-kaolin and (b) willow lignin-poly(ethylene)glycol diglycidyl ether (PEGDGE) (Nagardeolekar and others 2020; Used with permission from John Wiley and Sons through Copyright Clearance Center, Inc.).

cellulose ratio varied between 0.33 and 3. The swelling capacity of the resultant hydrogels in water/ethanol (19:1) medium was found to increase with increasing lignin content. The maximum swelling capacity after 24 h was reported to be 3,036% of initial dry weight of the hydrogel containing the highest amount of lignin. Higher lignin content was also found to result in a higher percentage release of absorbed polyphenols (Ciolacu and others 2012).

Recent research has demonstrated the suitability of the lignin-rich insoluble fraction of HW autohydrolysate (the Ins fraction; Fig. 1) to replace synthetic polymers as a renewable and environmentally friendly component of hydrogels and formaldehyde-free resins, allowing it to serve as an additional revenue source in biorefineries.

Lignin–Carbohydrate Complex Valorization Opportunities

Potential applications have been proposed for LCCs in the pharmaceutical, medical, and cosmetic fields (Zhao and others 2020). LCCs isolated from poplar (*Populus*×*euramericana*) by ball milling (total lignin 27%–32% dry weight; total sugar 61%–52% dry weight) were combined with PEGDGE in alkaline conditions to synthesize a porous hydrogel (Zhao and others 2017). The LCC–PEGDGE hydrogel was found to be useful as a biocompatible carrier for human hepatocytes in the biomedical field. LCCs isolated from pine (*Pinus* spp.) have been reported to possess antitumor, antimicrobial, antiparasitic, antiviral (including anti-HIV), and AO activities (the AO activity was synergistic with vitamin C) (Sakagami and others 2010). The authors hypothesized that the conjugation and sugar and polyphenolic structures found in LCCs were essential for biological activity. Additionally, the phenylpropanoid fraction is thought to be responsible for the antiviral activity and the carbohydrate fraction for immunopotentiation activity (Zhao and others 2020).

Potential Uses of the Low Molecular Weight Soluble Fraction of Lignin

Autohydrolysate produced at the end of HTP contains a wide range of LMW (poly)phenolic compounds: the Sol fraction (Fig. 1) (Leschinsky and others 2008a, 2009). The composition of the Sol fraction depends on the wood properties (species; origin; age; presence of bark, reaction wood, or knotwood; the heartwood/sapwood ratio) and HTP parameters, described as the severity through the effect of temperature and time (P-factor, S_O) (Abatzoglou and others 1992, Sixta 2006, Wood and others 2020). LMW (poly)phenolics encompass two categories of compounds: LDPs and (poly)phenolic extractives, e.g., flavonoids, lignans, and stilbenes (Mänttäri and others 2015, Conde and others 2013), the amount of which is highly dependent on the presence of extractive-rich tissues, such as bark, knotwood, and heartwood.

Most of the (poly)phenolic LMW compounds are toxic to downstream processes (EH and fermentation) and should be removed from the hydrolysate (Jönsson and others 1998, Kim and others 2013, Palmqvist and Hahn-Hägerdal 2000a). If separated, depending on their structure, these compounds may be considered to replace petrochemicals and promote the bioeconomy by adding value to wood-based biorefineries (Devaney and Iles 2019). A notable example is vanillin, which is a versatile platform chemical that is considered "natural" only if produced from vanilla beans and sells for ~$600/kg. Annual world production of natural vanillin is estimated at ~60 tonnes. Natural vanillin accounts for <1% of the total market volume but constitutes ~40% of the total market value. Most of the vanillin produced in the world today is labeled synthetic because it is made from crude oil via the guaiacol route (~85%) and from lignin–lignosulfonates by alkaline oxidation (~15%). The annual world production of synthetic vanillin is ~18,000 tonnes, and it sells for $12/kg (Fache and others 2016, Smolarski 2012). Vanillin produced from wood autohydrolysate may be favored over vanillin from lignosulfonates because it is sulfur-free and produced using water as the only solvent. Other high-value chemicals that may be recovered from wood autohydrolysates vary in selling price and include syringol ($33/kg) and syringaldehyde ($55/kg) from HWs (Devappa and others 2015) and ferulic acid ($85/kg) from both SWs and HWs, among other chemicals (Bugg and Rahmanpour 2015). In this context, the future of forest-based biorefineries depends on both the development of efficient separation processes and the valorization of these LMW (poly)phenolics.

Hardwoods

LMW phenolics extracted with organic solvents from autohydrolysates of HW species, such as eucalyptus, sugar maple, poplar, and willow, include numerous compounds: hydroxycinnamaldehydes (sinapaldehyde), hydroxycinnamic acids (ferulic acid, *p*-coumaric acid), hydroxycinnamyl alcohols (coniferyl alcohol), hydroxybenzoic acids (*p*-HBA, caffeic acid, vanillic acid, homovanillic acid, syringic acid, protocatechuic acid, salicylic acid, gentisic acid, gallic acid), hydroxybenzaldehydes (*p*-hydroxybenzaldehyde, vanillin, syringaldehyde), 1,2,3-trihydroxybenzene, guaiacol, syringol, *p*-hydroquinone, catechol, phenol, vanillyl alcohol, acetophenone (acetovanillone), and dihydroconiferyl alcohol. LMW polyphenolic extractives were also found in these extracts, specifically lignans, e.g., medioresinol and syringaresinol (Ares-Peón and others 2016; Devappa and others 2015; Garrote and others 2003, 2004; Gonzalez and others 2004; Goundalkar and others 2010; Jönsson and others 1998; Kim and others 2013; Vázquez and others 2005; Wang and others 2015). In addition, hydroxytyrosol, tyrosol, and oleuropein, which are bioactive phenolics present in olive oil, have been identified in autohydrolysates of olive tree pruned branches (Conde and others 2009). However, hydroxybenzaldehydes and hydroxybenzoic acids are typically the most abundant among numerous phenolics found in autohydrolysates of HW species (Ares-Peón and others 2016, Goundalkar and others 2010, Vázquez and others 2005).

Softwoods

LMW phenolics, which have been identified in organic extracts of autohydrolysates of SWs ((southern) pine, western hemlock, Norway spruce, and larch (PHWE)), include *p*-hydroxybenzyl alcohol, vanillyl alcohol, vanillin, homovanillin, vanillic acid, 3-hydroxybenzoic acid, dihydroxybenzoic acids, protocatechuic acid, dihydroconiferyl alcohol, coniferyl aldehyde, *p*-coumaric acid, ferulic acid, 1-guaiacylglycerol, and Hibbert's ketones: β-oxyconiferyl alcohol (G-CH_2COCH_2OH), vanilloyl methyl ketone (G-CO-CO-CH_3), and guaiacyl acetone (G-CH_2-CO-CH_3). Polyphenolic extractives stilbenes, lignans, flavonoids (taxifolin, quercetin), and gallic acid (from hydrolyzable tannins) have also been detected (Conde and others 2013, Goldschmid 1954, Mänttäri and others 2015, Örså and others 1997, Ravber and others 2015, Tran and Chambers 1986).

Although dissolution of nonphenolic nonpolar extractives during HTP of wood is not expected to a large degree, a limited dispersion of lipophilic extractives has also been reported. These include triglycerides, fatty acids (saturated C_9, C_{14}, C_{16}, and C_{18} and unsaturated 18:1 *cis*-9 and 18:2 *cis,cis* – 9,12 acids), resin acids (dehydroabietic acid), monoterpenoid alcohols (borneol and α-terpineol), monoterpenoid ketone (d-verbanone), steryl esters, and free sterols, which have been detected in autohydrolysates of wood (e.g., southern pine, Norway spruce, and eucalyptus) (Mänttäri and others 2015, Örså and others 1997, Tran and Chambers 1986, Vázquez and others 2005).

Many of these phenolics have been considered as promising value-added chemicals from biorefinery lignins, including

vanillin, vanillic acid, and syringaldehyde (Holladay and others 2007). Benzaldehydes may find use as platform chemicals for the production of active pharmaceutical ingredients. For example, dihydrocapsaicin and dihydrocapsiate with anti-inflammatory and anticancer activity may be produced from vanillin (Espro and others 2021) and the antibacterial drugs trimethoprim, bactrim, and biseptol may be produced from syringaldehyde (Ibrahim and others 2012). Vanillic acid has been proposed to possess numerous pharmacological effects including antihypertensive and anti-inflammatory (in ulcerative colitis) effects (Kumar and others 2011, Kim and others 2010). Paracetamol (acetaminophen; currently a petrochemical product produced from phenol) may also be produced from autohydrolysates containing *p*-HBA (*p*-HBA → *p*-hydroxybenzamide → *p*-aminophenol → paracetamol) (Ralph and others 2019). In this case, members of the *Salicaceae* family (willow, poplar) would be especially effective as feedstock because they contain lignin with *p*-hydroxybenzoates as readily hydrolyzable pendant groups (0.5%–2.9% of the dry weight of lignin) (Goacher and others 2021). Phenolics released during HTP of aspen (*P.* × *euramericana* Neva; 170 °C, 1 h) were remarkably abundant in *p*-HBA (>80%) (Wang and others 2015). In addition, *p*-HBA is used as a versatile platform chemical to produce a broad palette of products in the food, cosmetics, and pharmaceutical industries (Devappa and others 2015). Moreover, a recent study of novel PT processes has proposed the use of *p*-HBA (hydrogen bond donor) along with choline chloride (hydrogen bond acceptor) to prepare a deep eutectic solvent capable of reducing recalcitrance and facilitating enzymatic hydrolysis (>90% glucose yield in 72 h) of poplar. These results have demonstrated that wood-based biorefineries may be operated entirely with the use of renewable chemicals (Wang and others 2020b).

Antioxidizing Activity and Antimicrobial Activity

With their UV-absorption, radical scavenging, biocidal, and metal-chelating features, (poly)phenolic compounds found in trees serve protective functions against biotic (fungi, diseases) and abiotic (UV-irradiation, wounding, drought) factors (Pietarinen and others 2006a,b). Accordingly, (poly)phenolic compounds released during HTP may be considered for high-value applications that make use of these inherent bioactive properties, including antioxidizing activity (AOA) and antimicrobial activity (AMA).

(Poly)phenolic compounds/LDPs that typically appear in wood hydrolysates show a relatively high AOA and are widely considered renewable alternatives to synthetic AO such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and *tert*-butylhydroquinone (TBHQ). Although these synthetic agents are categorized as generally recognized as safe (GRAS; U.S. Federal Drug Administration Code of Federal Regulations, Title 21; April 1, 2020), suitable alternatives should be developed to adequately address the urgent need for decarbonization and to grow the bioeconomy (Devaney and Iles 2019).

Assays measuring AOA, such as radical scavenging methods [e.g., DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid))], have been conducted on phenolic compounds to elucidate the structure–AOA relationship. Various reference compounds for comparative purposes have been used such as Trolox (Hoffmann-La Roche Ltd., Basil, Switzerland) (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, water-soluble analogue of vitamin E, effective in both lipophilic and hydrophilic systems), ascorbic acid (vitamin C, 2-oxo-L-threo-hexono-1,4-lactone-2,3-enediol), and the synthetic AO agents BHT and BHA. Some general trends have been established, although the results of various tests might be contradictory because results depend on numerous factors including the method used (most importantly), the polarity of the medium (which affects the interaction of the AO with other compounds), the concentration of the AO (AOA increases with concentration), and the purity of the AO. The critical chemical feature of phenolic compounds in exhibiting radical quenching ability is the presence of nonetherified hydroxyl groups called free phenolic hydroxyl groups (PhOH) ($Ar\text{-}OH + X^{\cdot} \rightarrow AO^{\cdot} + XH$). *Ortho*-methoxy substitution of phenol improves AOA. Accordingly, guaiacol is a more potent AO agent than phenol and 2,6-dimethoxy phenolics exhibit a higher AOA than their 2-methoxy counterparts (AOA: syringol > guaiacol > phenol). Alkyl groups in the *para* position stabilize the phenoxy radical and increase AOA (AOA: 4-methyl guaiacol > guaiacol). This stabilization effect is increased with the presence of a conjugated double bond in these groups (e.g., the -CH=CH-COOH side chain in cinnamic acids provides greater efficiency than the COOH group in benzoic acids; AOA: ferulic acid > vanillic acid). Carboxyl derivatives present a lower AOA than aldehyde derivatives, i.e., benzoic acids are less potent AO agents than benzaldehydes (AOA: vanillic acid < vanillin, syringic acid < syringaldehyde). Also, hydroxyl groups in the aliphatic chain promote AOA (AOA: coniferyl alcohol ≈ 2× coniferyl aldehyde), whereas the α-carbonyl substitution in the side chain reduces AOA (AOA: guaiacol >> guaiacyl propanone-1). Monophenols are less efficient AO agents than polyphenols. Therefore, among hydroxybenzaldehydes, AOA decreases in the following order: syringaldehyde > protocatechuic aldehyde > vanillin. Among phenolic acids, AOA decreases in the following order: caffeic acid > sinapic acid > ferulic acid > *p*-coumaric acid; sinapic acid > syringic acid > vanillic acid. Experiments with lignin model compounds (LMCs) demonstrated that tetramers, trimers, and dimers are more potent AOs than monomers. Lignin studies indicated that an increase in the methoxy group content improves AOA whereas an increase in the molecular weight or polydispersity reduces AOA. The presence of nonlignin components (e.g., carbohydrates) has also been

shown to decrease the AOA of various lignins (Bortolomeazzi and others 2007, Bountagkidou and others 2010, Cuvelier and others 1992, Dizhbite 2004, Gonzalez and others 2004, Ibrahim and others 2012, Nenadis and others 2007).

The total phenol content (Folin Denis method expressed as gallic acid equivalents) and AOA (DPPH test) of ethyl acetate extracts of eucalyptus autohydrolysates produced at different severities have been measured. Although the yield of phenolics increased with severity, the AOA was higher if produced at less severe conditions. AOA of the extract produced at 180 °C was ~1.6 times higher than AOA of BHA. Eucalyptus extracts were more potent AOs than corncob extracts produced at the same relatively mild conditions. With increasing severity of HTP, AOA of eucalyptus extract decreased and at ~225 °C was equal to ~60% of BHA AOA, which was reaching that of corncob (Garrote and others 2003). Increased HTP severity has shown similar effects on the yield of phenolics and AOA of ethyl acetate extract of autohydrolysates in experiments using olive tree pruned branches. The highest AOA was observed at 170 °C, and AOA decreased at higher temperatures in contrast to the yield of phenolics, which increased with temperature (Conde and others 2009). Observed negative effects of an increase in the temperature of HTP on the AOA are in accordance with the thermal decomposition and/or reaction of extracted phenolics with other constituents of autohydrolysates, both of which reduce AOA of phenolics. Therefore, HTP at lower temperatures is expected to provide extracts with higher AOA (Moure and others 2001).

In the application as AOs, crude autohydrolysates would be more feasible and economically beneficial than individual phenolics separated from autohydrolysates. In addition, because of synergistic effects, the mixture of phenolics in autohydrolysates may show a greater AOA than individual compounds. However, antagonistic effects have also been reported (Moure and others 2001). Total phenolic content (TPC) and AOA (DPPH test) of crude autohydrolysates produced in PHWE (100 °C, 20 bar, 30 min) of larch wood fractions (sapwood, heartwood, bark, and branches) have been analyzed. The results indicated that fractions of higher TPC (heartwood and bark) presented a higher AOA in accordance with their higher contents of (poly)phenolics: vanillin, taxifolin, and quercetin (Ravber and others 2015). Crude residues obtained by freeze-drying poplar autohydrolysates produced from PHWE (180–200 °C, 10 MPa) exhibited excellent DPPH AOA, higher than both BHT and BHA. Moreover, the hydrolysate produced at 190 °C (5 min) was effective in protecting cherries from decay for 20 days, demonstrating its AMA capacity (Zhang and others 2020). This is in accordance with the observed broad AMA of various LMCs and LDPs reported earlier (Barber and others 2000, Cueva and others 2010, Rahouti and others 1999, Zemek and others 1979).

Hydroxycinnamic acids, hydroxycinnamaldehydes, and hydroxycinnamyl alcohols, all identified as LDPs in wood autohydrolysates, have been studied by measuring the minimal inhibitory concentration and minimum killing concentration against three yeasts (*Saccharomyces cerevisiae, Schizosaccharomyces pombe,* and *Sporobolomyces rosues*) and three bacteria (*E. coli* (gram negative), *Pseudomonas syringe* (gram negative), and *Bacillus subtilis* (gram positive)). It was concluded that hydroxycinnamaldehydes are the most effective AM compounds and are comparable with tolnaftate (synthetic thiocarbamate) in antifungal activity (coniferaldehyde > *p*-coumaraldehyde > sinapaldehyde) and exceed the antibacterial activity of eugenol (*p*-coumaraldehyde ≈ coniferaldehyde > sinapaldehyde) (Barber and others 2000). The results of these studies were in agreement with earlier studies of the AMA of 11 LMCs (Zemek and others 1979). The presence of OH-groups in the side chain was observed to have a negative effect, and hydroxycinnamyl alcohol and coniferyl alcohol had weakly inhibitory action against yeasts (*S. cerevisiae* and *Candida albicans*), bacteria (*E. coli, Bacillus licheniformis*, and *Micrococcus luteus*; gram positive), and fungus (*Aspergillus niger*). In addition, the superior AMA of isoeugenol compared with eugenol was emphasized and discussed in relation to the chemical structures of these two structural isomers (isoeugenol–propenyl-substituted guaiacol and eugenol–allyl guaiacol). Unlike eugenol, which lacks an extended conjugation of double bonds (Ar-$C_\alpha H_2$-$C_\beta H$=$C\gamma H_2$), isoeugenol possesses an extended conjugation of double bonds and a methyl group in the side chain (Ar-$C_\alpha H$=$C_\beta H$-$C_\gamma H_3$), which are considered critical in imparting AMA. This study also indicated that ferulic acid, syringaldehyde, and lignan-like compounds (β-β bound C_9 dimers) have AMAs that are lower than isoeugenol but corresponding to or better than that of eugenol (Zemek and others 1979).

The AMA of 13 phenolic acids including benzoic acid, 3-hydroxy benzoic acid, *p*-HBA, protocatechuic acid, and vanillic acid against bacteria (*E. coli, Pseudomonas aeruginosa, Lactobacillus* sp., and *Staphylococcus aureus*) and yeast (*C. albicans*) has been tested (Cueva and others 2010). All micro-organisms except *P. aeruginosa* showed various levels of susceptibility to the studied phenolic acids; the growth of *P. aeruginosa* was not affected by any of these phenolic acids. Gram positive *Lactiplantibacillus paraplantarum* and *S. aureus* bacteria were most susceptible to benzoic acids (*p*-HBA > 3-hydroxybenzoic acid > benzoic acid > vanillic acid > protocatechuic acid). Benzoic acids were also found more effective in inhibiting the growth of micro-organisms than phenylacetic and phenylpropionic acids (Cueva and others 2010).

It can be inferred that the efficiency of poplar PHWE autohydrolysates in protecting cherries from decay is a result of synergistic AMA of several LDPs (Zhang and others 2020) and that wood autohydrolysates should be explored as additives to impart protection against detrimental UV-irradiation, free radicals, and micro-organisms into various materials that lack resistance.

Low Molecular Weight Phenolics as Natural Laccase Mediators

LMW phenolics present in autohydrolysates may find use as laccase mediators because naturally occurring phenolics have been confirmed to promote laccase oxidation of aromatic compounds (Fig. 1). Hydroxybenzaldehydes (vanillin, syringaldehyde), acetophenones (acetovanillone, acetosyringone), hydroxycinnamic acids (*p*-coumaric acid, ferulic acid, sinapic acid), and *p*-HBA have been proposed as phenolic alternatives for synthetic laccase mediators, such as 1-hydroxybenzotriazole (HBT). Laccase-phenolic-based mediator systems have been studied for a variety of uses such as removal of pollutants and oxidation of toxic compounds (e.g., chlorinated phenols, fungicides–cyprodinil, halogenated pesticides), antibacterial agents (e.g., triclosan), pharmaceuticals (e.g., naproxene), decolorization of recalcitrant dyes, and laccase-assisted bleaching and reduction in pitch deposits during pulp production (specifically, syringaldehyde and acetosyringone). LMW phenolics meet most of the criteria of good redox mediators including LMW, high oxidation power, creation of stable radicals that do not deactivate enzyme, and regeneration without degradation. Moreover, in contrast to synthetic mediators, phenolic mediators increase the stability of laccase. Acetosyringone and syringaldehyde have been described as the most efficient laccase mediators (Cañas and Camarero 2010).

Lignin Consolidated Bioprocessing

Biocatalytic or biological funneling is related to the ability of selected micro-organisms to convert a broad range of lignin-derived aromatic compounds into a few main intermediates, such as protocatechuate; it may be decarboxylated to catechol via the expression of a protocatechuate decarboxylase (“upper pathways”). These intermediates are further metabolized by ring-opening dioxygenases, such as catechol 1,2-dioxygenase, which converts catechol to *cis,cis*-muconic acid following the β-ketoadipate pathway (“lower pathways”) (Fig. 12). In this manner, aromatic-originated carbon is included into the central carbon metabolism to provide carbon and energy (Beckham and others 2016, Ramírez-Morales and others 2021, Salvachúa and others 2015). Several aerobic and anaerobic bacteria and some oleaginous yeasts exhibit this aromatic catabolism activity (Beckham and others 2016). Some of these bacteria accumulate carbon-storage products either as triacylglycerides (TAGs) or PHAs, typically because of the deficiency of nutrients, e.g., nitrogen in excess of carbon. These carbon-storage products may be used for biofuel and biomaterial production, including higher-value medical applications. In this context, *Pseudomonas putida* is the most notable bacterium studied for the production of PHAs, including medium-length chain (mlc) PHAs from the mixture of three aromatic acids (*p*-coumaric, ferulic, and benzoic acid) (Ramírez-Morales and others 2021). Moreover, various bacteria, including *P. putida* can depolymerize HMW lignin and simultaneously catabolize LMW aromatics, paving the way to an entirely biologically based lignin conversion to chemicals and materials (Lin and others 2016, Salvachúa and others 2015). This one-pot process would be similar to the bioprocessing of polysaccharides to produce fuels, chemicals, and materials via SSF (lignin consolidated bioprocessing (L-CBP)) (Salvachúa and others 2015). L-CBP may be envisioned to work in concert with biological conversion of polysaccharides because some compounds that are intermediates in aromatic catabolism, and that are not readily produced from sugars, have the potential to become novel platform chemicals. An outstanding example of this type of compound is 2-pyrone-4,6-dicarboxylic acid (PDC), which may be produced from various LMW aromatic compounds by *Sphingobium* sp. strain SYK-6 and by mutant strain *P. putida* PpY1100. PDC is envisioned to become an important renewable platform chemical that may substitute for fossil-based terephthalic acid (precursor of polyethylene terephthalate) and has already been used to produce polyester and polyamide (Suzuki and others 2020).

White-rot fungi are known as the most efficient lignin-degrading organisms in nature, capable of converting lignin to CO_2 (Hatakka 1994). However, bacteria are more robust and tolerant to environmental changes, including pH and temperature, and are amenable to genetic manipulations. Therefore, although their lignin degradation capacity is weaker than that of white-rot fungi, bacteria are more desirable candidates to govern processes in L-CBP (Lin and others 2016, Salvachúa and others 2015). While various methods to improve bacterial aromatic catabolism are actively explored, understanding the mechanism of lignin biodegradation pathways remains an overarching goal to improve the conversion efficiency (Beckham and others 2016, Lin and others 2016, Salvachúa and others 2015).

The yield of TAG produced by *Rhodococcus opacus* growing on kraft lignin has been notably increased with the addition of exogenous laccase from *Trametes versicolor* (white-rot fungi Basidiomycota) demonstrating a concerted work of bacteria and ligninolytic enzymes. These results along with concepts encompassed in L-CBP have opened new avenues for biological valorization of lignin and LDPs, including those found in wood autohydrolysates (Beckham and others 2016, Ramírez-Morales and others 2021, Salvachúa and others 2015).

Biological methods in the valorization of lignin and LDPs to targeted LMW aromatics, TAGs, and PHAs are expected to be feasible if performed in water solution at high concentration (100–200 g/L) with a limited presence of toxic

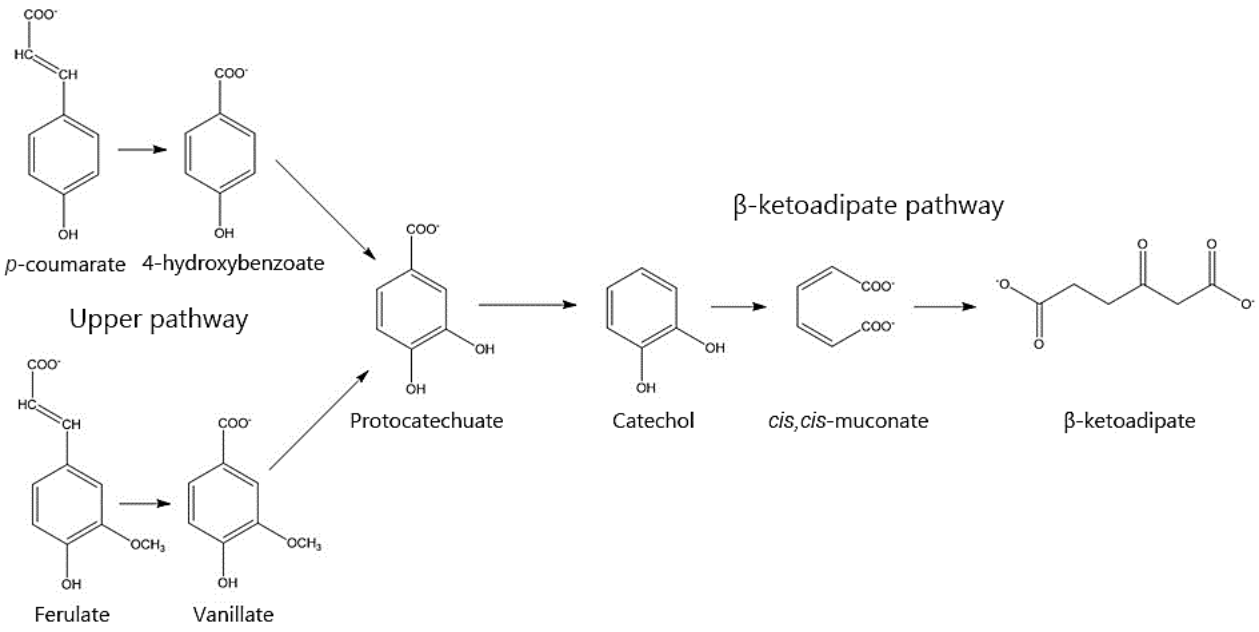


Figure 12—Biological conversion of *p*-coumarate and ferulate to β-ketoadipate.

aromatic compounds (e.g., benzoates in concentrations of ~7 g/L or higher are toxic to *P. putida*) (Beckham and others 2016). These requirements appear to be viable for HMW lignin degradation products present in autohydrolysates that have been recovered by acidification, centrifugation, and/or membrane filtration (the Ins fraction) (Bujanovic and others 2012; Corbett and others 2017; Dongre and others 2015; Elniski and others 2023; Goundalkar and others 2010; Leschinsky and others 2008a, 2009; Nagardeolekar and others 2020, 2024). However, they are more challenging to achieve for the plethora of LMW phenolic compounds (the Sol fraction) (Bujanovic and others 2012; Goundalkar and others 2010; Leschinsky and others 2008a, 2009) that are recovered by solvent extraction or adsorption followed by desorption with solvents. Their biological valorization remains dependent on the development of appropriate recovery methods.

Hydrothermal Treatment of Bark and Knotwood

The shift to a sustainable, low-carbon, circular bioeconomy requires a thorough consideration of wood waste, i.e. underutilized tree parts, such as bark and knotwood, which in fact present a wealth of valuable constituents, particularly (poly)phenols (Bianchi and others 2015; Devappa and others 2015; Dou and others 2018; García and others 2016a,b; Pietarinen and others 2006a,b). Using hydrothermal processes to extract these compounds would be environmentally desirable compared with the use of organic solvents. In addition, the relatively low severity of hydrothermal processes would help conserve the chemical structure of extracted compounds to maximize their potential utility in high-value applications.

Because of its lower content of polysaccharides and higher contents of ash and extractives, bark has conventionally been regarded as inferior in quality to xylem. On average, bark accounts for 9% to 15% of the total volume of the tree (Devappa and others 2015), but because the amount of bark depends greatly on the species, age, and location, there are examples showing that the stem volume of a tree may include 16% to 24% bark, as in the case of larch (Wagner and others 2019). In the wood-based industry, bark is mostly burned to produce heat and electricity because of its relatively high energy of combustion, or alternatively, it is used as soil amendment and ground cover. Nonetheless, bark is an abundant source of polyphenols, such as lignans, stilbenes, and flavonoids, including condensed tannins (CTs), which are oligomers built from flavan-3-ol or flavan-3,4-diol units condensed by 4 → 8 or 4 → 6 linkages (Devappa and others 2015; García and others 2016a,b; Pietarinen and others 2006a,b; Wagner and others 2019). Similarly, knots in trees are considered detrimental for pulping and pulp quality because of their shorter fibers and high content of extractives (greater consumption of pulping chemicals). Knots are also more difficult to chip because of their greater density. However, knotwood has been documented to provide a rich source of lignans (from SWs) and flavonoids (from HWs), based on a comprehensive study of 21 tree species (7 HWs and 14 SWs). Knotwood contains between 2.0% and 29.0% extractives (ASE; acetone/water 95:5 v/v) (HWs 5.7%; SWs 11.6%), which contain up to 60% lignans in SWs (*Picea glauca*, 9.2% extractives, 60% lignans) and up to 54% flavonoids in HWs (*Acacia crassicarpa*, 7.1% extractives, 54% flavonoids) (Pietarinen and others 2006a).

Extraction of (poly)phenols from bark and knotwood is usually performed using water in combination with organic solvents (e.g., acetone, ethanol, methanol, and ethyl acetate) and also using organic solvents only (e.g., ethyl acetate, petroleum ether, chloroform, and dichloromethane) (Pietarinen and others 2006a, Tanase and others 2019). Environmentally preferential hydrothermal processes (green extraction methods) are being considered to replace solvent-based processes. Studies have included experiments conducted at equilibrium pressure (HTP) and in pressurized systems (PHWE using ASE). Subsequently, hydrothermally treated bark or knotwood may be hydrolyzed and fermented to produce ethanol (Gomes and others 2021). However, the primary goal of these studies has been to recover (poly)phenols from readily available but currently underutilized bark and knotwood from the wood-based industry and to propose potential uses for these products.

CTs are a typical bark polyphenolic constituent that may account for up to ~56% of bark extractives (larch, with the extractive content between 2.9% and 11%). CTs may be used unmodified, or they can be modified to improve performance. They may be used in traditional applications such as leather tanning; adhesives; asphalt additives; dispersants; additives for tiles, floorings, and drilling muds; and pharmaceutical and alternative medicine uses, as well as in novel applications in several areas including purification technologies, AO films, cosmetics, and pharmaceuticals (García and others 2016b). In studies of bark, hot-water extracts from the bark of temperate SWs have been produced and analyzed for the content of CTs. It was concluded that larch provides yields that are most compatible with commercial sources of CTs (tropical species). The chemical structure and a higher content of carbohydrates differentiate these CTs from commercial CTs, opening new directions in applications (Bianchi and others 2015).

In addition to being a promising source of CTs, European larch bark contains (poly)phenolic compounds (benzaldehydes, benzoic acids, cinnamic acids, flavonoids, and stilbenoids) that can be extracted using PHWE (Ravber and others 2015). PHWE has been proposed for extracting (poly)phenolics from larch bark because water (at room temperature) is less efficient than methanol in the extraction of (poly)phenols that exhibit AMA (against gram positive *S. aureus*) (Wagner and others 2019). However, in ASE experiments using birch bark (*Betula pendula* and *B. pubescens*), ethanol and water were equally efficient at extracting compounds with high AOA (DPPH test). The AOA increased with increasing extraction temperature to up to 180 °C. These experiments have confirmed that in conjunction with high temperature and pressure, water acts as a good solvent for hydrophilic compounds ((poly)phenols) but is inefficient in the extraction of hydrophobic terpenoids (e.g., betulin, a pentacyclic triterpenoid with high bioactivity including AM, anti-inflammatory, anticancer, and anti-HIV properties), which are efficiently extracted with ethanol using ASE (Co and others 2009).

In contrast to PHWE, which has been recommended for extraction of (poly)phenolic antioxidants from birch and larch barks (Co and others 2009, Ravber and others 2015), mild HWE (60–100 °C) has been performed in studies using the bark of cultivated willow. The extracts contained picein (glucoside of piceol, *p*-hydroxyacetophenone) and (+)-catechin (flavan-3-ol), which are used in the food industry as antioxidants, and triandrin (C_γ glucoside of *p*-coumaryl alcohol), which has been suggested to assist in treating rheumatism. In addition, monosaccharides, mainly glucose and fructose were identified in these extracts. It was concluded that mild extraction conditions are essential to preserve the bioactivity of (poly)phenols because in more severe conditions (such as acid treatment), they undergo condensation reactions with HMF (Dou and others 2018).

PHWE has been performed under varying conditions to optimize extraction of (poly)phenols from aspen knotwood. Flavonoids, including dihydrokaempferol, naringenin (4′,5,7-trihydroxyflavanone), naringin (naringenin-7-rhamnoglucoside), and taxifolin, were identified and consisted of 4% of the dry weight of the knotwood (150 °C, 220 bar, 35 min). Detrimental effects of high temperatures on the stability of polyphenolic compounds were emphasized in this report, reaffirming previous findings. It was concluded that aspen knots present a more abundant source of naringenin than citrus fruits, which are traditionally suggested as a main source of this flavonoid with a range of bioactive properties from AMA to anti-estrogenic activity (Hartonen and others 2007).

Conclusion and Future Directions

A large variety of products can be produced from autohydrolysate (the spent liquor of wood hydrothermal pretreatment). The relatively mild conditions used in this process retain the potentially valuable structural features of hemicelluloses and lignin even after dissolution. These structural features include reactive sites for cross-linking and functionalization, aromaticity, phenolic functionalities with antioxidant properties, and structures that can be further upgraded to commodity or fine chemicals. Low-intensity pretreatments that avoid extensive degradation of these native structures preserve the opportunity to add important and much-needed value to wood biorefineries, making the industry more attractive and promoting growth of the bioeconomy while providing a more environmentally friendly process for extracting these products.

Literature Cited

Abatzoglou, N.; Chornet, E.; Belkacemi, K.; Overend, R.P. 1992. Phenomenological kinetics of complex systems: the development of a generalized severity parameter and its application to lignocellulosics fractionation. Chemical Engineering Science. 47: 1109–1122. https://doi.org/10.1016/0009-2509(92)80235-5

Agarwal, U.P.; Ralph, S.A.; Padmakshan, D.; Liu, S.; Karlen, S.D.; Foster, C.; Ralph, J. 2015. Estimation of S/G ratio in woods using 1064 nm FT-Raman spectroscopy. In: Proceedings of the 18th ISWFPC. Vienna, Austria. 2015. 333–336. http://www.fs.usda.gov/treesearch/pubs/49384. (Accessed January 14, 2022.)

Al-Rudainy, B.; Galbe, M.; Arcos Hernandez, M.; Jannasch, P.; Wallberg, O. 2019. Impact of lignin content on the properties of hemicellulose hydrogels. Polymers.1: 35. https://doi.org/10.3390/polym11010035

Amidon, T.E.; Wood, C.D.; Shupe, A.M.; Wang, Y.; Graves, M.; Liu, S. 2008. Biorefinery: conversion of woody biomass to chemicals, energy and materials. Journal of Biobased Materials and Bioenergy. 2: 100–120. https://doi.org/10.1166/jbmb.2008.302

Amidon, T.E.; Bujanovic, B.; Liu, S.; Howard, J.R. 2011. Commercializing biorefinery technology: a case for the multi-product pathway to a viable biorefinery. Forests. 2: 929–947. https://doi.org/10.3390/f2040929

Ares-Peón, I.A.; Garrote, G.; Domínguez, H.; Parajó, J.C. 2016. Phenolics production from alkaline hydrolysis of autohydrolysis liquors. CyTA Journal of Food. 14: 255–265. https://doi.org/10.1080/19476337.2015.1094516

Baktash, M.M.; Ahsan, L.; Ni, Y. 2015. Production of furfural from an industrial pre-hydrolysis liquor. Separation and Purification Technology. 149: 407–412. https://doi.org/10.1016/j.seppur.2015.06.003

Barber, M.S.; McConnell, V.S.; DeCaux, B.S. 2000. Antimicrobial intermediates of the general phenylpropanoid and lignin specific pathways. Phytochemistry. 54: 53–56. https://doi.org/10.1016/S0031-9422(00)00038-8

Barbosa, B.M.; Colodette, J.L.; Longue Júnior, D.; Gomes, F.J.B.; Martino, D.C. 2014. Preliminary studies on furfural production from lignocellulosics. Journal of Wood Chemistry and Technology. 34: 178–190. http://dx.doi.org/10.1080/02773813.2013.844167

Beckham, G.T.; Johnson, C.W.; Karp, E.M.; Salvachúa, D.; Vardon, D.R. 2016. Opportunities and challenges in biological lignin valorization. Current Opinion in Biotechnology. 42: 40–53. https://doi.org/10.1016/j.copbio.2016.02.030

Bhatia, L.; Johri, S.; Ahmad, R. 2012. An economic and ecological perspective of ethanol production from renewable agro waste: a review. AMB Express. 2: 65. https://doi.org/10.1186/2191-0855-2-65

Bhutto, A.W.; Qureshi, K.; Harijan, K.; Abro, R.; Abbas, T.; Bazmi, A.A.; Karim, S.; Yu, G. 2017. Insight into progress in pre-treatment of lignocellulosic biomass. Energy. 122: 724–745. https://doi.org/10.1016/j.energy.2017.01.005

Bianchi, S.; Kroslakova, I.; Janzon, R.; Mayer, I.; Saake, B.; Pichelin, F. 2015. Characterization of condensed tannins and carbohydrates in hot water bark extracts of European softwood species. Phytochemistry. 120: 53–61. https://doi.org/10.1016/j.phytochem.2015.10.006

Biedrzycka, E.; Bielecka, M. 2004. Prebiotic effectiveness of fructans of different degrees of polymerization. Trends in Food Science & Technology. 15: 170–175. https://doi.org/10.1016/j.tifs.2003.09.014

Bortolomeazzi, R.; Sebastianutto, N.; Toniolo, R.; Pizzariello, A. 2007. Comparative evaluation of the antioxidant capacity of smoke flavouring phenols by crocin bleaching inhibition, DPPH radical scavenging and oxidation potential. Food Chemistry. 100: 1481–1489. https://doi.org/10.1016/j.foodchem.2005.11.039

Bouchard, J.; Nguyen, T.S.; Chornet, E.; Overend, R.P. 1990. Analytical methodology for biomass pretreatment — Part 1: solid residues. Biomass. 23: 243–261. https://doi.org/10.1016/0144-4565(90)90035-I

Bouchard, J.; Nguyen, T.S.; Chornet, E.; Overend, R.P. 1991. Analytical methodology for biomass pretreatment. Part 2: characterization of the filtrates and cumulative product distribution as a function of treatment severity. Bioresource Technology. 36: 121–131. https://doi.org/10.1016/0960-8524(91)90169-K

Bountagkidou, O.G.; Ordoudi, S.A.; Tsimidou, M.Z. 2010. Structure–antioxidant activity relationship study of natural hydroxybenzaldehydes using *in vitro* assays. Food Research International. 43: 2014–2019. https://doi.org/10.1016/j.foodres.2010.05.021

Bozell, J.J.; Petersen, G.R. 2010. Technology development for the production of biobased products from biorefinery carbohydrates—the US Department of Energy's "Top 10" revisited. Green Chemistry. 12: 539–554. https://doi.org/10.1039/b922014c

Bu, L.; Tang, Y.; Gao, Y.; Jian, H.; Jiang, J. 2011. Comparative characterization of milled wood lignin from furfural residues and corncob. Chemical Engineering Journal. 175: 176–184. https://doi.org/10.1016/j.cej.2011.09.091

Bugg, T.D.; Rahmanpour, R. 2015. Enzymatic conversion of lignin into renewable chemicals. Current Opinion in Chemical Biology. 29: 10–17. https://doi.org/10.1016/j.cbpa.2015.06.009

Bujanovic, B.M.; Goundalkar, M.J.; Amidon, T.E. 2012. Increasing the value of a biorefinery based on hot-water extraction: lignin products. TAPPI Journal. 11: 19–26. https://doi.org/10.32964/10.32964/TJ11.1.19

Cañas, A.I.; Camarero, S. 2010. Laccases and their natural mediators: biotechnological tools for sustainable eco-friendly processes. Biotechnology Advances. 28: 694–705. https://doi.org/10.1016/j.biotechadv.2010.05.002

Castro, E.; Conde, E.; Moure, A.; Falqué, E.; Cara, C.; Ruiz, E.; Domínguez, H. 2008. Antioxidant activity of liquors from steam explosion of *Olea europea* wood. Wood Science and Technology. 42: 579–592. http://dx.doi.org/10.1007/s00226-007-0169-y

Chandra, R.P.; Bura, R.; Mabee, W.E.; Berlin, A.; Pan, X.; Saddler, J.N. 2007. Substrate pretreatment: the key to effective enzymatic hydrolysis of lignocellulosics? In: Olsson, L., ed. Biofuels. Berlin Heidelberg: Springer-Verlag: 67–93. https://doi.org/10.1007/10_2007_064

Chen, Z.; Wan, C. 2017. Biological valorization strategies for converting lignin into fuels and chemicals. Renewable and Sustainable Energy Reviews. 73: 610-621. https://doi.org/10.1016/j.rser.2017.01.166

Chen, X.; Lawoko, M.; van Heiningen, A. 2010. Kinetics and mechanism of autohydrolysis of hardwoods. Bioresource Technology. 101: 7812–7819. https://doi.org/10.1016/j.biortech.2010.05.006

Chen, X.; Kuhn, E.; Wang, W.; Park, S.; Flanegan, K.; Trass, O.; Tenlep, L.; Tao, L.; Tucker, M. 2013. Comparison of different mechanical refining technologies on the enzymatic digestibility of low severity acid pretreated corn stover. Bioresource Technology. 147: 401–408. https://doi.org/10.1016/j.biortech.2013.07.109

Chua, M.G.S.; Wayman, M. 1979. Characterization of autohydrolysis aspen (*P. tremuloides*) lignins. Part 1. composition and molecular weight distribution of extracted autohydrolysis lignin. Canadian Journal of Chemistry. 5: 1141–1149. https://doi.org/10.1139/v79-187

Ciolacu, D.; Oprea, A.M.; Anghel, N.; Cazacu, G.; Cazacu, M. 2012. New cellulose–lignin hydrogels and their application in controlled release of polyphenols. Materials Science and Engineering: C. 32: 452–463. https://doi.org/10.1016/j.msec.2011.11.018

Co, M.; Koskela, P.; Eklund-Åkergren, P.; Srinivas, K.; King, J.W.; Sjöberg, P.J.R.; Turner, C. 2009. Pressurized liquid extraction of betulin and antioxidants from birch bark. Green Chemistry. 11: 668. https://doi.org/10.1039/b819965e

Colodette, J.L.; Longue, D.; Pedrazzi, C.; Oliveira, R.C.; Gomide, J.L.; Gomes, F.J.B. 2011. Pulpability and bleachability of xylan-depleted *Eucalyptus* wood chips. Industrial & Engineering Chemical Research. 50: 1847–1852. https://doi.org/10.1021/ie101799y

Conde, E.; Cara, C.; Moure, A.; Ruiz, E.; Castro, E.; Domínguez, H. 2009. Antioxidant activity of the phenolic compounds released by hydrothermal treatments of olive tree pruning. Food Chemistry. 114: 806–812. https://doi.org/10.1016/j.foodchem.2008.10.017

Conde, E.; Fang, W.; Hemming, J.; Willför, S.; Moure, A.; Domínguez, H.; Parajó, J.C. 2013. Water-soluble components of *Pinus pinaster* wood. BioResources. 8: 2047–2063. https://doi.org/10.15376/biores.8.2.2047-2063

Corbett, D.B. 2016. Recovery of lignin from the hot water extracts of sugar maple: valorization by catalytic fast pyrolysis. Syracuse, NY: State University of New York College of Environmental Science and Forestry. 130 p. M.S. thesis.

Corbett, D.B.; Kohan, N.; Machado, G.; Jing, C.; Nagardeolekar, A.; Bujanovic, B.M. 2015. Chemical composition of apricot pit shells and effect of hot-water extraction. Energies. 8: 9640–9654. https://doi.org/10.3390/en8099640

Corbett, D.B.; Mante, O.D.; Bujanovic, B. 2017. Toward valorization of lignin: characterization and fast pyrolysis of lignin recovered from hot-water extracts of electron-beam irradiated sugar maple. TAPPI Journal. 16: 213–226. http://dx.doi.org/10.32964/TJ16.4.213

Corbett, D.B.; Hong, C.; Venditti, R.; Jameel, H.; Park, S. 2019. Hydrophobic resin treatment of hydrothermal autohydrolysate for prebiotic applications. RSC Advances. 9: 31819–31827. https://doi.org/10.1039/C9RA06018A

Crittenden, R.G.; Playne, M.J. 1996. Production, properties and applications of food-grade oligosaccharides. Trends in Food Science & Technology. 7: 353–361. https://doi.org/10.1016/S0924-2244(96)10038-8

Cueva, C.; Moreno-Arribas, M.V.; Martín-Álvarez, P.J.; Bills, G.; Vicente, M.F.; Basilio, A.; Rivas, C.L.; Requena, T.; Rodríguez, J.M.; Bartolomé, B. 2010. Antimicrobial activity of phenolic acids against commensal, probiotic and pathogenic bacteria. Research in Microbiology. 161: 372–382. https://doi.org/10.1016/j.resmic.2010.04.006

Cuvelier, M.-E.; Richard, H.; Berset, C. 1992. Comparison of the antioxidative activity of some acid-phenols: structure-activity relationship. Bioscience, Biotechnology & Biochemistry. 56: 324–325. https://doi.org/10.1271/bbb.56.324

Dai, J.; McDonald, A.G. 2014. Production of fermentable sugars and polyhydroxybutyrate from hybrid poplar: response surface model optimization of a hot-water pretreatment and subsequent enzymatic hydrolysis. Biomass and Bioenergy. 71: 275–284. https://doi.org/10.1016/j.biombioe.2014.09.030

del Río, P.G.; Gullón, B.; Wu, J.; Saddler, J.; Garrote, G.; Romaní, A. 2022. Current breakthroughs in the hardwood biorefineries: hydrothermal processing for the co-production of xylooligosaccharides and bioethanol. Bioresource Technology. 343: 126100. https://doi.org/10.1016/j.biortech.2021.126100

Devaney, L.; Iles, A. 2019. Scales of progress, power and potential in the US bioeconomy. Journal of Cleaner Production. 233: 379–389. https://doi.org/10.1016/j.jclepro.2019.05.393

Devappa, R.K.; Rakshit, S.K. Dekker, R.F.H. 2015. Forest biorefinery: potential of poplar phytochemicals as value-added co-products. Biotechnology Advances. 33: 681–716. https://doi.org/10.1016/j.biotechadv.2015.02.012

Dizhbite, T. 2004. Characterization of the radical scavenging activity of lignins—natural antioxidants. Bioresource Technology. 95: 309–317. https://doi.org/10.1016/j.biortech.2004.02.024

Djajadi, D.T.; Hansen, A.R.; Jensen, A.; Thygesen, L.G.; Pinelo, M.; Meyer, A.S.; Jørgensen, H. 2017. Surface properties correlate to the digestibility of hydrothermally pretreated lignocellulosic Poaceae biomass feedstocks. Biotechnology for Biofuels. 10: 49. https://doi.org/10.1186/s13068-017-0730-3

Dongre, P. 2018. Characterization and utilization of hot-water extracted lignin for formaldehyde-free resin applications. Syracuse, NY: State University of New York College of Environmental Science and Forestry. 147 p. Ph.D. dissertation.

Dongre, P.; Bujanovic, B. 2019. Lignin-based resins for kraft paper applications. TAPPI Journal. 18: 666–675. https://doi.org/10.32964/TJ18.11.666

Dongre, P.; Driscoll, M.; Amidon, T.; Bujanovic, B. 2015. Lignin-furfural based adhesives. Energies. 8: 7897–7914. https://doi.org/10.3390/en8087897

Dou, J.; Xu, W.; Koivisto, J.J.; Mobley, J.K.; Padmakshan, D.; Kögler, M.; Xu, C.; Willför, S.; Ralph, J.; Vuorinen, T. 2018. Characteristics of hot water extracts from the bark of cultivated willow (*Salix* sp.). ACS Sustainable Chemistry & Engineering. 6: 5566–5573. https://doi.org/10.1021/acssuschemeng.8b00498

Elniski, A.; Dongre, P.; Bujanovic, B.M. 2023. Lignin use in enhancing the properties of willow pellets. Forests. 14(10): 2041. https://doi.org//10.3390/f14102041

Eisenbies, M.H.; Volk, T.A.; Amidon, T.E.; Shi, S. 2019. Influence of blending and hot water extraction on the quality of wood pellets. Fuel. 241: 1058–1067. http://dx.doi.org/10.1016/j.fuel.2018.12.120

Espro, C.; Paone, E.; Mauriello, F.; Gotti, R.; Uliassi, E.; Bolognesi, M.L.; Rodríguez-Padrón, D.; Luque, R. 2021. Sustainable production of pharmaceutical, nutraceutical and bioactive compounds from biomass and waste. Chemical Society Reviews. 50: 11191–11207. https://doi.org/10.1039/D1CS00524C

Fache, M.; Boutevin, B.; Caillol, S. 2016. Vanillin production from lignin and its use as a renewable chemical. ACS Sustainable Chemistry & Engineering. 4: 35–46. https://doi.org/10.1021/acssuschemeng.5b01344

Figueiredo, P.; Lintinen, K.; Hirvonen, J.T.; Kostiainen, M.A.; Santos, H.A. 2018. Properties and chemical modifications of lignin: towards lignin-based nanomaterials for biomedical applications. Progress in Materials Science. 93: 233–269. https://doi.org/10.1016/j.pmatsci.2017.12.001

Frazer, F.R.; McCaskey, T.A. 1989. Wood hydrolyzate treatments for improved fermentation of wood sugars to 2,3-butanediol. Biomass. 18: 31–42. https://doi.org/10.1016/0144-4565(89)90079-6

Gairola, K.; Smirnova, I. 2012. Hydrothermal pentose to furfural conversion and simultaneous extraction with SC-CO_2- kinetics and application to biomass hydrolysates. Bioresource Technology. 123: 592–598. https://doi.org/10.1016/j.biortech.2012.07.031

García, D.E.; Carrasco, J.C.; Salazar, J.P.; Pérez, M.A.; Cancino, R.A.; Riquelme, S. 2016a. Bark polyflavonoids from *Pinus radiata* as functional building-blocks for polylactic acid (PLA)-based green composites. Express Polymer Letters. 10: 835–848. https://doi.org/10.3144/expresspolymlett.2016.78

García, D.E.; Glasser, W.G.; Pizzi, A.; Paczkowski, S.P.; Laborie, M.-P. 2016b. Modification of condensed tannins: from polyphenol chemistry to materials engineering. New Journal of Chemistry. 40: 36–49. https://doi.org/10.1039/C5NJ02131F

Garrote, G.; Dominguez, H.; Parajo, J.C. 1999. Mild autohydrolysis: an environmentally friendly technology for xylooligosaccharide production from wood. Journal of Chemical Technology & Biotechnology. 74: 1101–1109. https://doi.org/10.1002/(SICI)1097-4660(199911)74:11%3C1101::AID-JCTB146%3E3.0.CO;2-M

Garrote, G.; Cruz, J.M.; Domínguez, H.; Parajó, J.C. 2003. Valorisation of waste fractions from autohydrolysis of selected lignocellulosic materials. Journal of Chemical Technology and Biotechnology. 78: 392–398. https://doi.org/10.1002/jctb.760

Garrote, G.; Cruz, J.M.; Moure, A.; Domínguez, H.; Parajó, J.C. 2004. Antioxidant activity of byproducts from the hydrolytic processing of selected lignocellulosic materials. Trends in Food Science & Technology. 15: 191–200. https://doi.org/10.1016/j.tifs.2003.09.016

Ge, J.; Wu, Y.; Han, Y.; Qin, C.; Nie, S.; Liu, S.; Wang, S.; Yao, S. 2020. Effect of hydrothermal pretreatment on the demineralization and thermal degradation behavior of Eucalyptus. Bioresource Technology. 307: 123246. https://doi.org/10.1016/j.biortech.2020.123246

Geng, W. 2019. Hemicellulose extraction of biomass to develop high valued hemicellulose-based products. Raleigh, NC: North Carolina State University. Ph.D. thesis.

Gil-Chávez, G.J.; Padhi, S.S.P.; Pereira, C.V.; Guerreiro, J.N.; Matias, A.A.; Smirnova, I. 2019. Cytotoxicity and biological capacity of sulfur-free lignins obtained in novel biorefining process. International Journal of Biological Macromolecules. 136: 697–703. https://doi.org/10.1016/j.ijbiomac.2019.06.021

Giummarella, N.; Lawoko, M. 2017. Structural insights on recalcitrance during hydrothermal hemicellulose extraction from wood. ACS Sustainable Chemistry & Engineering. 5: 5156–5165. https://doi.org/10.1021/acssuschemeng.7b00511

Giummarella, N.; Lindgren, C.; Lindström, M.; Henriksson, G. 2016a. Lignin prepared by ultrafiltration of black liquor: investigation of solubility, viscosity, and ash content. BioResources. 11: 3494–3510. http://dx.doi.org/10.15376/biores.11.2.3494-3510

Giummarella, N.; Zhang, L.; Henriksson, G.; Lawoko, M. 2016b. Structural features of mildly fractionated lignin carbohydrate complexes (LCC) from spruce. RSC Advances. 6: 42120–42131. https://doi.org/10.1039/C6RA02399A

Giummarella, N.; Pu. Y.; Ragauskas, A.J.; Lawoko, M. 2019. A critical review on the analysis of lignin carbohydrate bonds. Green Chemistry: 21 (7): 1573–1595. https://doi.org/10.1039/C8GC03606C

Goacher, R.E.; Mottiar, Y.; Mansfield, S.D. 2021. ToF-SIMS imaging reveals that *p*-hydroxybenzoate groups specifically decorate the lignin of fibres in the xylem of poplar and willow. Holzforschung. 75: 452–462. https://doi.org/10.1515/hf-2020-0130

Goksu, E.; Karamanlioglu, U.; Bakir, U.; Yilmaz, L.; Yilmazer, U. 2008. Production and characterization of films from cotton stalk xylan. Journal of Agriculture and Food Chemistry. 55: 10685–10691. https://doi.org/10.1021/jf071893i

Goldschmid, O. 1954. Determination of phenolic hydroxyl content of lignin preparations by ultraviolet spectrophotometry. Analytical Chemistry. 26: 1421–1423. https://doi.org/10.1021/ac60093a009

Gomes, D.G.; Michelin, M.; Romaní, A.; Domingues, L.; Teixeira, J.A. 2021. Co-production of biofuels and value-added compounds from industrial *Eucalyptus globulus* bark residues using hydrothermal treatment. Fuel. 285: 119265. https://doi.org/10.1016/j.fuel.2020.119265

Gong, C.; Bujanovic, B.M. 2014. Impact of hot-water extraction on acetone-water oxygen delignification of *Paulownia spp*. and lignin recovery. Energies. 7: 857–873. https://doi.org/10.3390/en7020857

Gonzalez, J.; Cruz, J.M.; Domínguez, H.; Parajó, J.C. 2004. Production of antioxidants from *Eucalyptus globulus* wood by solvent extraction of hemicellulose hydrolysates. Food Chemistry. 84: 243–251. https://doi.org/10.1016/S0308-8146(03)00208-5

Goundalkar, M.J. 2014. Investigating the effect of hot-water extraction on phenolic constituents of selected hardwoods: a study aided by 2D NMR and GC-MS. Syracuse, NY: State University of New York College of Environmental Science and Forestry. 180 p. Ph.D. dissertation http://search.proquest.com/pqdtglobal/docview/1625969956/abstract/944952D4D6004007PQ/2

Goundalkar, M.J.; Bujanovic, B.; Amidon, T.E. 2010. Analysis of non-carbohydrate based low-molecular weight organic compounds dissolved during hot-water extraction of sugar maple. Cellulose Chemistry and Technology. 44: 27–33.

Guo, X.; Fu, Y.; Zhang, F.; Li, X.; Liu, N. 2021. Change of structural features and relocalization of chemical components in the autohydrolyzed poplar wood chips enhance the accessibility of remaining components. Industrial Crops and Products. 167: 113508. https://doi.org/10.1016/j.indcrop.2021.113508

Gütsch, J.S.; Sixta, H. 2012. Regeneration of spent activated charcoals used for lignin removal from prehydrolysis-kraft prehydrolyzates. Industrial & Engineering Chemistry Research. 51: 8624–8630. https://doi.org/10.1021/ie3006116

Gütsch, J.S.; Nousiainen, T.; Sixta, H. 2012. Comparative evaluation of autohydrolysis and acid-catalyzed hydrolysis of *Eucalyptus globulus* wood. Bioresource Technology. 109: 77–85. https://doi.org/10.1016/j.biortech.2012.01.018

Hansen, N.M.L.; Plackett, D. 2008. Sustainable films and coatings from hemicelluloses: a review. Biomacromolecules. 9: 1493–1505. https://doi.org/10.1021/bm800053z

Hartman, J.; Albertsson, A.-C.; Lindblad, M.S.; Sjöberg, J. 2006. Oxygen barrier materials from renewable sources: material properties of softwood hemicellulose-based films. Journal of Applied Polymer Science. 100: 2985–2991. https://doi.org/10.1002/app.22958

Hartonen, K.; Parshintsev, J.; Sandberg, K.; Bergelin, E.; Nisula, L.; Riekkola, M.-L. 2007. Isolation of flavonoids from aspen knotwood by pressurized hot water extraction and comparison with other extraction techniques. Talanta. 74: 32–38. https://doi.org/10.1016/j.talanta.2007.05.040

Hatakka, A. 1994. Lignin-modifying enzymes from selected white-rot fungi: production and role from in lignin degradation. FEMS Microbiology Reviews. 13: 125–135. https://doi.org/10.1111/j.1574-6976.1994.tb00039.x

Hatfield, R.; Fukushima, R.S. 2005 Can lignin be accurately measured? 2005. Crop Science. 45: 832–839. https://doi.org/10.2135/cropsci2004.0238

Hiltunen, S.; Heiskanen, I.; Backfolk, K. 2018. Effect of hydrothermal treatment of microfibrillated cellulose on rheological properties and formation of hydrolysis products. Cellulose. 25: 4653–4662. https://doi.org/10.1007/s10570-018-1884-2

Hoffman, A.S. 2012. Hydrogels for biomedical applications. Advanced Drug Delivery Reviews. 64: 18–23. https://doi.org/10.1016/j.addr.2012.09.010

Holladay, J.E.; Bozell, J.J.; White, J.F.; Johnson, D. 2007. Top value-added chemicals from biomass: volume II—results of screening for potential candidates from biorefinery lignin. PNNL-16983. Richland, WA: Pacific Northwest National Laboratory (under contract for U.S. Department of Energy). https://www.pnnl.gov/main/publications/external/technical_reports/PNNL-16983.pdf

Hong, C.; Corbett, D.; Venditti, R.; Jameel, H.; Park, S. 2019. Xylooligosaccharides as prebiotics from biomass autohydrolyzate. LWT. 111: 703–710. https://doi.org/10.1016/j.lwt.2019.05.098

Hörhammer, H.; Walton, S.; van Heiningen, A. 2011. A larch based biorefinery: pre-extraction and extract fermentation to lactic acid. Holzforschung. 65: 491–496. https://doi.org/10.1515/hf.2011.085

Hu, F.; Ragauskas, A. 2012. Pretreatment and lignocellulosic chemistry. BioEnergy Research. 5: 1043–1066. https://doi.org/10.1007/s12155-012-9208-0

Huang, C.; Jeuck, B.; Du, J.; Yong, Q.; Chang, H.; Jameel, H.; Phillips, R. 2016. Novel process for the coproduction of xylo-oligosaccharides, fermentable sugars, and lignosulfonates from hardwood. Bioresource Technology. 219: 600–607. https://doi.org/10.1016/j.biortech.2016.08.051

Ibn Yaich, A.; Edlund, U.; Albertsson, A.-C. 2012. Wood hydrolysate barriers: performance controlled via selective recovery. Biomacromolecules. 13: 466–473. https://doi.org/10.1021/bm201518d

Ibrahim, M.N.M.; Balakrishnan, R.S.; Shamsudeen, S.; Bahwani, S.A.; Adam, F. 2012. A concise review of the natural existance, synthesis, properties, and applications of syringaldehyde. BioResources. 7: 4377–4399. https://doi.org/10.15376/biores.7.3.4377-4399

Jang, S.-K.; Kim, J.-H.; Choi, J.-H.; Cho, S.-M.; Kim, J.-C.; Kim, H.; Choi, I.-G. 2021. Evaluation of xylooligosaccharides production for a specific degree of polymerization by liquid hot water treatment of tropical hardwood. Foods. 10: 463. https://doi.org/10.3390/foods10020463

Jara, R. 2010. The removal of wood components from hardwood by hot water. Orono, ME: University of Maine. 186 p. Ph.D. dissertation. https://digitalcommons.library.umaine.edu/etd/225

Jofre, F.M.; Weber Bordini, F.; de Andrade Bianchini, I.; de Souza Queiroz, S.; da Silva Boaes, T.; Hernández-Pérez, A.F.; das Graças de Almeida Felipe, M. 2022. Xylitol and sorbitol: production routes, challenges and opportunities in biorefineries integration. Chapter 8 In: Chandel, A.K.; Segato, F., eds. Production of top 12 biochemicals selected by USDOE from renewable resources: status and innovation. Amsterdam, the Netherlands: Elsevier: 233-268. https://doi.org/10.1016/B978-0-12-823531-7.00013-5

Jönsson, L.J.; Martín, C. 2016. Pretreatment of lignocellulose: formation of inhibitory by-products and strategies for minimizing their effects. Bioresource Technology. 199: 103–112. https://doi.org/10.1016/j.biortech.2015.10.009

Jönsson, L.J.; Palmqvist, E.; Nilvebrant, N.-O.; Hahn-Hägerdal, B. 1998. Detoxification of wood hydrolysates with laccase and peroxidase from the white-rot fungus *Trametes versicolor*. Applied Microbiology and Biotechnology. 49: 691–697. https://doi.org/10.1007/s002530051233

Kabbour, M.; Luque, R. 2020. Chapter 10 - furfural as a platform chemical: from production to applications. In: Saravanamurugan, S.; Pandey, A.; Li, H.; Riisager, A., eds. Biomass, biofuels, biochemicals: recent advances in development of platform chemicals. Amsterdam, Netherlands: Elsevier: 283–297. https://doi.org/10.1016/B978-0-444-64307-0.00010-X

Kilpeläinen, P.; Leppänen, K.; Spetz, P.; Kitunen, V.; Ilvesniemi, H.; Pranovich, A.; Willför, S. 2012. Pressurised hot water extraction of acetylated xylan from birch sawdust. Nordic Pulp & Paper Research Journal. 27: 680–688. https://doi.org/10.3183/npprj-2012-27-04-p680-688

Kim, S.-J.; Kim, M.-C.; Um, J.-Y.; Hong, S.-H. 2010. The beneficial effect of vanillic acid on ulcerative colitis. Molecules. 15: 7208–7217. https://doi.org/10.3390/molecules15107208

Kim, Y.; Kreke, T.; Hendrickson, R.; Parenti, J.; Ladisch, M.R. 2013. Fractionation of cellulase and fermentation inhibitors from steam pretreated mixed hardwood. Bioresource Technology. 135: 30–38. https://doi.org/10.1016/j.biortech.2012.10.130

Klass, D.L., ed. 1998. Biomass for renewable energy, fuels, and chemicals. San Diego, CA: Academic Press. 651 p. https://doi.org/10.1016/B978-0-12-410950-6.X5000-4

Klinke, H.B.; Thomsen, A.B.; Ahring, B.K. 2004. Inhibition of ethanol-producing yeast and bacteria by degradation products produced during pre-treatment of biomass. Applied Microbiology and Biotechnology. 66: 10–26. https://doi.org/10.1007/s00253-004-1642-2

Ko, J.K.; Kim, Y.; Ximenes, E.; Ladisch, M.R. 2015a. Effect of liquid hot water pretreatment severity on properties of hardwood lignin and enzymatic hydrolysis of cellulose. Biotechnology and Bioengineering. 112: 252–262. https://doi.org/10.1002/bit.25349

Ko, J.K.; Um, Y.; Park, Y.-C.; Seo, J.-H.; Kim, K.H. 2015b. Compounds inhibiting the bioconversion of hydrothermally pretreated lignocellulose. Applied Microbiology and Biotechnology. 99: 4201–4212. https://doi.org/10.1007/s00253-015-6595-0

Koivula, E.; Kallioinen, M.; Preis, S.; Testova, L.; Sixta, H.; Mänttäri, M. 2011. Evaluation of various pretreatment methods to manage fouling in ultrafiltration of wood hydrolysates. Separation and Purification Technology. 83: 50–56. https://doi.org/10.1016/j.seppur.2011.09.006

Kučerová, V.; Výbohová, E.; Čaňová, I.; Ďurkovič, J. 2016. The effects of both insoluble lignin and the macromolecular traits of cellulose on the content of saccharides within solids during hydrothermal pretreatment of hybrid poplar wood. Industrial Crops and Products. 91: 22–31. http://dx.doi.org/10.1016/j.indcrop.2016.06.021

Kumar, S.; Prahalathan, P.; Raja, B. 2011. Antihypertensive and antioxidant potential of vanillic acid, a phenolic compound in L-NAME-induced hypertensive rats: a dose-dependence study. Redox Report. 16: 208–215. https://doi.org/10.1179/1351000211Y.0000000009

Kyyrö, S.; Altgen, M.; Rautkari, L. 2020. Pressurized hot water extraction of Scots pine sapwood: effect of wood size on obtained treatment products. Biomass Conversion and Biorefinery. 12: 5019–5029. https://doi.org/10.1007/s13399-020-00927-3

Lee, J.-W.; Jeffries, T.W. 2011. Efficiencies of acid catalysts in the hydrolysis of lignocellulosic biomass over a range of combined severity factors. Bioresource Technology. 102: 5884–5890. https://doi.org/10.1016/j.biortech.2011.02.048

Lehto, J.T.; Alén, R.J. 2012. Purification of hardwood-derived autohydrolysates. BioResources. 7: 1813–1823. https://doi.org/10.15376/biores.7.2.1813-1823

Leppänen, K.; Spetz, P.; Pranovich, A.; Hartonen, K.; Kitunen, V.; Ilvesniemi, H. 2011. Pressurized hot water extraction of Norway spruce hemicelluloses using a flow-through system. Wood Science and Technology. 45: 223–236. https://doi.org/10.1007/s00226-010-0320-z

Leschinsky, M.; Zuckerstätter, G.; Weber, H.K.; Patt, R.; Sixta, H. 2008a. Effect of autohydrolysis of *Eucalyptus globulus* wood on lignin structure. Part 1: comparison of different lignin fractions formed during water prehydrolysis. Holzforschung. 62: 642–652. https://doi.org/10.1515/HF.2008.117

Leschinsky, M.; Zuckerstaetter, G.; Weber, H.K.; Patt, R.; Sixta, H. 2008b. Effect of autohydrolysis of *Eucalyptus globulus* wood on lignin structure. Part 2: influence of autohydrolysis intensity. Holzforschung. 62(6): 653–658. http://dx.doi.org/10.1515/HF.2008.133

Leschinsky, M.; Weber, H.K.; Patt, R.; Sixta, H. 2009. Formation of insoluble components during autohydrolysis of *Eucalyptus globulus*. Lenzinger Berichte. 87: 16–25.

Lessard, J.; Morin, J.-F.; Wehrung, J.-F.; Magnin, D.; Chornet, E. 2010. High yield conversion of residual pentoses into furfural via zeolite catalysis and catalytic hydrogenation of furfural to 2-methylfuran. Topics in Catalysis. 53: 1231–1234. https://doi.org/10.1007/s11244-010-9568-7

Li, J.; Gellerstedt, G. 2008. Improved lignin properties and reactivity by modifications in the autohydrolysis process of aspen wood. Industrial Crops and Products. 27: 175–181. https://doi.org/10.1016/j.indcrop.2007.07.022

Lin, L.; Cheng, Y.; Pu, Y.; Sun, S.; Li, X.; Jin, M.; Pierson, E.A.; Gross, D.C.; Dale, B.E.; Dai, S.Y.; Ragauskas, A.J.; Yuan, J.S. 2016. Systems biology-guided biodesign of consolidated lignin conversion. Green Chemistry. 18: 5536–5547. https://doi.org/10.1039/C6GC01131D

Liu, L.; Chang, H.; Jameel, H.; Park, S. 2018. Furfural production from biomass pretreatment hydrolysate using vapor-releasing reactor system. Bioresource Technology. 252: 165–171. https://doi.org/10.1016/j.biortech.2018.01.006

Lloyd, T.A.; Wyman, C.E. 2005. Combined sugar yields for dilute sulfuric acid pretreatment of corn stover followed by enzymatic hydrolysis of the remaining solids. Bioresource Technology. 96: 1967–1977. https://doi.org/10.1016/j.biortech.2005.01.011

Lora, J.H.; Wayman, M. 1980. Autohydrolysis of aspen milled wood lignin. Canadian Journal of Chemistry. 58: 669–676. http://dx.doi.org/10.1139/v80-102

Ma, J.; Ji, Z.; Chen, J.C.; Zhou, X.; Kim, Y.S.; Xu, F. 2015. The mechanism of xylans removal during hydrothermal pretreatment of poplar fibers investigated by immunogold labeling. Planta. 242: 327–337. https://doi.org/10.1007/s00425-015-2313-5

Mäki-Arvela, P.; Salmi, T.; Holmbom, B.; Willför, S.; Murzin, D.Yu. 2011. Synthesis of sugars by hydrolysis of hemicelluloses—a review. Chemical Reviews. 111: 5638–5666. https://doi.org/10.1021/cr2000042

Maleki, L.; Edlund, U.; Albertsson, A.-C. 2014. Unrefined wood hydrolysates are viable reactants for the reproducible synthesis of highly swellable hydrogels. Carbohydrate Polymers. 108: 281–290. https://doi.org/10.1016/j.carbpol.2014.02.060

Mandalika, A.; Runge, T. 2012. Enabling integrated biorefineries through high-yield conversion of fractionated pentosans into furfural. Green Chemistry. 14: 3175–3184. https://doi.org/10.1039/C2GC3575C

Mante, O.D.; Amidon, T.E.; Stipanovic, A.; Babu, S.P. 2014. Integration of biomass pretreatment with fast pyrolysis: an evaluation of electron beam (EB) irradiation and hot-water extraction (HWE). Journal of Analytical and Applied Pyrolysis. 110: 44–54. https://doi.org/10.1016/j.jaap.2014.08.004

Mänttäri, M.; Al Manasrah, M.; Strand, E.; Laasonen, H.; Preis, S.; Puro, L.; Xu, C.; Kisonen, V.; Korpinen, R.; Kallioinen, M. 2015. Improvement of ultrafiltration performance by oxidation treatment in the recovery of galactoglucomannan from wood autohydrolyzate. Separation and Purification Technology. 149: 428–436. https://doi.org/10.1016/j.seppur.2015.06.001

Mariscal, R.; Maireles-Torres, P.; Ojeda, M.; Sádaba, I.; López Granados, M. 2016. Furfural: a renewable and versatile platform molecule for the synthesis of chemicals and fuels. Energy & Environmental Science. 9: 1144–1189. https://doi.org/10.1039/C5EE02666K

Martín, C.; Dixit, P.; Momayez, F.; Jönsson, L.J. 2022. Hydrothermal pretreatment of lignocellulosic feedstocks to facilitate biochemical conversion. Frontiers in Bioengineering and Biotechnology. 10: 846592. https://doi.org/10.3389/fbioe.2022.846592

Martínez-Abad, A.; Giummarella, N.; Lawoko, M.; Vilaplana, F. 2018. Differences in extractability under subcritical water reveal interconnected hemicellulose and lignin recalcitrance in birch hardwoods. Green Chemistry. 20: 2534–2546. https://doi.org/10.1039/C8GC00385H

Míguez, B.; Gullón, P.; Cotos-Yáñez, T.; Massot-Cladera, M.; Pérez-Cano, F.J.; Vila, C.; Alonso, J.L. 2021. Manufacture and prebiotic potential of xylooligosaccharides derived from *Eucalyptus nitens* wood. Frontiers in Chemical Engineering. 3: 670440. https://doi.org/10.3389/fceng.2021.670440

Mittal, A.; Chatterjee, S.G.; Scott, G.M.; Amidon, T.E. 2009a. Modeling xylan solubilization during autohydrolysis of sugar maple and aspen wood chips: reaction kinetics and mass transfer. Chemical Engineering Science. 64: 3031–3041. https://doi.org/10.1016/j.ces.2009.03.011

Mittal, A.; Chatterjee, S.G.; Scott, G.M.; Amidon, T.E. 2009b. Modeling xylan solubilization during autohydrolysis of sugar maple wood meal: reaction kinetics. Holzforschung. 63: 307–314. https://doi.org/10.1515/HF.2009.054

Moure, A.; Cruz, J.M.; Franco, D.; Domínguez, J.M.; Sineiro, J.; Domínguez, H.; José Núñez, M.; Parajó, J.C. 2001. Natural antioxidants from residual sources. Food Chemistry. 72: 145–171. https://doi.org/10.1016/S0308-8146(00)00223-5

Mussatto, S.I.; Mancilha, I.M. 2007. Non-digestible oligosaccharides: a review. Carbohydrate Polymers. 68: 587–597. https://doi.org/10.1016/j.carbpol.2006.12.011

Nagardeolekar, A.; Ovadias, M.; Wang, K.-T.; Bujanovic, B. 2020. Willow lignin recovered from hot-water extraction for the production of hydrogels and thermoplastic blends. ChemSusChem. 13: 4702–4721. http://dx.doi.org/10.1002/cssc.202001259

Nagardeolekar, A.; Dongre, P.; Bujanovic, B.M. 2024. Lignin-based hydrogels for the delivery of bioactive chaga mushroom extract. Polymers. 16(6): 807. https://doi.org/10.3390/polym16060807

Narron, R.H.; Chang, H.; Jameel, H.; Park, S. 2017. Soluble lignin recovered from biorefinery pretreatment hydrolyzate characterized by lignin–carbohydrate complexes. ACS Sustainable Chemistry & Engineering. 5: 10763–10771. https://doi.org/10.1021/acssuschemeng.7b02716

Nenadis, N.; Lazaridou, O.; Tsimidou, M.Z. 2007. Use of reference compounds in antioxidant activity assessment. Journal of Agricultural and Food Chemistry. 55: 5452–5460. https://doi.org/10.1021/jf070473q

Nilvebrant, N.-O.; Reimann, A.; Larsson, S.; Jönsson, L.J. 2001. Detoxification of lignocellulose hydrolysates with ion-exchange resins. Applied Biochemistry and Biotechnology. 91: 35–49. https://doi.org/10.1007/978-1-4612-0217-2_3

Nitsos, C.K.; Matis, K.A.; Triantafyllidis, K.S. 2013. Optimization of hydrothermal pretreatment of lignocellulosic biomass in the bioethanol production process. ChemSusChem. 6: 110–122. https://doi.org/10.1002/cssc.201200546

Nitzsche, R.; Gröngröft, A.; Kraume, M. 2019. Separation of lignin from beech wood hydrolysate using polymeric resins and zeolites—determination and application of adsorption isotherms. Separation and Purification Technology. 209: 491–502. https://doi.org/10.1016/j.seppur.2018.07.077

Obst, J.R.; Landucci, L.L. 1986. Quantitative ^{13}C NMR of lignins—methoxyl:aryl ratio. Holzforschung. 40(Suppl.): 87–92.

Örså, F.; Holmbom, B.; Thornton, J. 1997. Dissolution and dispersion of spruce wood components into hot water. Wood Science and Technology. 31: 279–290. https://doi.org/10.1007/BF00702615

Otto, W.; Drahoslav, L. 1965. Cross-linked hydrophilic polymers and articles made therefrom. U.S. Patent 3220960A. https://patents.google.com/patent/US3220960A/en

Palmqvist, E.; Hahn-Hägerdal, B. 2000a. Fermentation of lignocellulosic hydrolysates. I: inhibition and detoxification. Bioresource Technology. 74: 17–24. http://dx.doi.org/10.1016/S0960-8524(99)00160-1

Palmqvist, E.; Hahn-Hägerdal, B. 2000b. Fermentation of lignocellulosic hydrolysates. II: inhibitors and mechanisms of inhibition. Bioresource Technology. 74: 25–33. http://dx.doi.org/10.1016/S0960-8524(99)00161-3

Pan, W.; Perrotta, J.A.; Stipanovic, A.J.; Nomura, C.T.; Nakas, J.P. 2012. Production of polyhydroxyalkanoates by *Burkholderia cepacia* ATCC 17759 using a detoxified sugar maple hemicellulosic hydrolysate. Journal of Industrial Microbiology and Biotechnology. 39: 459–469. https://doi.org/10.1007/s10295-011-1040-6

Parajó, J.C.; Garrote, G.; Cruz, J.M.; Dominguez, H. 2004. Production of xylooligosaccharides by autohydrolysis of lignocellulosic materials. Trends in Food Science & Technology. 15: 115–120. https://doi.org/10.1016/j.tifs.2003.09.009

Penttilä, P.A.; Kilpeläinen, P.; Tolonen, L.; Suuronen, J.-P. Sixta, H.; Willför, S.; Serimaa, R. 2013. Effects of pressurized hot water extraction on the nanoscale structure of birch sawdust. Cellulose. 20: 2335–2347. https://doi.org/10.1007/s10570-013-0001-9

Pielhop, T.; Larrazábal, G.O.; Rudolf von Rohr, P. 2016. Autohydrolysis pretreatment of softwood—enhancement by phenolic additives and the effects of other compounds. Green Chemistry. 18: 5239–5247. https://doi.org/10.1039/C6GC01447J

Pielhop, T.; Reinhard, C.; Hecht, C.; Del Bene, L.; Studer, M.H.; Rudolf von Rohr, P. 2017. Application potential of a carbocation scavenger in autohydrolysis and dilute acid pretreatment to overcome high softwood recalcitrance. Biomass and Bioenergy. 105: 164–173. https://doi.org/10.1016/j.biombioe.2017.07.005

Pietarinen, S.P.; Willför, S.M.; Ahotupa, M.O.; Hemming, J.E.; Holmbom, B.R. 2006a. Knotwood and bark extracts: strong antioxidants from waste materials. Journal of Wood Science. 52: 436–444. https://doi.org/10.1007/s10086-005-0780-1

Pietarinen, S.P.; Willför, S.M.; Vikström, F.A.; Holmbom, B.R. 2006b. Aspen knots, a rich source of flavonoids. Journal of Wood Chemistry and Technology. 26: 245–258. https://doi.org/10.1080/02773810601023487

Pilato, L. 2013. Phenolic resins: 100 years and still going strong. Reactive and Functional Polymers. 73: 270–277. http://dx.doi.org/10.1016/j.reactfunctpolym.2012.07.008

Pontes, R.; Romaní, A.; Michelin, M.; Domingues, L.; Teixeira, J.; Nunes, J. 2021. L-lactic acid production from multi-supply autohydrolyzed economically unexploited lignocellulosic biomass. Industrial Crops and Products. 170: 113775. https://doi.org/10.1016/j.indcrop.2021.113775

Pu, Y.; Treasure, T.; Gonzalez, R.; Venditti, R.A.; Jameel, H. 2013. Autohydrolysis pretreatment of mixed softwood to produce value prior to combustion. BioEnergy Research. 6: 1094–1103. https://doi.org/10.1007/s12155-013-9343-2

Ragauskas, A.J.; Beckham, G.T.; Biddy, M.J.; Chandra, R.; Chen, F.; Davis, M.F.; Davison, B.H.; Dixon, R.A.; Gilna, P.; Keller, M.; Langan, P.; Naskar, A.K.; Saddler, J.N.; Tschaplinski, T.J.; Tuskan, G.A.; Wyman, C.E. 2014. Lignin valorization: improving lignin processing in the biorefinery. Science. 344: 1246843. https://doi.org/10.1126/science.1246843

Rahmati, S.; Doherty, S.; Dubal, D.; Atanda, L.; Moghaddam, L.; Sonar, P.; Hessel, V.; Ostrikov, K. 2020. Pretreatment and fermentation of lignocellulosic biomass: reaction mechanisms and process engineering. Reaction Chemistry & Engineering. 5: 2017–2047. https://doi.org/10.1039/D0RE00241K

Rahouti, M.; Steiman, R.; Seigle-Murandi, F.; Christov, L.P. 1999. Growth of 1044 strains and species of fungi on 7 phenolic lignin model compounds. Chemosphere. 38: 2549–2559. https://doi.org/10.1016/S0045-6535(98)00462-7

Ralph, J.; Karlen, S.; Mobley, J. 2019. Synthesis of paracetamol (acetaminophen) from biomass-derived p-hydroxybenzamide. U.S. Patent 10,286,504.

Ramírez-Morales, J.E.; Czichowski, P.; Besirlioglu, V.; Regestein, L.; Rabaey, K.; Blank, L.M.; Rosenbaum, M.A. 2021. Lignin aromatics to PHA polymers: nitrogen and oxygen are the key factors for *Pseudomonas*. ACS Sustainable Chemistry & Engineering. 9: 10579–10590. https://doi.org/10.1021/acssuschemeng.1c02682

Ranjan, R.; Thust, S.; Gounaris, C.E.; Woo, M.; Floudas, C.A.; von Keitz, M.; Valentas, K.J.; Wei, J.; Tsapatsis, M. 2009. Adsorption of fermentation inhibitors from lignocellulosic biomass hydrolyzates for improved ethanol yield and value-added product recovery. Microporous and Mesoporous Materials. 122: 143–148. https://doi.org/10.1016/j.micromeso.2009.02.025

Ravber, M.; Knez, Ž.; Škerget, M. 2015. Isolation of phenolic compounds from larch wood waste using pressurized hot water: extraction, analysis and economic evaluation. Cellulose. 22: 3359–3375. https://doi.org/10.1007/s10570-015-0719-7

Renders, T.; Van den Bosch, S.; Koelewijn, S.-F.; Schutyser, W.; Sels, B.F. 2017. Lignin-first biomass fractionation: the advent of active stabilisation strategies. Energy & Environmental Science. 10: 1551–1557. https://doi.org/10.1039/C7EE01298E

Rissanen, J.V.; Grénman, H.; Xu, C.; Willför, S.; Murzin, D.Y.; Salmi, T. 2014. Obtaining spruce hemicelluloses of desired molar mass by using pressurized hot water extraction. ChemSusChem. 7: 2947–2953. https://doi.org/10.1002/cssc.201402282

Rodríguez-López, J.; Sánchez, A.J.; Gómez, D.M.; Romaní, A.; Parajó, J.C. 2012. Fermentative production of fumaric acid from *Eucalyptus globulus* wood hydrolyzates. Journal of Chemical Technology and Biotechnology. 87: 1036–1040. https://doi.org/10.1002/jctb.2729

Romaní, A.; Garrote, G.; Alonso, J.L.; Parajó, J.C. 2010. Experimental assessment on the enzymatic hydrolysis of hydrothermally pretreated *Eucalyptus globulus* wood. Industrial and Engineering Chemistry Research. 49: 4653–4663. https://doi.org/10.1021/ie100154m

Rowell, R.M. 2007. Composite materials from forest biomass: a review of current practices, science, and technology. In: Argyropoulos, D.S., ed. Materials, chemicals, and energy from biomass energy. ACS Symposium Series Vol. 954. Washington, DC: Oxford University Press: 76–92. Chapter 5. https://doi.org/10.1021/bk-2007-0954.ch005

Ruiz, H.A.; Conrad, M.; Sun, S.-N.; Sanchez, A.; Rocha, G.J.M.; Romaní, A.; Castro, E.; Torres, A.; Rodríguez-Jasso, R.M.; Andrade, L.P.; Smirnova, I.; Sun, R.-C.; Meyer, A.S. 2020. Engineering aspects of hydrothermal pretreatment: from batch to continuous operation, scale-up and pilot reactor under biorefinery concept. Bioresource Technology. 299: 122685. https://doi.org/10.1016/j.biortech.2019.122685

Saadatmand, S.; Edlund, U.; Albertsson, A.-C.; Danielsson, S.; Dahlman, O. 2012. Prehydrolysis in softwood pulping produces a valuable biorefinery fraction for material utilization. Environmental Science & Technology. 46: 8389–8396. https://doi.org/10.1021/es301699n

Sakagami, H.; Kushida, T.; Oizumi, T.; Nakashima, H.; Makino, T. 2010. Distribution of lignin–carbohydrate complex in plant kingdom and its functionality as alternative medicine. Pharmacology & Therapeutics. 128: 91–105. https://doi.org/10.1016/j.pharmthera.2010.05.004

Salamanca-Cardona, L.; Ashe, C.S.; Stipanovic, A.J.; Nomura, C.T. 2014. Enhanced production of polyhydroxyalkanoates (PHAs) from beechwood xylan by recombinant *Escherichia coli*. Applied Microbiology and Biotechnology. 98: 831–842. https://doi.org/10.1007/s00253-013-5398-4

Salvachúa, D.; Karp, E.M.; Nimlos, C.T.; Vardon, D.R.; Beckham, G.T. 2015. Towards lignin consolidated bioprocessing: simultaneous lignin depolymerization and product generation by bacteria. Green Chemistry. 17: 4951–4967. https://doi.org/10.1039/C5GC01165E

Samanta, A.K.; Jayapal, N.; Jayaram, C.; Roy, S.; Kolte, A.P.; Senani, S.; Sridhar, M. 2015. Xylooligosaccharides as prebiotics from agricultural by-products: production and applications. Bioactive Carbohydrates and Dietary Fibre. 5: 62–71. https://doi.org/10.1016/j.bcdf.2014.12.003

Samuel, R.; Foston, M.; Jaing, N.; Cao, S.; Allison, L.; Studer, M.; Wyman, C.; Ragauskas, A.J. 2011. HSQC (heteronuclear single quantum coherence) ^{13}C–^{1}H correlation spectra of whole biomass in perdeuterated pyridinium chloride–DMSO system: an effective tool for evaluating pretreatment. Fuel. 90: 2836–2842. https://doi.org/10.1016/j.fuel.2011.04.021

Samuel, R.; Cao, S.; Das, B.K.; Hu, F.; Pu, Y.; Ragauskas, A.J. 2013. Investigation of the fate of poplar lignin during autohydrolysis pretreatment to understand the biomass recalcitrance. RSC Advances. 3: 5305–5309. https://doi.org/10.1039/c3ra40578h

Santos, R.B.; Capanema, E.A.; Balakshin, M.Yu.; Chang, H.; Jameel, H. 2012. Lignin structural variation in hardwood species. Journal of Agricultural and Food Chemistry. 60: 4923–4930. https://doi.org/10.1021/jf301276a

Santos, T.M.; Alonso, M.V.; Oliet, M.; Domínguez, J.C.; Rigual, V.; Rodriguez, F. 2018. Effect of autohydrolysis on *Pinus radiata* wood for hemicellulose extraction. Carbohydrate Polymers. 194(2): 85–293. https://doi.org/10.1016/j.carbpol.2018.04.010

Schwartz, T.J.; Lawoko, M. 2010. Removal of acid-soluble lignin from biomass extracts using amberlite XAD-4 resin. BioResources. 5: 2337–2347. https://doi.org/10.15376/biores.5.4.2337-2347

Shinde, S.D.; Meng, X.; Kumar, R.; Ragauskas, A.J. 2018. Recent advances in understanding the pseudo-lignin formation in a lignocellulosic biorefinery. Green Chemistry. 20: 2192–2205. https://doi.org/10.1039/C8GC00353J

Shu, R.; Li, R.; Lin, B.; Wang, C.; Cheng, Z.; Chen, Y. 2020. A review on the catalytic hydrodeoxygenation of lignin-derived phenolic compounds and the conversion of raw lignin to hydrocarbon liquid fuels. Biomass and Bioenergy. 132. 105432. https://doi.org/10.1016/j.biombioe.2019.105432

Shuai, L.; Amiri, M.T.; Questell-Santiago, Y.M.; Héroguel, F.; Li, Y.; Kim, H.; Meilan, R.; Chapple, C.; Ralph, J.; Luterbacher, J.S. 2016. Formaldehyde stabilization facilitates lignin monomer production during biomass depolymerization. Science. 354: 329–333. https://doi.org/10.1126/science.aaf7810

Singh, R.D.; Banerjee, J.; Arora, A. 2015. Prebiotic potential of oligosaccharides: a focus on xylan derived oligosaccharides. Bioactive Carbohydrates and Dietary Fibre. 5: 19–30. https://doi.org/10.1016/j.bcdf.2014.11.003

Sixta, H. 2006. Handbook of pulp. Vol. 1. Weinheim, Germany: Wiley-VCH. https://doi.org/10.1002/9783527619887

Sjöström, E. 1993. Wood chemistry: fundamentals and applications, second edition. London: Academic Press.

Skog, K.E.; Stanturf, J.A. 2011. Forest biomass sustainability and availability. In: Zhu, J. (J.Y.); Zhang, X.; Pan, X. (Jun), eds. Sustainable production of fuels, chemicals, and fibers from forest biomass. ACS Symposium Series Vol. 1067. Washington, DC: American Chemical Society: 3–25. Chapter 1. https://doi.org/10.1021/bk-2011-1067.ch001

Smolarski, N. 2012. High-value opportunities for lignin: unlocking its potential. San Antonio, TX: Frost & Sullivan. 15 p. http://www.greenmaterials.fr/wp-content/uploads/2013/01/High-value-Opportunities-for-Lignin-Unlocking-its-Potential-Market-Insights.pdf

Song, T.; Pranovich, A.; Sumerskiy, I.; Holmbom, B. 2008. Extraction of galactoglucomannan from spruce wood with pressurised hot water. Holzforschung. 62: 659–666. https://doi.org/10.1515/HF.2008.131

Soto, M.L.; Moure, A.; Domínguez, H.; Parajó, J.C. 2011. Recovery, concentration and purification of phenolic compounds by adsorption: a review. Journal of Food Engineering. 105: 1–27. https://doi.org/10.1016/j.jfoodeng.2011.02.010

Su, T.; Zhao, D.; Khodadadi, M.; Len, C. 2020. Lignocellulosic biomass for bioethanol: recent advances, technology trends, and barriers to industrial development. Current Opinion in Green Sustainable Chemistry. 24: 56–60. https://doi.org/10.1016/j.cogsc.2020.04.005

Sun, S.; Cao, X.; Sun, S.; Xu, F.; Song, X.; Sun, R-C.; Jones, G.L. 2014. Improving the enzymatic hydrolysis of thermo-mechanical fiber from *Eucalyptus urophylla* by a combination of hydrothermal pretreatment and alkali fractionation. Biotechnology for Biofuels and Bioproducts. 7: 116. https://doi.org/10.1186/s13068-014-0116-8

Suzuki, Y.; Okamura-Abe, Y.; Nakamura, M.; Otsuka, Y.; Araki, T.; Otsuka, H.; Navarro, R.R.; Kamimura, N.; Masai, E.; Katayama, Y. 2020. Development of the production of 2-pyrone-4,6-dicarboxylic acid from lignin extracts, which are industrially formed as by-products, as raw materials. Journal of Bioscience and Bioengineering. 130: 71–75. https://doi.org/10.1016/j.jbiosc.2020.02.002

Tan, L.; Liu, Z.; Zhang, T.; Wang, Z.; Liu, T. 2020. Enhanced enzymatic digestibility of poplar wood by quick hydrothermal treatment. Bioresource Technology. 302: 122795. https://doi.org/10.1016/j.biortech.2020.122795

Tanase, C.; Coșarcă, S.; Muntean, D.-L. 2019. A critical review of phenolic compounds extracted from the bark of woody vascular plants and their potential biological activity. Molecules. 24: 1182. https://doi.org/10.3390/molecules24061182

Teleman, A. 2009. Hemicelluloses and pectins. In: Ek, M.; Gellerstedt, G.; Henriksson, G., eds. Wood Chemistry and Wood Biotechnology. Stockholm, Sweden: De Gruyter: 101–120. https://doi.org/10.1515/9783110213409.101

Teo, C.C.; Tan, S.N.; Yong, J.W.H.; Hew, C.S.; Ong, E.S. 2010. Pressurized hot water extraction (PHWE). Journal of Chromatography A. 1217: 2484–2494. https://doi.org/10.1016/j.chroma.2009.12.050

Thakur, V.K.; Thakur, M.K. 2015. Recent advances in green hydrogels from lignin: a review. International Journal of Biological Macromolecules. 72 (January): 834–847. https://doi.org/10.1016/j.ijbiomac.2014.09.044

Tian, G.; Chu, Y.; Chen, X.; Zhong, X.; Wang, Z.; Zhang, T. 2021. Separation and characterization of lignin and sugars in the hydrolysate of hot water extraction of poplar wood by membrane filtration and activated carbon adsorption. BioResources. 16: 6613–6628. https://doi.org/10.15376/biores.16.4.6613-6628

Tran, A.V.; Chambers, R.P. 1986. Lignin and extractives derived inhibitors in the 2,3-butanediol fermentation of mannose-rich prehydrolysates. Applied Microbiology and Biotechnology. 23: 191–197. https://doi.org/10.1007/BF00261912

Tunc, M.S.; van Heiningen, A.R.P. 2008. Hemicellulose extraction of mixed southern hardwood with water at 150 °C: effect of time. Industrial & Engineering Chemistry Research. 47: 7031–7037. https://doi.org/10.1021/ie8007105

Tunc, M.S.; van Heiningen, A.R. 2009. Autohydrolysis of mixed southern hardwoods: effect of P-factor. Nordic Pulp and Paper Research Journal. 24: 46–51. http://dx.doi.org/10.3183/NPPRJ-2009-24-01-p046-051

Tunc, M.S.; Lawoko, M.; van Heiningen, A.R.P. 2010. Understanding the limitations of removal of hemicelluloses during autohydrolysis of a mixture of southern hardwoods. BioResources. 5: 356–371. https://doi.org/10.15376/biores.5.1.356-371

Vázquez, M.J.; Garrote, G.; Alonso, J.L.; Domínguez, H.; Parajó, J.C. 2005. Refining of autohydrolysis liquors for manufacturing xylooligosaccharides: evaluation of operational strategies. Bioresource Technology. 96: 889–896. https://doi.org/10.1016/j.biortech.2004.08.013

Vegas, R.; Luque, S.; Alvarez, J.R.; Alonso, J.L.; Domínguez, H.; Parajó, J.C. 2006. Membrane-assisted processing of xylooligosaccharide-containing liquors. Journal of Agriculture and Food Chemistry. 54: 5430–5436. https://doi.org/10.1021/jf060525w

Vegas, R.; Alonso, J.L.; Domínguez, H.; Parajó, J.C. 2008. Enzymatic processing of rice husk autohydrolysis products for obtaining low molecular weight oligosaccharides. Food Biotechnology. 22: 31–46. https://doi.org/10.1080/08905430701863811

Vila, C.; Campos, A.R.; Cristovao, C.; Cunha, A.M.; Santos, V.; Parajó, J.C. 2008. Sustainable biocomposites based on autohydrolysis of lignocellulosic substrates. Composites Science and Technology. 68: 944–952. https://doi.org/10.1016/j.compscitech.2007.08.006

Virtanen, T.; Lahti, J.; Kalliola, A.; Tamminen, T.; Mänttäri, M.; Kallioinen, M. 2020. Influence of laccase treatment on fouling layer formation in ultrafiltration of birch hot-water extract. Separation and Purification Technology. 240: 116558. https://doi.org/10.1016/j.seppur.2020.116558

Vithanage, L.N.G.; Barbosa, A.M.; Kankanamge, G.R.N.; Rakshit, S.K.; Dekker, R.F.H. 2016. Valorization of hemicelluloses: production of bioxylitol from poplar wood prehydrolyzates by *Candida guilliermondii* FTI 20037. BioEnergy Research. 9: 181–197. https://doi.org/10.1007/s12155-015-9673-3

Wagner, K.; Roth, C.; Willför, S.; Musso, M.; Petutschnigg, A.; Oostingh, G.J.; Schnabel, T. 2019. Identification of antimicrobial compounds in different hydrophilic larch bark extracts. BioResources. 14: 5807–5815. https://doi.org/10.15376/biores.14.3.5807-5815

Walton, S.L.; Bischoff, K.M.; van Heiningen, A.R.P.; van Walsum, G.P. 2010. Production of lactic acid from hemicellulose extracts by *Bacillus coagulans* MXL-9. Journal of Industrial Microbiology and Biotechnology. 37: 823–830. https://doi.org/10.1007/s10295-010-0727-4

Wang, Z.; Wang, X.; Jiang, J.; Fu, Y.; Qin, M. 2015. Fractionation and characterization of saccharides and lignin components in wood prehydrolysis liquor from dissolving pulp production. Carbohydrate Polymers. 126: 185–191. https://doi.org/10.1016/j.carbpol.2015.03.0012

Wang, P.; Fu, Y.; Shao, Z.; Zhang, F.; Qin, M. 2016. Structural changes to aspen wood lignin during autohydrolysis pretreatment. BioResources. 11: 4086–4103. https://doi.org/10.15376/biores.11.2.4086-4103

Wang, C.; Li, H.; Li, M.; Bian, J.; Sun, R. 2017. Revealing the structure and distribution changes of *Eucalyptus* lignin during the hydrothermal and alkaline pretreatments. Scientific Reports. 7: 593. https://doi.org/10.1038/s41598-017-00711-w

Wang, X.; Hou, Q.; Zhang, X.; Zhang, Y.; Liu, W.; Xu, C.; Zhang, F. 2020a. Color evolution of poplar wood chips and its response to lignin and extractives changes in autohydrolysis pretreatment. International Journal of Biological Macromolecules. 157: 673–679. https://doi.org/10.1016/j.ijbiomac.2019.11224

Wang, Y.; Meng, X.; Jeong, K.; Li, S.; Leem, G.; Kim, K.H.; Pu, Y.; Ragauskas, A.J.; Yoo, C.G. 2020b. Investigation of a lignin-based deep eutectic solvent using *p*-hydroxybenzoic acid for efficient woody biomass conversion. ACS Sustainable Chemistry & Engineering. 8: 12542–12553. https://doi.org/10.1021/acssuschemeng.0c03533

Werpy, T.; Petersen, G.; Aden, A.; Bozell, J.; Holladay, J.; White, J.; Manheim, A. 2004. Top value added chemicals from biomass: Volume 1—Results of screening for potential candidates from sugars and synthesis gas. Washington, DC: U.S. Department of Energy, Office of Science and Technical Information. https://doi.org/10.2172/15008859

Westerberg, N.; Sunner, H.; Helander, M.; Henriksson, G.; Lawoko, M.; Rasmuson, A. 2012. Separation of galactoglucomannans, lignin, and lignin-carbohydrate complexes from hot-water-extracted Norway Spruce by cross-flow filtration and adsorption chromatography. BioResources. 7: 4501–4516. https://doi.org/10.15376/biores.7.4.4501-4516

Wichterle, O.; Lím, D. 1960. Hydrophilic gels for biological use. Nature. 185: 117–118. https://doi.org/10.1038/185117a0

Witzler, M.; Alzagameem, A.; Bergs, M.; Khaldi-Hansen, B.E.; Klein, S.E.; Hielscher, D.; Kamm, B.; Kreyenschmidt, J.; Tobiasch, E.; Schulze, M. 2018. Lignin-derived biomaterials for drug release and tissue engineering. Molecules. 23: 1885. https://doi.org/10.3390/molecules23081885

Wojtasz-Mucha, J.; Hasani, M.; Theliander, H. 2021. Dissolution of wood components during hot water extraction of birch. Wood Science and Technology. 55: 811–835. https://doi.org/10.1007/s00226-021-01283-9

Wood, C.D. 2016. Novel solvent and catalyst system for production of furfural from biomass-derived xylose. Syracuse, NY: State University of New York College of Environmental Science and Forestry. 174 p. Ph.D. dissertation. http://www.proquest.com/docview/1875126866/abstract/BCB60D4240724830PQ/1

Wood, C.D.; Amidon, T.E.; Volk, T.A.; Emerson, R.M. 2020. Hot water extraction: short rotation willow, mixed hardwoods, and process considerations. Energies. 13: 2071. https://doi.org/10.3390/en13082071

Xing, R.; Qi, W.; Huber, G.W. 2011. Production of furfural and carboxylic acids from waste aqueous hemicellulose solutions from the pulp and paper and cellulosic ethanol industries. Energy & Environmental Science. 4: 2193–2205. http://dx.doi.org/10.1039/C1EE01022K

Yang, S.; Zhang, Y.; Yuan, T.-Q.; Sun, R.-C. 2015. Lignin–phenol–formaldehyde resin adhesives prepared with biorefinery technical lignins. Journal of Applied Polymer Science. 132(36). https://doi.org/10.1002/app.42493

Yasarla, L.R.; Ramarao, B.V. 2012. Dynamics of flocculation of lignocellulosic hydrolyzates by polymers. Industrial & Engineering Chemistry Research. 51: 6847–6861. http://dx.doi.org/10.1021/ie202567c

Ye, P.; Cheng, L.; Ma, H.; Bujanovic, B.; Goundalkar, M.J.; Amidon, T.E. 2012. Biorefinery with water. In: Xie, H.; Gathergood, N., eds. The role of green chemistry in biomass processing and conversion. New York: John Wiley & Sons, Inc.: 135–180. https://doi.org/10.1002/9781118449400.ch4

Yelle, D.J.; Kaparaju, P.; Hunt, C.G.; Hirth, K.; Kim, H.; Ralph, J.; Felby, C. 2013. Two-dimensional NMR evidence for cleavage of lignin and xylan substituents in wheat straw through hydrothermal pretreatment and enzymatic hydrolysis. BioEnergy Research. 6: 211–221. https://doi.org/10.1007/s12155-012-9247-6

Zemek, J.; Košíková, B.; Augustin, J.; Joniak, D. 1979. Antibiotic properties of lignin components. Folia Microbiologica. 24: 483–486. https://doi.org/10.1007/BF02927180

Zhang, Y.; Wang, S.; Xu, W.; Cheng, F.; Pranovich, A.; Smeds, A.; Willför, S.; Xu, C. 2019. Valorization of lignin–carbohydrate complexes from hydrolysates of Norway spruce: efficient separation, structural characterization, and antioxidant activity. ACS Sustainable Chemistry & Engineering. 7: 1447–1456. https://doi.org/10.1021/acssuschemeng.8b05142

Zhang, T.; Zhou, H.; Fu, Y.; Zhao, Y.; Yuan, Z.; Shao, Z.; Wang, Z.; Qin, M. 2020. Short-time hydrothermal treatment of poplar wood for the production of a lignin-derived polyphenol antioxidant. ChemSusChem. 13: 4478–4486. https://doi.org/10.1002/cssc.202000534

Zhao, H.; Feng, Q.; Xie, Y.; Li, J.; Chen, X. 2017. Preparation of biocompatible hydrogel from lignin-carbohydrate complex (LCC) as cell carriers. BioResources. 12: 8490–8504. https://doi.org/10.15376/biores.12.4.8490-8504

Zhao, Y.; Shakeel, U.; Rehman, M.S.U.; Li, H.; Xu, X.; Xu, J. 2020. Lignin-carbohydrate complexes (LCCs) and its role in biorefinery. Journal of Cleaner Production. 253: 120076. https://doi.org/10.1016/j.jclepro.2020.120076

Zhu, J.Y.; Pan, X.; Zalesny, R.S. 2010. Pretreatment of woody biomass for biofuel production: energy efficiency, technologies, and recalcitrance. Applied Microbiology and Biotechnology. 87: 847–857. https://doi.org/10.1007/s00253-010-2654-8

Zhu, C.; Krumm, C.; Facas, G.G.; Neurock, M.; Dauenhauer, P.J. 2017. Energetics of cellulose and cyclodextrin glycosidic bond cleavage. Reaction Chemistry & Engineering. 2: 201–214. https://doi.org/10.1039/C6RE00176A

Zhu Ryberg, Y.Z.; Edlund, U.; Albertsson, A.-C. 2011. Conceptual approach to renewable barrier film design based on wood hydrolysate. Biomacromolecules. 12: 1355–1362. https://doi.org/10.1021/bm200128s