In: Sustainable Biorefining of Woody Biomass to Biofuels and Biochemicals, First Edition. 410pp. 978-0-323-91187-0, pp 215-262, https://doi.org/10.1016/B978-0-323-91187-0.00003-5; 2023

CHAPTER

9 Hydrothermal pretreatment of woody biomass and potential non-fuel applications of the solubilized constituents

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9.1 Introduction

Predictions as to the quantity of lignocellulosic biomass (LCB) available 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 US, the forest-based biomass supply physically available in 2011 was estimated to be ~45 million dry tonnes per year depending on market economics with a feasible increase to as much as 225 million dry tonnes achievable with longer-term industry growth (Skog and Stanturf, 2011). In contrast, the USDOE's "Billion Ton Study" predicted a potential supply of 1.3 billion dry tonnes 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 and grasses, account for additional potentially available biomass not included in the estimates above. Both woody and nonwoody LCB types have certain advantages; however, woody biomass is likely the best candidate for near-term implementation: it allows for year-round harvesting and supply, and it has environmental benefits, a relatively higher density, a higher lignin content, and a lower inorganic content compared to agricultural biomass (Zhu et al., 2010). Most importantly, it is not in conflict with food and feed production. For these reasons, this chapter will focus on wood as the cornerstone of lignocellulosic-based second-generation (2G) biorefineries.

[a]Biljana Bujanovic part of contribution is in the Public Domain.

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Biochemical pathways have been considered more appropriate for commercial 2G biorefineries as they are more environmentally compliant, sustainable, and cost-effective than chemical or thermochemical pathways (Bhutto et al., 2017). Generally speaking, in this design, the use of polysaccharides is emphasized, and lignin is utilized as a (low value) fuel. This chapter, however, concentrates on potential opportunities to diversify the product portfolio of wood-based biorefineries that employ 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/autohydrolyzate and used either as recovered (e.g., acetic acid) or after chemical or biological conversion, offering a broad palette of potential value-added products (Fig. 9.1). Efforts toward valorization of the wood degradation

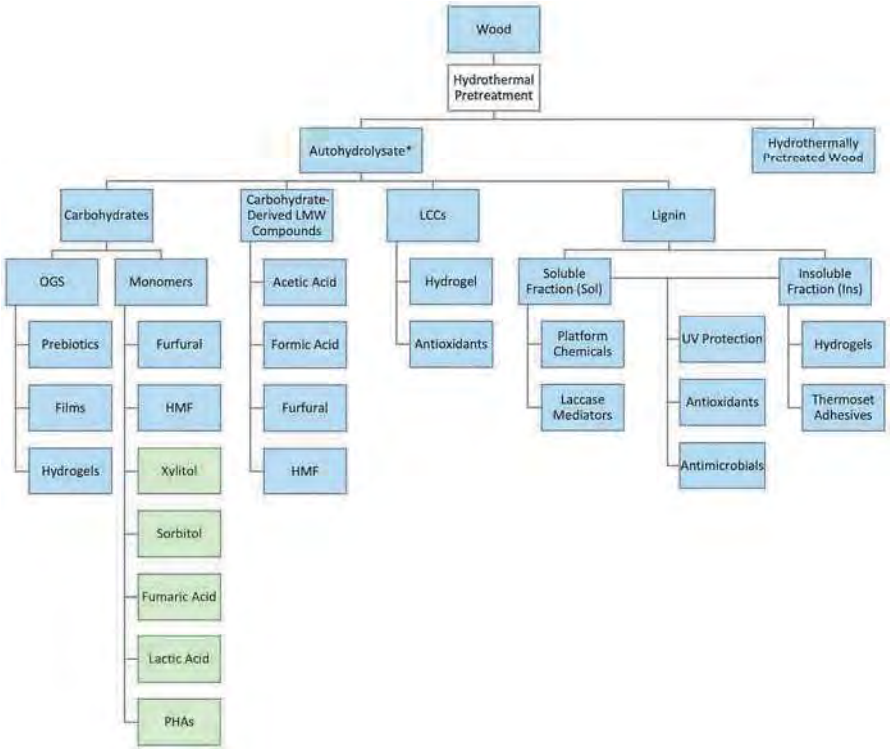


FIGURE 9.1

Potential non-fuel products from wood autohydrolyzate. Products of biological conversion are shown in green. *For some applications, autohydrolyzate may be used without treatment or separation (raw/crude). Note that this scheme is not inclusive of all potential non-fuel products that have been suggested in the literature. *HMF*, hydroxymethylfurfural; *LCC*, lignin—carbohydrate complex; *OGS*, oligosaccharides/short-chain hemicelluloses; *PHAs*, polyhydroxyalkanoates.

products present in autohydrolyzate aim to provide additional revenues to advance the implementation of biorefineries and contribute to the growing bioeconomy (Bhutto et al., 2017; Bujanovic et al., 2012; Devaney and Iles, 2019; Ragauskas et al., 2014).

9.2 Pretreatment

The goal of pretreatment (PT) is to reduce the recalcitrance of wood by disrupting its tight composite structure to enable access of enzymes to the polysaccharide/cellulose component for the release of sugars/glucose (Fig. 9.2). 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 et al., 2007). In addition, 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 as 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 et al., 2019; Martínez-Abad et al., 2018).

Pretreatment should be conducted with minimal degradation of sugars since sugar degradation products are notorious enzyme inhibitors and are not fermentable.

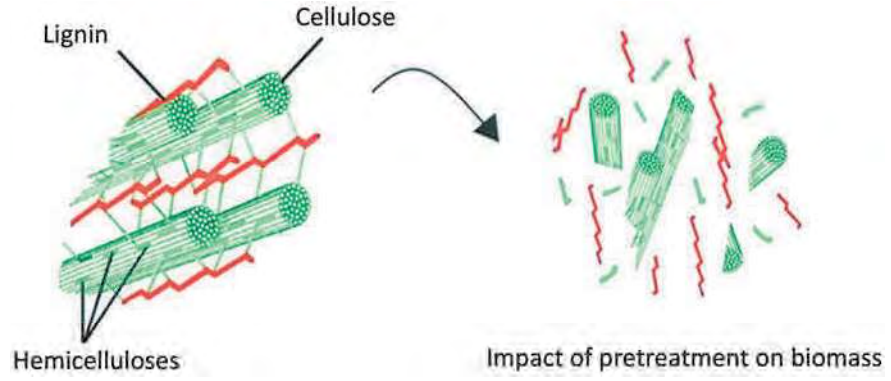


FIGURE 9.2

Graphical representation of the goals of pretreatment (PT): disrupt the native lignocellulosic structure, remove hemicelluloses and lignin, reduce cellulose degree of polymerization and crystallinity, and increase cellulose accessibility to enzymes.

Courtesy of Rahmati et al. (2020).

In this regard, PT has a large impact on the success of downstream operations, including the efficiency of EH and fermentation yields. PT should also be cost-effective regarding both capital and operational requirements, with low energy demand, including low demand for comminution. Currently, PT is considered as a major barrier to advancing 2G biorefineries, as PT costs may account for anywhere from 11% to more than 40% of the total cost of biofuel production (Bhutto et al., 2017; Su et al., 2020).

Multiple PT technologies have been suggested, including physical, chemical, and biological PT methods. Hydrothermal, dilute-acid, and ammonia-based processes have already been implemented at the 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 (Bhutto et al., 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 feature (galacto)glucomannans (GGMs) and G-lignin (guaiacyl lignin), which in the mild acid conditions of HTP are more stable and more prone to condensation, respectively, compared to glucuronoxylans (GXs) and SG-lignin (syringyl−guaiacyl lignin), which are predominant in HWs (Leschinsky et al., 2008b; Pu et al., 2013; Ye et al., 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 et al., 2010; Ye et al., 2012). Even though the removal of hemicelluloses/xylans has been shown to be more complete in acid PT than in HTP (e.g., 99% vs. 75% removal of starting xylan in poplar (*Populus trichocarpa x deltoides*) (Samuel et al., 2011)), some benefits favor the use of HTP. 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 assists with 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 (Eq. 9.2) up to 600) (Ye et al., 2012).

9.3 Hydrothermal pretreatment

Autohydrolysis is initiated by deacetylation of hemicelluloses leading to a decrease in pH. Subsequently, acid-labile glycosidic bonds are cleaved, 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 removal of a majority of hemicelluloses leads to an overall increase in the porosity and surface area of the hydrothermally treated wood. This improves the accessibility of cellulose

as demonstrated by the improvements in EH of wood after HTP (Guo et al., 2021; Romaní et al., 2010; Tan et al., 2020).

The extent of degradation of hemicelluloses depends on the severity of HTP (temperature and time of the process; Eqs. 9.1 and 9.2), although the removal of xylans usually exceeds 70% of the xylans originally present in HWs (Amidon et al., 2008; Narron et al., 2017). More severe conditions may lead to almost complete removal of xylans, for example, HTP of beech at 220°C for 15 min (Nitsos et al., 2013) and HTP of eucalyptus at 240°C for 30 min (Sun et al., 2014) (Fig. 9.4). However, HTP at higher temperatures promotes degradation of sugars resulting in a higher concentration of furfural in the autohydrolyzate (Fig. 9.5). For example, the furfural concentration in beech autohydrolyzate was found to increase dramatically with an increase in temperature; almost a ninefold increase from 190°C to 220°C after 15 min of HTP (Nitsos et al., 2013).

Evidently, the effects of HTP depend strongly on the employed conditions, and therefore, they are tunable based on desired outcomes. 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, that is, 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. 9.1) and the "P-factor" (Eq. 9.2) (Sixta, 2006). These two factors consider the treatment time and temperature, similar to the H-factor. 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, that is, cleavage of the glycosidic bonds in the main xylan chain, is the predominant reaction during HTP (Sixta, 2006; Wood et al., 2020).

$$S_O = \log R_0,\ R_0 = \int_{t_0}^{t} e^{\left(\frac{T_r - T_{ref}}{\omega}\right)} d \tag{9.1}$$

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 ω is an empirical constant (most often 14.75°C) (Garrote et al., 2004).

$$P = \int_{t_0}^{t} e^{\left(40.48 - \frac{15106}{T}\right)} dt \tag{9.2}$$

Where t is the reaction time (h) and T is the reaction temperature (K) (Wood et al., 2020).

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

of wood constituents, hemicelluloses, lignin, hydrophilic extractives, and inorganics from HWs (Amidon et al., 2008; Bujanovic et al., 2012; Corbett et al., 2017; Dongre et al., 2015; Gong and Bujanovic, 2014; Goundalkar et al., 2010; Mante et al., 2014; Nagardeolekar et al., 2020; Wood et al., 2020; Ye et al., 2012). Subcritical water extraction or pressurized hot-water extraction (PHWE) is a form of hydrothermal PT that involves pressurization of the reaction vessel beyond the saturated vapor pressure of water (Kyyrö et al., 2020; Martínez-Abad et al., 2018). PHWE is more often performed on SWs, since HTP performed on SWs has been shown to be less effective in reducing recalcitrance than when performed on HWs.

9.3.1 Removal of hemicelluloses

The reactions of hemicelluloses, particularly of HW glucuronoxylans (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, ~60–75% of the acetyl groups present in native HWs are cleaved (Mittal et al., 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 3.8–3.2 (Ye et al., 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. 9.3) (Ma et al., 2015; Zhu et al., 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 have 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 et al., 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%–30% OD wood, 7Ac/10Xylp) (Sjöström, 1993) (Fig. 9.4). It should be noted that the increase in severity promotes degradation of XOS leading to a reduced recovery of XOS (Mittal et al., 2009a). Even under the relatively mild

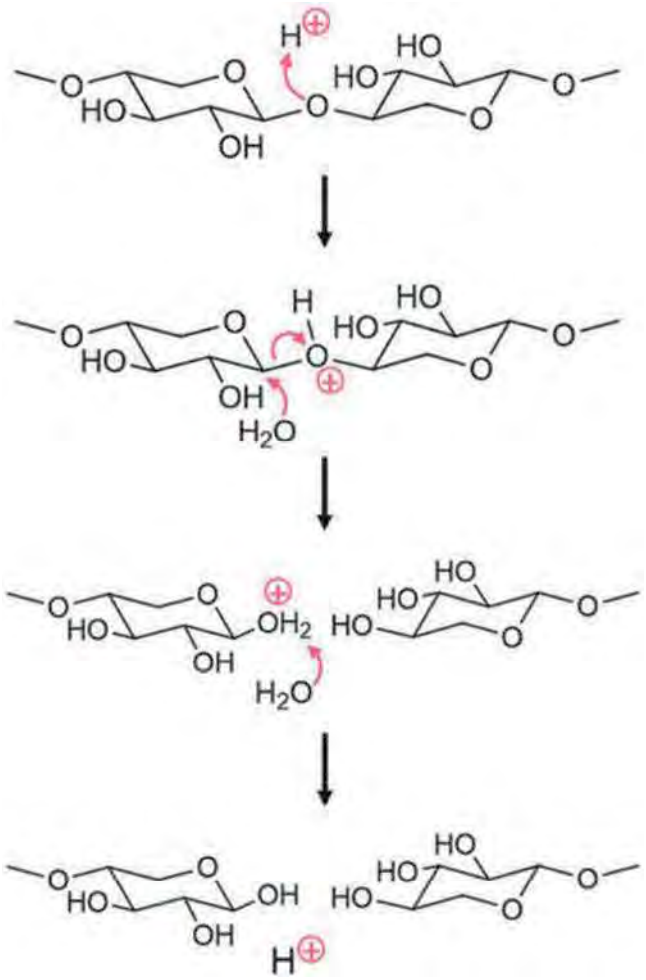


FIGURE 9.3

Hydronium ion catalyzed cleavage of the glycosidic bond between XylP units in xylan.

Adapted from Zhu et al. (2017).

conditions of HTP, degradation of XOS to xylose and further to furfural occurs (P-factors >600) (Fig. 9.5). Likewise, glucomannans may be converted to HMF. These carbohydrate degradation products are well-known inhibitors of EH (Palmqvist and Hahn-Hägerdal, 2000a, 2000b). A decrease in the content of cellulose may also be observed (Nitsos et al., 2013; Sun et al., 2014). Therefore, HTP conditions must be tuned properly to minimize degradation reactions while maximizing the fermentable sugars and cellulose accessibility to further treatments (Nitsos et al., 2013; Sun et al., 2014; Wood et al., 2020; Ye et al., 2012).

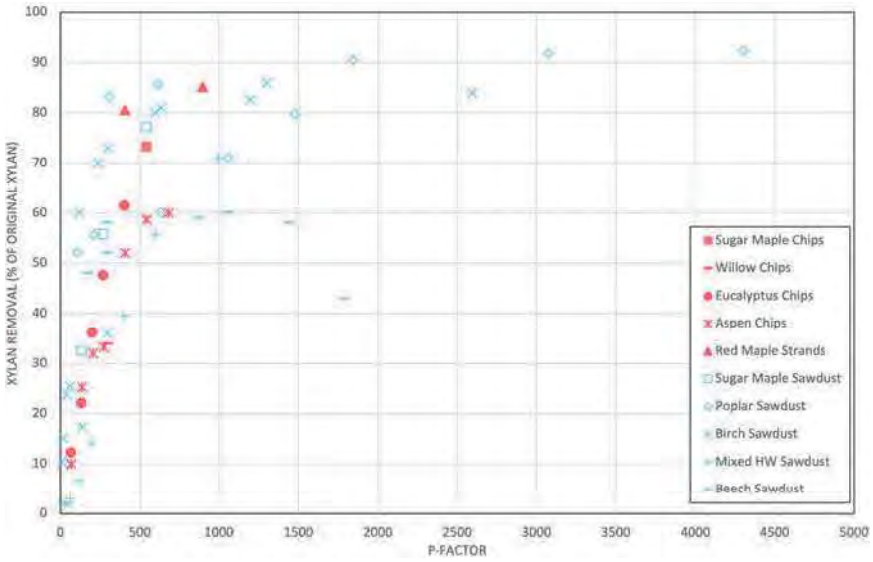


FIGURE 9.4

Removal of xylans during hydrothermal pretreatment (HTP) of hardwoods (HWs). Xylan removal (%) = [(xylan in original wood (g) − xylan remaining in pretreated wood (g))/ xylan in original wood (g)]*100.

Data from: Beech Sawdust (Nitsos et al., 2013); Willow Chips (Nagardeolekar et al., 2020); Birch sawdust (Kilpeläinen et al., 2012; Wojtasz-Mucha et al., 2021); Red Maple Strands (Jara, 2010); Eucalyptus Chips (Colodette et al., 2011); Poplar Sawdust (Kučerová et al., 2016); Sugar Maple Sawdust (Mittal et al., 2009b); Sugar Maple Chips (Amidon et al., 2008); Aspen Chips (Mittal et al., 2009a); Mixed HW Sawdust (Tunc and van Heiningen, 2008).

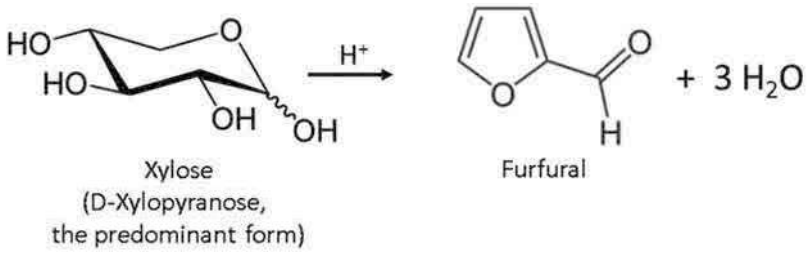


FIGURE 9.5

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

9.3.2 Effects on cellulose

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 et al., 2014). It has been proposed that under less severe conditions, the tightly packed nature of the crystalline regions of cellulose in the cell wall protects cellulose from degradation. In addition, even amorphous regions are fairly resistant to degradation as the glycosidic bonds in cellulose cleave ~1500 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 hydrolyzate liquor mainly originates from the hydrolysis of hexosans/(G)GMs (Amidon et al., 2008).

Hydrothermal treatment of microfibrillated cellulose was studied at temperatures up to 150°C, and 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 et al., 2018). In acidic conditions, cellulose degradation proceeds preferentially in the amorphous regions resulting in a net increase in cellulose crystallinity of the residual solids (Hiltunen et al., 2018). Cellulose-derived compounds removed during HTP have received limited attention in the context of high-value applications as HTP is tailored to maintain cellulose yields and avoid the production of degradation compounds to maximize yields during EH (Chandra et al., 2007).

9.3.3 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 delignification degree (DD); lignin removed during HTP calculated as a percent of the total lignin present in starting/native wood). 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. 9.6). Two distinct fractions of dissolved lignin may be recovered from the hydrolyzate: a soluble (Sol) and an insoluble (Ins) fraction (Leschinsky et al., 2008a, 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 MW (LMW) lignin, and lignin monomers. Nevertheless, the content of lignin in the extracted wood increases, due to 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 (AS) lignin (Hatfield and Fukushima, 2005), are removed to different extents during HTP. For example, the HTP of

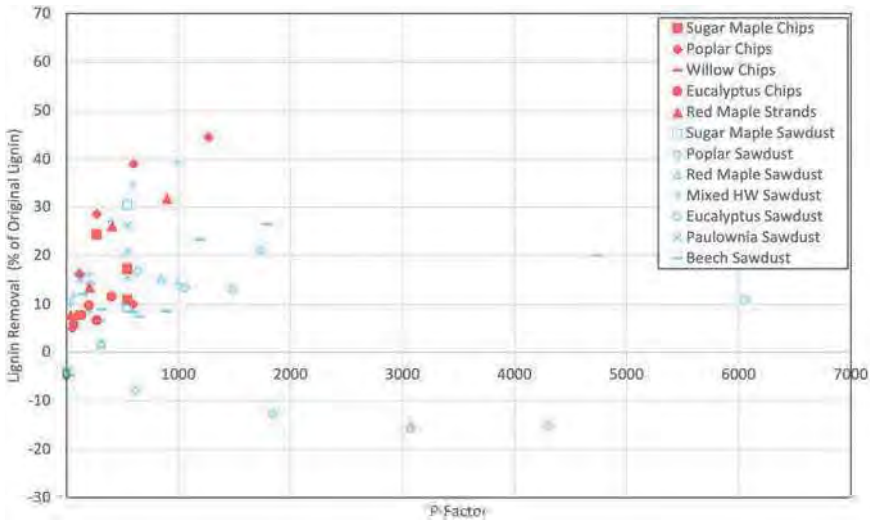


FIGURE 9.6

Delignification of hardwoods during hydrothermal pretreatment (HTP): DD versus P-factor: delignification degree (DD, %) refers to the amount of removed lignin expressed as a percent of the amount of lignin present in native wood.

Data from: Sugar maple chips (Amidon et al., 2008; Corbett et al., 2017; Mittal et al., 2009a); poplar chips (Wang et al., 2016); willow chips (Nagardeolekar et al., 2020); eucalyptus chips (Colodette et al., 2011); red maple strands (Jara, 2010), sugar maple sawdust (Gong and Bujanovic, 2014); poplar sawdust (Kučerová et al., 2016); red maple sawdust (Narron et al., 2017); mixed HW sawdust (Tunc et al., 2010; Tunc and van Heiningen, 2008); eucalyptus sawdust (Gütsch et al., 2012); Paulownia sawdust (Gong and Bujanovic, 2014); and beech sawdust (Nitsos et al., 2013).

eucalyptus (*Eucalyptus globulus*) at 200°C for 20 min led to the removal of ∼80% of the starting AS lignin (a fourfold increase from ∼20% at 150°C for 30 min), whereas Klason lignin removal was much lower (ranged from ∼2% at 150°C for 30 min to ∼9% at 200°C for 20 min) (Gütsch et al., 2012). Accordingly, the main portion of lignin removed appears to be lignin originally present in wood as AS lignin. In terms of the total DD, it 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, which take place through the formation of carbon–carbon bonds and surpass lignin degradation reactions at high temperatures/high severities (Garrote et al., 2004; Leschinsky et al., 2009; Li and Gellerstedt, 2008; Wang et al., 2016; Wang et al., 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 1478) in contrast to HTP performed at similar severity but at a higher temperature (195°C for 30 min; P-factor 1843), which resulted in a "gain" in lignin amount (negative DD of 12.7%; Fig. 9.6).

In 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 improves (selectivity: $\frac{hemicelluloses\ removal\ (\%\ of\ original\ hemicelluloses)}{DD\ (\%\ of\ original\ lignin)}$). For example, 100 min of HTP of mixed southern HWs at 150°C and 170°C resulted in 8.57% and 14.47% delignification, respectively, whereas the removal of xylans reached 12.27% and 67.57% in the respective experiments; that is, a 20°C increase in temperature resulted in ~3.3× increase in selectivity (Tunc et al., 2010). Due to the relatively selective removal of hemicelluloses, the content of lignin in the hydrothermally pretreated wood increases and leads to an overall increase in the energy of combustion (Ge et al., 2020; Nagardeolekar et al., 2020). This increase aids in the use of hydrothermally pretreated lignocellulosics/wood for the production of fuel pellets (Eisenbies et al., 2019).

The lignin reactions proposed to take place in mild acid conditions during HTP follow two competing reaction mechanisms leading to lignin depolymerization; acidolytic or homolytic cleavage of the β-O-4 bonds (Fig. 9.7, Routes 1 and 3, respectively) and lignin—lignin condensation/repolymerization through C_2/

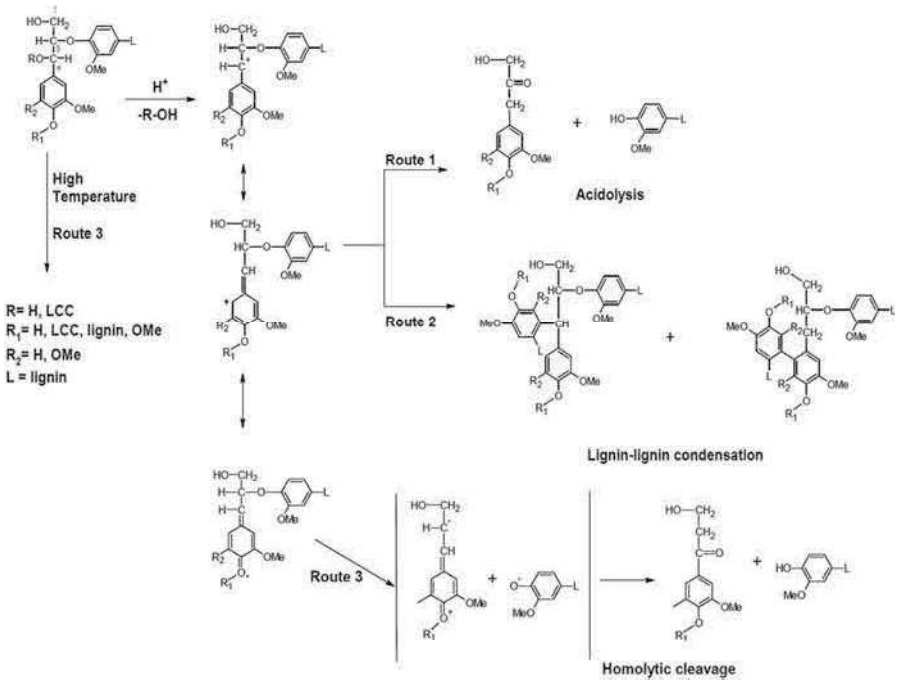


FIGURE 9.7

Proposed degradation of the β-O-4 bond between lignin C9 units in the mildly acidic conditions of autohydrolysis.

Modified from Bujanovic et al. (2012).

C_6-C_α crosslinking (Fig. 9.7, Route 2). The chain of lignin reactions is initiated by the formation of resonance-stabilized benzylium carbocations after proton-induced elimination of water or alcohol (Fig. 9.7). The relative importance of these reactions depends on the conditions of HTP and the structure of lignin in employed wood. Lignin repolymerization, noticed mostly at higher temperatures (DD < 0 in Fig. 9.6), may be reduced with the addition of strong nucleophiles (Li and Gellerstedt, 2008; Lora and Wayman, 1980). 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 (Pielhop et al., 2017). Accordingly, stronger inhibition of EH was demonstrated from lignin derived from more severely pretreated HWs (Ko et al., 2015a). Additionally, lignin derived from more severely pretreated HWs was shown to have higher T_g, implying that more advanced condensation reactions took place during HTP (Ko et al., 2015a).

The disruption of the wood cell wall with increased HTP severity has been observed by confocal Raman microscopy and scanning electron microscopy (SEM). HT-pretreated samples showed a gradual decrease in lignin content in all regions (Raman microscopy), although the delignification rate decreased from the secondary wall (S-wall) layers (highest lignin dissolution) to the cell corner areas (lowest lignin dissolution). This effect may be attributed to the greater content of syringyl (S) units inherently present in lignin in the S-wall layers resulting in greater reactivity of this lignin (Sjöström, 1993). Results have also shown that HTP of HWs leads to the removal of lignin enriched in S units compared to original lignin in the wood, leading to a decrease in S/G ratio in the extracted material (Bujanovic et al., 2012; Corbett et al., 2017; Goundalkar, 2014; Wang et al., 2020a).

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 et al., 2017). However, these droplets may also originate from acid-catalyzed condensation reactions of dehydrated carbohydrates, which yield polyphenolic compounds resembling lignin—pseudo-lignin (Shinde et al., 2018). In recent studies of autohydrolysis of poplar, the hydrophobicity of poplar chips was evaluated by measurements of external and internal surface contact angles (Guo et al., 2021). With an increase in the 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 et al., 2021). In general, surface hydrophobicity—wettability—has been recognized as an effective means to predict the success of EH (Ruiz et al., 2020) with enzymatic digestibility typically correlated negatively with contact angle (higher digestibility at reduced hydrophobicity in accordance with lower overall lignin content) (Djajadi et al., 2017).

9.3.4 Effects on LCC

LC bonds, which are inherently involved in creating the composite entangled structure of wood, are also proposed to contribute vitally to the native recalcitrance of wood (Chen et al., 2010). They lead to longer treatments and/or higher temperature requirements for the removal of lignin and hemicelluloses (Chen et al., 2010). During HTP of 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, while the latter (xylans incorporated into the 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 et al., 2010).

Differences in relative stability between the three types of LC bonds (benzyl ether, BE; benzyl ester, γE; and phenyl glycoside, PG) have been demonstrated by their extractability during HTP (Giummarella et al., 2016a, 2019; Martínez-Abad et al., 2018). Hot-water extracts of birch obtained at 160°C for 2 h contained PG and BE linkages, while spruce extracts obtained at the same conditions contained only the PG linkages (Giummarella and Lawoko, 2017). Further, for both birch and spruce, BE and γE LC bonds were found in higher amounts in the hot-water extracted wood as compared to native wood, indicating their relative resistance toward extraction and/or autohydrolytic cleavage. The relative stability of γ-esters to extraction is hypothesized to stem from their increased hydrophobicity compared to 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 et al., 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, while 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 et al., 2016b). On the other hand, 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 (Narron et al., 2017). In a similar study, hydrophobic resin retentate isolated from spruce autohydrolysates was found to contain three types of LC linkages with ester linkages as the most prominent (Zhang et al., 2019a).

These reports demonstrate how LCCs can hinder the separation between lignin and carbohydrates from the hydrolysates and substantially complicate attempts at the valorization of the individual components once extracted (Corbett et al., 2019; Narron et al., 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 hydrolyzate to suit the application needs.

9.3.5 Removal of inorganics—deashing

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 et al., 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 et al., 2021). ~45% of the ash originally present in willow with bark (commercial cultivars) was removed during HWE at 160°C for 2 h (Nagardeolekar et al., 2020).

The deashing 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 (Ca, Mg) metals. These metals influence the thermal decomposition of biomass and affect the composition of produced bio-oils. In studies of catalytic fast pyrolysis studies of sugar maple (*A. saccharum*), HWE (160°C, 2 h) reduced char/coke formation and increased the yield of benzene, toluene, and xylene (46%, 35%, and 26%, respectively) in the pyrolysis oils (Mante et al., 2014). Additionally, ash removal during HTP can be expected to have beneficial impacts on the wood pelletizing industry due to reduced degradation of process metal, higher energy content, and decreased fouling of furnaces at end-use (Eisenbies et al., 2019).

9.3.6 Challenges with implementation of HTP

While the advantages of HTP relative to other PT technologies are evident, there are limitations/challenges that must be considered and overcome. Water and energy consumption must be reduced. High water demand could be mitigated by maximizing solids loading (>25% w/w (del Río et al., 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 utilizing screw conveyance reactors have been trialed at a pilot scale (Corbett et al., 2019). Engineering obstacles related to mass and energy transfer must be overcome.

Though it is not unique to the HTP approach, the separation of dissolved constituents after HTP is an additional challenge. 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 upon throughout the rest of this chapter in the context of the specific products or categories of chemicals being discussed.

Another important consideration is that while 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 PT technologies could be considered; for example, mild HTP to promote XOS production followed by a delignification technology to maximize cellulose conversion to glucose (del Río et al., 2022).

These challenges have hindered efforts to commercialize HTP, particularly when utilizing woody biomass as a feedstock. To date, only two purportedly commercial-scale implementations of HTP have been undertaken (Inbicon and Clariant) and both utilize wheat straw, not woody biomass (Martín et al., 2022). Nonetheless, HTP remains a popular strategy touted by both researchers and entrepreneurs due to its relatively low capital costs, benign environmental profile, and potential to produce a diverse range of value-added co-products.

9.4 Potential non-fuel applications—carbohydrates

9.4.1 Materials

The carbohydrates present in wood autohydrolyzate 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 HTP, these may include some degree of branching, abundant hydroxyl groups, high hydrophilicity, biodegradability, and nontoxicity (Maleki et al., 2014). The large number of hydroxyl groups, in particular, provides an excellent opportunity for various chemical manipulations, including functionalization (Hansen and Plackett, 2008) and crosslinking reactions (Maleki et al., 2014).

Traditional preparation of hemicelluloses for materials applications involves high-specificity extraction techniques; for example, 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 autohydrolyzate, the presence of at least some lignin is a near certainty. A notable portion of hemicelluloses in wood autohydrolyzate remains covalently attached to lignin fragments via LC bonds (LCC) (Corbett et al., 2019; Maleki et al., 2014; Narron et al., 2017). Although chromatographic, resin, or enzyme-based purification of these hemicelluloses is possible, such upgrading strategies reduce economic feasibility (Al-Rudainy et al., 2019; Maleki et al., 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 et al., 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 et al., 2006; Ibn Yaich et al., 2012; Maleki et al., 2014; Saadatmand et al., 2012; Zhu Ryberg et al., 2011).

Albertsson and co-workers have studied the utilization of noncellulosic polysaccharides obtained from wood hydrolyzate produced via HTP of both SWs and HWs (Hartman et al., 2006; Ibn Yaich et al., 2012; Maleki et al., 2014; Saadatmand et al., 2012; Zhu Ryberg et al., 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 et al., 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 of 1 kDa; MWCO) (Maleki et al., 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, Qeq, from 50 to 270 g water/g dry hydrogel) were produced with potential applications including absorbing materials and drying or water-retaining agents (Maleki et al., 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 et al., 2019; Goksu et al., 2008). Goksu et al. successfully formed films from xylan–lignin mixtures extracted from cotton stalks in an alkaline solution. The films were produced with no crosslink or plasticizer additive. It was reported that coherent films could be formed only if lignin was still present in the film-forming solution (Goksu et al., 2008). Al-Rudainy et al. 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, likely due to reduced crosslinking as a result of consumption of the crosslinking agent (epichlorohydrin) by lignin (Al-Rudainy et al., 2019).

9.4.2 Platform chemicals

Carbohydrates present in autohydrolysates have also been explored for conversion into platform chemicals for the production of high-value non-fuel 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 et al., 2004). Although they can be produced during HTP, as they are products of acid-catalyzed dehydration of pentoses/hexoses (Fig. 9.5), it has been demonstrated that this conversion is minimal at P-factors <600 (Tunc and van Heiningen, 2009, 2008). Chemical conversion of dissolved pentoses/xylose into furfural (Fig. 9.1) is one of the most common valorization strategies due to the wide range of chemicals into which furfural can be further upgraded including dihydropyran, tetrahydrofuran (THF), 2-methylfuran, furfuryl alcohol, and furoic acid (Amidon et al., 2008; Barbosa et al., 2014; Gairola and Smirnova, 2012; Kabbour and Luque, 2020; Mandalika and Runge, 2012; Mariscal et al., 2016; Werpy et al., 2004; Wood, 2016; Xing et al., 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 et al., 2021).

Various thermocatalytic systems have been proposed to improve upon 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, although the technology would benefit from further efforts toward improving catalyst regeneration (Lessard et al., 2010).

Most studies utilize 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 et al., 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 (Baktash et al., 2015; Liu et al., 2018). Liu et al. studied batch, biphasic, and vapor release reaction systems that relied only on the soluble acids present in mixed southern HW autohydrolysates (Liu et al., 2018). Acetic acid and formic acid were present in the starting liquor at 3–7 g/L and 0.5–0.6 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 et al., 2018). Baktash et al. 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 (Baktash et al., 2015).

Acetic acid is inherently present in autohydrolysates produced from wood, particularly due to deacetylation of acetylated hemicelluloses. Acetic acid is a well-known inhibitor of EH and fermentation and should be removed from autohydrolysates before further processing (Ye et al., 2012). After removal and concentration, acetic acid becomes a significant potential revenue source for a multiproduct biorefinery (Amidon et al., 2011). Membrane filtration is the most promising technology for the separation of acetic acid from autohydrolysates due to its relatively low cost, simplicity, and noninvasiveness (Amidon et al., 2008; Liu et al., 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 et al., 2008).

9.4.3 Food and nutrition

By far, the most plentiful dissolved constituents in autohydrolysates are mono- and OGS derived from hemicelluloses. Dissolved noncellulosic polysaccharides can account for 60%–70% of the total solids in autohydrolysates (Vegas et al., 2006). Near complete removal of hemicelluloses from solid biomass can be achieved; Fig. 9.4 shows the removal of xylans versus severity (P-factor). PT severity also determines the extent to which hemicelluloses are hydrolyzed; completely to monomers or partially to OGS (Vázquez et al., 2005). These dissolved carbohydrates, including xylose, mannose, galactose, glucose, and their OGS, have prospective applications as functional additives to food.

9.4.3.1 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, immune function, and 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 et al., 2019; Mussatto and Mancilha, 2007). OGS have been shown to act as effective prebiotics (Hong et al., 2019; Mussatto and Mancilha, 2007).

OGS 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 OGS and so are often included as OGS (Crittenden and Playne, 1996). OGS considered for prebiotic applications include galactooligosaccharides (GOS), fructooligosaccharides (FOS), xylooligosaccharides (XOS), inulin, lactulose, and polydextrose. OGS 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. 9.1). XOS are particularly attractive since their sweet taste allows for applications as artificial sweeteners (Samanta et al., 2015). In addition to potential applications in human health supplements, OGS such as XOS have applications as animal feed additives (Samanta et al., 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 et al., 2021; Mittal et al., 2009a; Parajó et al., 2004). Up to 75% of the starting xylan in sugar maple was dissolved during HTP at 175°C for 2 h (P-factor ~1750). In the same study, a maximum XOS yield of 51% was achieved at less severe conditions (125°C, 4.5 h, P-factor ~57) (Mittal et al., 2009a). OGS accounting for >60% of the starting xylan in *E. globulus* were identified in autohydrolysates produced at varying conditions from 145°C to 190°C. XOS concentration in the autohydrolyzate was highly dependent on reaction time and temperature and was maximized after ~1 h at 160°C but after only ~5 min at 190°C (Garrote et al., 1999). In studies of the tropical HW *E. pellita*, 77.6% of original xylan was removed in HTP at 170°C for 50 min. XOS accounted for 76.5% of the total sugars in the autohydrolyzate (Jang et al., 2021). The nonspecific hydrolysis and solubilization taking place during HTP result in a heterogeneous mixture of OGS (often still substituted with sidechains 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. 9.5).

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 et al., 2015). DP has also been shown to influence downstream processing strategies; while lower DP is preferred due to enhanced prebiotic activity, complete hydrolysis to monosaccharides must be avoided (Corbett et al., 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. Commercial endo-xylanases are available and have been shown to effectively reduce DP of xylans in autohydrolyzate without producing xylose (Biedrzycka and Bielecka, 2004; Garrote et al., 1999; Martínez-Abad et al., 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 et al., 2008). Often a combination of strategies is employed. 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 IRA-96). Processing increased the substituted XOS content from 57% to 83% (percent of nonvolatile components in solution). However, the overall yield of XOS was only 38% (based on hemicelluloses in the starting wood), implying significant losses during production and/or processing (Míguez et al., 2021). Hydrophobic resin (XAD 16N) was utilized to process sweetgum autohydrolyzate (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 autohydrolyzate lost during resin treatment (Huang et al., 2016).

Isolation/purification of XOS from autohydrolyzate 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* autohydrolyzate was measurable by analytical chromatography only after complete EH to xylose (Corbett et al., 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 et al., 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 even though 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.

9.4.3.2 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 et al., 2021; Mäki-Arvela et al., 2011) (Fig. 9.1). Xylitol and sorbitol are produced by hydrogenation (Raney nickel catalyst) or enzymatic transformation of xylose or glucose, respectively; D-xylose isomerase of *Bacillus coagulans* or using *Mycobacterium smegmatis* or yeast *Candida guilliermondii*.

Xylitol and sorbitol were included in the top 12 value-added products from biomass in the original US DOE report in 2004 (Werpy et al., 2004) and confirmed in a reinstated list by Bozell and Petersen in 2010 (Bozell and Petersen, 2010). The global market of xylitol is estimated to exceed 260k tons. It is considered as 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 et al., 2021). DuPont produces xylitol of 99% purity under the brand name Xivia by catalytic reduction of xylose derived from wood-based xylans (Espro et al., 2021). Sorbitol is a sweetener, humectant, pharmaceutical excipient, emulsifier for food, pharmaceuticals, and cosmetics and 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 et al., 2021).

9.4.4 Fermentation

Fermentation of the carbohydrate constituents dissolved in autohydrolyzate is a thoroughly examined subject. Although, fuels such as ethanol and butanol are the most commonly cited fermentation products, a variety of non-fuel products can be produced from autohydrolysates via fermentation, including LMW products, such as lactic and fumaric acid and polymers, such as polyhydroxyalkanoates (PHAs) (Fig. 9.1).

Lactic acid (LA; included in the top 30 potential building-block chemicals from biomass in 2004 (Werpy et al., 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, other fine and commodity chemicals, and a building block for biodegradable polymers (polylactic acid; PLA) (Bozell and Petersen, 2010; Pontes et al., 2021; Walton et al., 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 and optical purity is necessary for the production of high-quality PLA thermoplastics. Lactic acid 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 et al., 2021), and yeasts; for example fermentation of xylose to LA by yeast *Pichia stipitis* (Bozell and Petersen, 2010).

Both pentose and hexose sugars present in wood autohydrolyzate have been successfully converted to LA in laboratory studies. The monomeric sugars from mixed HW autohydrolyzate were converted to LA at 94% yield by *Bacillus coagulans* MXL-9 (following complete hydrolysis in sulfuric acid and neutralization) (Walton et al., 2010). Similarly, LA was produced at high yield from the sugars in Siberian larch autohydrolyzate by the same organism (Hörhammer et al., 2011). *B. coagulans* is a moderately thermophilic LAB that is capable of surviving at pHs as low as 4. The conditions of optimal temperature and pH are similar to those suggested for

hydrolytic enzymes and are thus well-suited for simultaneous saccharification and fermentation (SSF). Most strains of *B. coagulans* can utilize xylose, while all can utilize glucose (Walton et al., 2010). More recently, LA production by *L. rhamnosus* was studied using a mixture of wood species (majority eucalyptus and pine) following HTP (Pontes et al., 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 et al. studied the production of LA from eucalyptus autohydrolyzate (Vila et al., 2008). The autohydrolyzate was subjected to secondary hydrolysis with sulfuric acid prior to fermentation of the resulting monomers by *L. 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 et al., 2008).

Fumaric acid is traditionally utilized in the food industry as an acidulent for food and beverages but has other applications in the chemical, pharmaceutical, and cosmetic industries (Rodríguez-López et al., 2012). *E. globulus* chips were subjected to autohydrolysis followed by EH and then subjected to fermentation by *Rhizopus arrhizus* DSM 5772 for the production of fumaric acid. Membrane filtration (MWCO 1 kDa) and anion exchange processing (Amberlite IRA-96) 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 hydrolyzate were relatively unsuccessful (Rodríguez-López et al., 2012).

Polyhydroxyalkanoates are a class of biodegradable polyesters that exhibit properties similar to petroleum-derived thermoplastics and that can be produced by a variety of microorganisms under the correct nutritional conditions (Pan et al., 2012). The most commonly studied PHA is the homopolymer polyhydroxybutyrate (PHB), but other PHA homopolymers and co-polymers 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 different PHAs. While 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%–50% of the total costs of conventional production (Dai and McDonald, 2014; Pan et al., 2012). Efforts to produce PHAs from autohydrolyzate have been motivated by a need for a lower-cost carbon source (Pan et al., 2012).

Autohydrolyzate 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 due to 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 et al., 2012). Only a small

difference in xylose consumption was observed between the control and the detoxified substrates (Pan et al., 2012). In a different approach, recombinant *Escherichia coli* was engineered to produce endoxylanase to enable the consumption of xylan directly (Salamanca-Cardona et al., 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 to control) by allowing the accumulation of biomass-derived sugars before their uptake by microbes (Salamanca-Cardona et al., 2014).

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

9.5 Potential non-fuel applications—lignin

9.5.1 Recovery of insoluble (Ins) and soluble (Sol) lignin

Autohydrolyzate produced at the end of HTP contains 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 hydrolyzate is decreased. This scaling/incrustation tendency causes issues in the processing of hydrolysates (Gütsch and Sixta, 2012; Leschinsky et al., 2008b). The precipitate contains lignin of relatively HMW—insoluble fraction (Ins), whereas (poly)phenolics of LMW remain soluble—soluble fraction (Sol) (Fig. 9.1) (Bujanovic et al., 2012; Gütsch et al., 2012; Leschinsky et al., 2008b, 2009). The total amount of (poly)phenolics depends on the properties of wood and HTP conditions. For example, 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 et al., 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 et al., 2008b). This material presents a broad range of potential applications and represents an important avenue to improve the economics of biorefineries (Bujanovic et al., 2012). Moreover, HMW lignin and LMW (poly)phenolics interfere with EH and fermentation and should be removed from autohydrolyzate. HMW lignin poses a physical barrier restricting the access of enzymes to cellulose and by nonproductive adsorption of enzymes (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 microorganisms 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 microorganisms have been the focus of numerous efforts to improve the efficiency of fermentation processes (Klinke et al., 2004; Ko et al., 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 hydrolyzate (Gütsch et al., 2012; Leschinsky et al., 2009) or after acidification (of the hydrolyzate). For example, in HWE experiments (160°C, 2 h), the Ins fraction was recovered from the hot-water extract after acidification to pH 2 (Corbett et al., 2017). The Ins fractions recovered by centrifugation of either crude or acidified hydrolyzate contain lignin as the dominant component (Leschinsky et al., 2009); for example, 83% lignin content was found in the Ins fraction recovered from sugar maple (Corbett et al., 2017).

In pilot-scale HWE experiments performed in a 65 ft^3-digester using willow 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 et al., 2015; Nagardeolekar et al., 2020). Ins fractions containing relatively high lignin content were also recovered by membrane separation of autohydrolysates in laboratory conditions (Bujanovic et al., 2012; Goundalkar et al., 2010; Tian et al., 2021). For example, the Ins 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 et al., 2012).

Although successfully used in small-scale investigations, membrane separation is known to cause issues in commercial-scale applications due to frequent fouling. However, the fouling tendency of membranes may be reduced by various PTs of autohydrolyzate, such as centrifugation, pH adjustment, and oxidation, including the gas-phase pulsed corona discharge oxidation and laccase-catalyzed treatment of autohydrolysates (Mänttäri et al., 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 et al., 2011; Mänttäri et al., 2015; Virtanen et al., 2020).

Adsorption on ion-exchange resins (Nilvebrant et al., 2001), Amberlite/XAD resins (Lehto and Alén, 2012; Narron et al., 2017; Nitzsche et al., 2019; Schwartz and Lawoko, 2010; Vithanage et al., 2016; Zhang et al., 2019b), minerals such as siliceous materials, clay, and natural zeolites (Nitzsche et al., 2019; Ranjan et al., 2009; Soto et al., 2011) and activated carbon (Kim et al., 2013; Tian et al., 2021) have been used to separate lignin/LDPs from carbohydrates in the spent liquor produced by HTP. One important advantage of activated carbon is its higher effective surface area compared to resins and its diverse feedstock base, which includes coal and also renewable alternatives such as agriculture residues and lignin (Soto et al., 2011). However, the adsorbed compounds have been found to desorb more readily from XAD resins than from activated carbon (Zhang et al., 2019b). In

addition, in the adsorption of (poly)phenolics, activated carbon is considered less selective than XAD resins due to the simultaneous adsorption of carbohydrates. However, XAD16N was also shown to adsorb a relatively high amount of XOS accounting for more than 40% of the mass of adsorbate (Corbett et al., 2019; Narron et al., 2017). High-temperature adsorption onto activated charcoal (HiTAC process) has been recommended for use promptly after HTP, at the hydrolyzate 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 et al., 1991; Conde et al., 2009; Garrote et al., 2003; Goldschmid, 1954; Gonzalez et al., 2004; Lehto and Alén, 2012; Mänttäri et al., 2015; Tran and Chambers, 1986; Vázquez et al., 2005) or to separate the Sol fraction in sequence with the removal of the Ins fraction (Goundalkar et al., 2010; Leschinsky et al., 2009). 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 et al., 2017; Dongre et al., 2015; Leschinsky et al., 2009; Nagardeolekar et al., 2020) or microfiltration/ultrafiltration (Bujanovic et al., 2012; Goundalkar et al., 2010; Tian et al., 2021) as it may lead to low recovery yields (Kim et al., 2013) if conducted individually. The ethyl acetate extraction of crude hydrolyzate was conducted with no loss of carbohydrates and the removal of half of the dissolved lignin in the case of birch HTP (*Betula pendula*) (Lehto and Alén, 2012). Up to 65% of the lignin-related phenolics present in autohydrolyzate 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 et al., 1991).

9.5.2 Potential uses of the insoluble ("Ins") 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 1000 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 $0.30 per pound, this would be over $6 million in potential revenues per year (Ye et al., 2012). Consequently, it is becoming increasingly clear that to render the biorefinery focused on carbohydrate-based products economically feasible, lignin utilization for high-value products is imperative, warranting a departure from the traditional view of considering lignin as an energy source or waste.

Since the quality of lignin is generally not the focus of the PT process, 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. Here, we discuss the application of the Ins fraction in thermoset and hydrogel applications (Fig. 9.1). The properties of the Ins faction recovered from the autohydrolyzate (HTP, 160°C, 2 h) of HWs are listed in Tables 9.1–9.4.

9.5.2.1 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).

Table 9.1 Lignin content (acid-insoluble lignin (AISL) and acid-soluble lignin (ASL)) and ash content of the Ins fraction recovered from HTP (160°C, 2 h) based on oven-dried mass.

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

NHW, *mixed northern hardwoods;* SM, *Sugar maple;* W, *willow, and kraft lignin (KL; reference) (Dongre, 2018).*
[a] *Dongre (2018).*
[b] *Corbett et al. (2017).*

Table 9.2 Carbohydrate composition of Ins fraction recovered from HTP (160°C, 2 h) based on oven-dried mass.

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

NHW, *mixed northern hardwoods;* SM, *Sugar maple;* W, *willow, and kraft lignin (KL; reference).*
ND, *not detected.*
[a] *(Corbett et al., 2017).*
[b] *Method according to Corbett et al. (2017).*

Table 9.3 Free phenolic hydroxyl groups (PhOH) content and S/G ratios of the Ins fraction recovered from HTP (160°C and 2 h). Reference values of PhOH and S/G ratios from literature are also provided.

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

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

Table 9.4 Thermal properties and molecular weight distribution (number average (Mn), weight average (Mw) and polydispersity (PD)) of the Ins fraction recovered from HTP (160°C and 2 h).

Lignin sample ID	Glass transition temperature (Tg, °C)	Thermal degradation temperature (Td, °C)	Molecular weight distribution			References
			Mn	Mw	PD	
W	159	358	2186	5962	2.7	Dongre and Bujanovic (2019)
SM	143	356	–	–	–	Dongre (2018)
	–	–	1825	3058	1.7	Dongre et al. (2015)
	–	–	1600	4960	3.1	Bujanovic et al. (2012)

SM, *Sugar maple;* W, *willow.*

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 eco-friendly lignin–furfural resins (Dongre et al., 2015; Dongre and Bujanovic, 2019). This lignin contained xylose-derived carbohydrates (Table 9.2), which can convert to furfural in acid conditions; that is, furfural may be produced in situ. Furfural is a good crosslinking agent for lignin such that it can serve as a nontoxic substitute for formaldehyde, which is carcinogenic (Fig. 9.8). Glass fiber filters were impregnated with the synthesized resins and subjected to mechanical testing yielding a tensile index and Young's modulus comparable to those of a commercial PF resin. 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 seemed to govern the mechanical properties. It was also observed that resins produced from wood lignin with molecular weights within a narrow range (M_n: 6000–9000 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, that is, excessive or limited mobility compromises the tensile properties (Dongre, 2018). It must be noted that this observation is based on a limited amount of data, and further

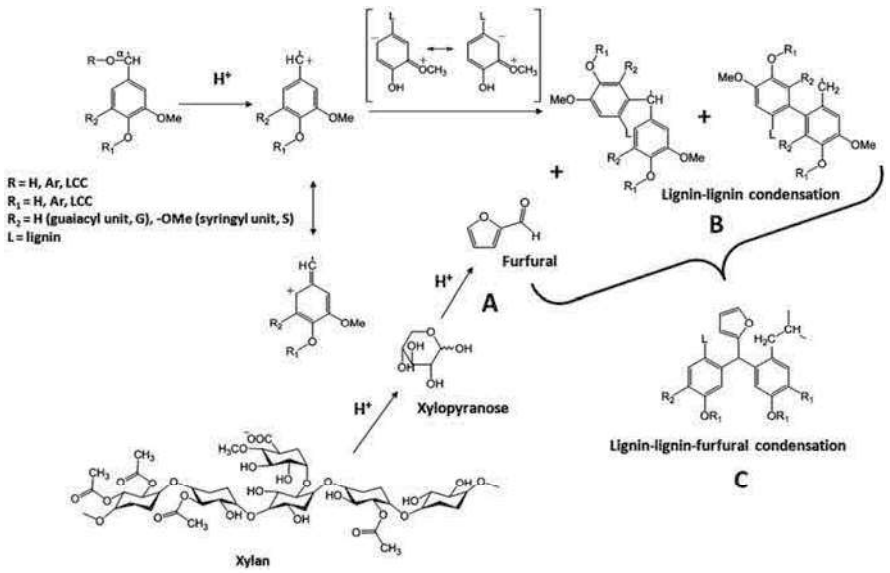


FIGURE 9.8

Proposed mechanism of lignin–lignin and lignin–furfural condensation products in acid conditions; in situ, xylan-containing lignin (Bu et al., 2011; Samuel et al., 2013; Yang et al., 2015).

Reprinted from (Dongre et al., 2015), open access.

research is required. Lignin-PF resins prepared from HTP-derived poplar lignin exhibited poorer bonding strength, lower formaldehyde emissions, and higher curing temperature (Yang et al., 2015).

9.5.2.2 Lignin-based hydrogels

Hydrogels are three-dimensional networks of chemically or physically crosslinked 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 since then due to 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 synthesis, recent research has focused on using biopolymers as sustainable alternatives. As the most abundant aromatic biopolymer, lignin has been a popular choice for this purpose due to its unique properties (Figueiredo et al., 2018; Thakur and Thakur, 2015). Apart from being nontoxic, biocompatible, and biodegradable, the antioxidant (AO) and antimicrobial 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 et al., 2019; Witzler et al., 2018). The relative hydrophobicity of lignin may also contribute to an increased shelf-life as compared to 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 autohydrolyzate by mild acid precipitation (Tables 9.1—9.4) (Nagardeolekar et al., 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. 9.9—9.11).

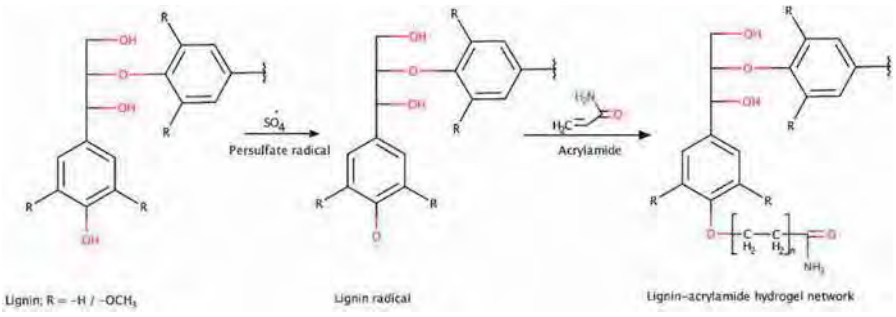


FIGURE 9.9

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

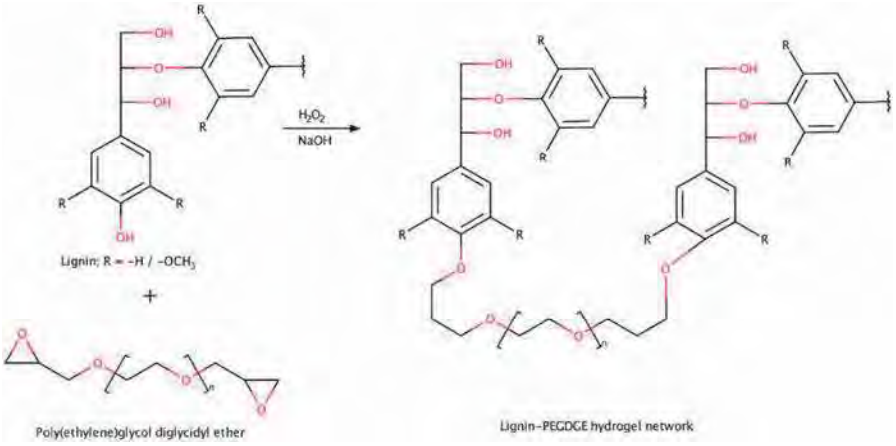


FIGURE 9.10

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

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 crosslinked 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% initial dry weight of hydrogel) than those containing SW kraft lignin (461%—529% initial dry weight of hydrogel). The better sorption profile of the W lignin over kraft lignin was hypothesized to stem from its less condensed S/G nature (S/G = 1.25 (Dongre et al., 2015)) and lower PhOH content (2.28 mmol/g (Nagardeolekar et al., 2020)) as compared to the more condensed G-type SW kraft lignin and its higher PhOH content (4.93 mmol/g (Nagardeolekar et al., 2020)). As a result, the hydrogel network formed by kraft lignin was thought to be highly crosslinked and dense, hence less permeable to solutions than the hydrogel network formed by the willow lignin; willow Ins fraction.

The overall beneficial effects of incorporation of lignin into the hydrogel matrix were also observed in a separate study of steam explosion lignin isolated from aspen wood (Ciolacu et al., 2012). In this 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 crosslinked with cellulose in an alkaline medium to synthesize a bio-based hydrogel network. Epichlorohydrin was used as the crosslinker with the hydroxyl groups on lignin and cellulose acting as the crosslinking sites to form the hydrogel matrix. The lignin to cellulose ratio varied

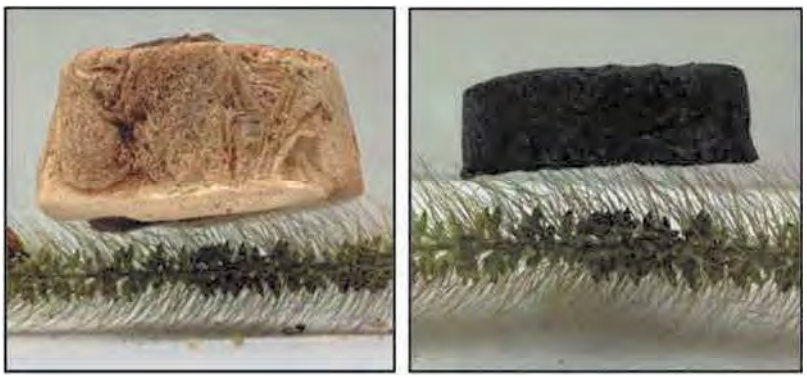


FIGURE 9.11

Lightweight and low-density ($\rho \leq 0.21$ g/cm^3) willow lignin-acrylamide-kaolin (a), and willow lignin—poly(ethylene)glycol diglycidyl ether (PEGDGE) (b), hydrogels after lyophilization, balanced on a spike of bristlegrass.

Reprinted with permission from Nagardeolekar et al. (2020).

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 3036% of the initial dry weight of the hydrogel containing the highest amount of lignin. Higher lignin content was also found to result in a higher percent release of absorbed polyphenols (Ciolacu et al., 2012).

It may be concluded that the lignin-rich insoluble fraction of HW autohydrolyzate (the Ins fraction; Fig. 9.1) may serve as an additional revenue source in biorefineries, as recent research has demonstrated its suitability to replace synthetic polymers as a renewable and environmentally friendly component of hydrogels and formaldehyde-free resins.

9.5.2.3 LCC valorization opportunities

Potential applications have been proposed for LCCs in the pharmaceutical, medical, and cosmetic fields (Zhao et al., 2020). LCCs isolated from poplar (*P. 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 et al., 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) activities and antioxidant activity synergistic with vitamin C (Sakagami et al., 2010). The combination of sugar and polyphenolic structures found in LCCs were hypothesized by the authors to be 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 et al., 2020).

9.5.3 Potential uses of the LMW ("Sol") fraction of lignin

Autohydrolyzate produced at the end of HTP contains a wide range of LMW (poly) phenolic compounds: the Sol fraction (Fig. 9.1) (Leschinsky et al., 2008b, 2009). The composition of the Sol fraction depends on the wood properties (e.g., species, origin, and age) and HTP parameters (Sixta, 2006; Wood et al., 2020). LMW (poly)phenolics encompass two categories of compounds, LDPs and (poly)phenolic extractives, for example, flavonoids, lignans, and stilbenes (Conde et al., 2013; Mänttäri et al., 2015), 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 hydrolyzate (Jönsson et al., 1998; Kim et al., 2013; Palmqvist and Hahn-Hägerdal, 2000a). If separated, depending on their structure, these compounds may be considered to replace petrochemicals and promote the bioeconomy through value addition 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. The majority of vanillin produced in the world today (>90%) is labeled "synthetic," as it is made from crude oil via the guaiacol route (~85%) and from lignin/lignosulfonates by alkaline oxidation (~15%) and sells for $12/kg (Fache et al., 2016; Smolarski, 2012). Vanillin produced from wood autohydrolyzate may be favored compared to vanillin from lignosulfonates, as it is sulfur-free and produced using water as the only solvent. Other high-value chemicals which may be recovered from wood autohydrolysates vary in selling price and include syringol ($15/lb) and syringaldehyde ($25/lb) from HWs (Devappa et al., 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.

LMW phenolics extracted with organic solvents from autohydrolysates of HW species include numerous compounds; however, hydroxybenzaldehydes and hydroxybenzoic (*p*-HBA) acids are typically the most abundant (Ares-Peón et al., 2016; Goundalkar et al., 2010; Vázquez et al., 2005). A limited dispersion of lipophilic extractives has also been reported, although dissolution of nonphenolic nonpolar extractives during HTP of wood is not expected to a large degree (Mänttäri et al., 2015; Örså et al., 1997; Tran and Chambers, 1986; Vázquez et al., 2005).

Many of these phenolics have been considered as promising value-added chemicals from biorefinery lignins, including vanillin, vanillic acid, and syringaldehyde (Holladay et al., 2007). Benzaldehydes may find use as platform chemicals for the production of active pharmaceutical ingredients (Ibrahim et al., 2012). Vanillic acid has been proposed to possess numerous pharmacological effects including antihypertensive and anti-inflammatory (in ulcerative colitis) effects (Kim et al., 2010; Kumar et al., 2011). 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 et al., 2019). In this case, members of Salicaceae family (willow, poplar) would be especially effective as feedstock as they contain lignin with p-hydroxybenzoates as readily hydrolyzable pendant groups (0.5%-2.9% of the dry weight of lignin) (Goacher et al., 2021). Phenolics released during HTP of aspen (P. *x euramericana* Neva; 170°C, 1 h) were remarkably abundant in *p-HBA* (>80%) (Wang et al., 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 et al., 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 based entirely on the use of renewable chemicals (Wang et al., 2020b).

9.5.3.1 Antioxidizing activity (ADA) and antimicrobial activity (AMA)

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 et al., 2006a, 2006b). Accordingly, (poly)phenolic compounds released during HTP may be considered for high-value applications that make use of these inherent bioactive properties, including antioxidizing (AOA) and antimicrobial activities (AMA).

(Poly)phenolic compounds/LDPs which typically appear in wood hydrolysates show a relatively high AOA and are widely considered renewable alternatives to synthetic antioxidants such as BHT (butylated hydroxytoluene), BHA (butylated hydroxyanisole), and tert-butylhydroquinone (TBHQ). Although these synthetic agents are categorized as generally recognized as safe, suitable alternatives should be developed to adequately address the urgent need for decarbonization and to grow the bioeconomy (Devaney and Iles, 2019).

Various assays measuring AOA, including radical scavenging methods (e.g., DPPH (2,2-diphenyl-1-picrylhydrazyl)), have been conducted on phenolic compounds to elucidate the structure-AOA relationship. Different reference compounds have been used for comparative purposes, such as Trolox (water-soluble analog of vitamin E), ascorbic acid (vitamin C), and the synthetic AO agents BHT and BHA. Although the results of various tests might be contradictory, some general trends have been established. 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-OH+ X- ----* AO" + 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, that is, benzoic acids are less potent AO agents than benzaldehydes (AOA: vanillic acid < vanillin, syringic acid < syringaldehyde). Monophenols are less efficient AO agents than polyphenols. Experiments with lignin model compounds demonstrated that tetramers, trimers, and dimers are more potent antioxidants than monomers. Lignin studies indicated that an increase in the methoxy group content improves AOA, whereas an increase in the MW or polydispersity reduces AOA. The presence of nonlignin components (e.g., carbohydrates) has also been shown to decrease the AOA of various lignins (Bortolomeazzi et al., 2007; Bountagkidou et al., 2010; Cuvelier et al., 1992; Dizhbite, 2004; Gonzalez et al., 2004; Ibrahim et al., 2012; Nenadis et al., 2007).

The total phenol content (TPC) and AOA (DPPH test) of ethyl acetate extracts of eucalyptus (*E. globulus*) 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. 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 et al., 2001).

In the application as antioxidants, crude autohydrolysates would be more feasible and economically beneficial than individual phenolics separated from autohydrolysates. In addition, due to synergistic effects, the mixture of phenolics in autohydrolysates may show a greater AOA than individual compounds. It must be noticed, however, that antagonistic effects have also been reported (Moure et al., 2001). TPC and AOA (DPPH test) of crude autohydrolysates produced in PHWE (100°C, 20 bar, 30 min) of larch wood fractions 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 certain (poly)phenolics (Ravber et al., 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 hydrolyzate produced at 190°C (5 min) was effective in protecting cherries from decay for 20 days, demonstrating its antimicrobial capacity (Zhang et al., 2020).

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 et al., 2020) and that wood autohydrolysates should be explored as additives to impart protection against detrimental UV-irradiation, free radicals, and microorganisms into various materials that lack resistance.

9.5.3.2 LMW phenolics as natural laccase mediators

LMW phenolics present in autohydrolysates may find use as laccase mediators as naturally occurring phenolics have been confirmed to promote laccase oxidation of aromatic compounds (Fig. 9.1). 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 (chlorinated phenols, fungicides/cyprodinil, halogenated pesticides), as antibacterial agents (such as triclosan), as pharmaceuticals (naproxene), as decolorization of recalcitrant dyes, and in 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 low MW, high oxidation power, creation of stable radicals which 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).

9.6 Conclusion

There is a large variety of products that can be produced from autohydrolyzate (the spent liquor of wood HTP). This is due, in large part, to the relatively mild conditions employed, which allow the potentially valuable structural features of hemicelluloses and lignin to remain even after dissolution. These structural features include reactive sites for crosslinking and functionalization, aromaticity, phenolic functionalities with antioxidant properties, and structures that can be further upgraded to commodity or fine chemicals. In contrast to high-intensity PTs that substantially degrade these native structures, HTP enables opportunities to add considerable and much-needed value to wood biorefineries, promoting their implementation and the growth of the bioeconomy.

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WOODHEAD PUBLISHING
An imprint of Elsevier

Woodhead Publishing is an imprint of Elsevier
50 Hampshire Street, 5th Floor, Cambridge, MA 02139, United States
The Boulevard, Langford Lane, Kidlington, OX5 1GB, United Kingdom

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ISBN: 978-0-323-91187-0

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Publisher: Megan Ball
Acquisitions Editor: Peter Adamson
Editorial Project Manager: Helena Beauchamp
Production Project Manager: Sharmila Kirouchenadassou
Cover Designer: Miles Hitchen

Typeset by TNQ Technologies

