

Topochemistry of delignification behaviors of various lignocelluloseics by thermochemical treatment

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Abstract

Lignin in lignocellulosic biomass exhibits pronounced heterogeneity in spatial distribution, chemical structure, and concentration within the cell wall. This heterogeneity plays a key role in governing delignification behavior during thermochemical treatments and should be understood from a topochemical perspective. Hydrothermal treatment generally results in preferential delignification from the secondary wall, while lignin in the compound middle lamella remains resistant due to its condensed structure. In contrast, subcritical phenolic and supercritical alcoholic solvent systems promote solvolytic reactions, enabling delignification to proceed even in more recalcitrant regions. Other treatments, including steam explosion, ionic liquids, and deep eutectic solvents, further demonstrate that solvent properties strongly influence lignin redistribution and the formation of secondary products. Overall, delignification is governed by the interplay of structural heterogeneity, linkage-dependent reactivity, solvent characteristics, and mass transport processes, providing key insights for the rational design of lignin valorization strategies.

Keywords: Delignification; Sub-/supercritical fluids; Thermochemical treatment; Topochemistry

INTRODUCTION

Lignocellulosic biomass has attracted considerable attention as an alternative to fossil resources toward the sustainable society. Among the major constituents of plant cell walls, lignin is the second most abundant natural polymer after cellulose and represents the largest renewable source of aromatic carbon on Earth. Owing to its aromatic backbone, high carbon content, and diverse functional groups, lignin has considerable potential as a feedstock for value-added chemicals, advanced materials, and bio-based products. However, the efficient utilization of lignin remains challenging because of its inherent structural complexity and heterogeneity. Variations in lignin composition, interunit linkages, and spatial distribution within the cell wall significantly influence its reactivity, extraction behavior, and subsequent conversion pathways. Therefore, efficient lignin valorization requires the selective depolymerization and extraction of lignin from lignocellulosic cell walls while minimizing undesirable side reactions and preserving targeted structural features. Accordingly, understanding and controlling lignin depolymerization and extraction remain important challenges.

Traditionally, delignification has been understood mainly from the perspective of chemical reaction mechanisms, such as ether bond cleavage and recondensation. However, in practice, these reactions occur within the hierarchical and heterogeneous structure of the cell wall, and are thus spatially non-uniform and strongly coupled with mass transfer processes. From this viewpoint, delignification should be regarded as a topochemical phenomenon governed by the spatial heterogeneity of the reaction field. The term “topochemistry,” as used in the title of this article, represents a conceptual framework that integrates morphological and chemical perspectives. Although this term may not be widely familiar, it has a long history. In wood science, the concept of “topochemical reactions” was introduced as early as the 1930s in studies on the reactivity of cellulose derivatives ^{1,2)}. Subsequently, with the rapid

development of the pulp and paper industry, advances in chemical pulping technologies led to extensive research on lignin degradation behavior, and the concept of the “topochemistry of delignification” became widely established³⁻⁶.

Recent advances in lignin valorization have driven the development of thermochemical conversion technologies, including organosolv processes and sub- and supercritical fluid treatments, leading to a resurgence of studies on the topochemistry of delignification. Furthermore, from the perspective of polysaccharide utilization, lignin distribution and its redistribution behavior within the cell wall have been recognized as key factors influencing enzymatic saccharification, particularly in terms of substrate accessibility⁷⁻⁹. With the expansion of biorefinery research, target biomass resources have extended beyond softwoods and hardwoods to include monocotyledonous plants such as grasses and palms, necessitating a deeper understanding of lignin distribution and reaction behavior across diverse lignocellulosic systems.

Despite the accumulation of these studies, most findings have been reported under specific processing conditions and material systems, and comprehensive reviews that systematically organize the topochemistry of delignification in thermochemical treatments remain limited. Therefore, this review first provides a concise overview of the heterogeneity of lignin distribution within lignocellulosic cell walls. Subsequently, focusing primarily on thermochemical treatments, particularly sub- and supercritical solvent systems, the topochemistry of delignification is discussed from a unified perspective as a coupled process of chemical reactions and mass transfer.

Heterogeneity of Lignin Distribution in Lignocellulosic Cell Walls

Lignin is heterogeneously distributed within the cell wall, and its chemical structure and concentration vary depending on the cell type and its morphological region. Such heterogeneity provides the basis for understanding delignification behavior during thermochemical treatments, as discussed below. Detailed descriptions of lignin distribution and structural heterogeneity within the cell wall have already been comprehensively reviewed elsewhere¹⁰⁻¹², therefore, this section focuses on key aspects directly relevant to delignification behavior.

From the perspective of spatial distribution, lignin is known to be highly concentrated in the intercellular layer and the compound middle lamella (CML, the structural region comprising the intercellular layer and primary walls of adjacent cells), while its concentration is relatively lower in the secondary wall, particularly in the S₂ layer^{13, 14}. This distribution is consistent with its roles in cell adhesion and mechanical support, while also influencing accessibility of reagents and solvents to lignin within the cell wall, as well as mass transport within the reaction field. Furthermore, the xylem is composed of various cell types, including fibers, vessel elements, and ray and axial parenchyma, each exhibiting differences in wall thickness and ultrastructure, resulting in heterogeneity in lignin distribution even at the cellular level¹⁴.

From a chemical perspective, the reactivity of lignin is strongly governed by its linkage structure. Ether linkages, such as the β -O-4 linkage, are relatively susceptible to cleavage, whereas carbon-carbon linkages, such as β - β , β -5, and 5-5 linkages, representing condensed structures, exhibit high stability¹⁵. These linkage types are not uniformly distributed within the cell wall; in general, the CML is enriched in condensed linkages compared with the secondary wall¹⁶⁻¹⁸. Such structural differences are critical in determining the spatial progression of delignification during thermochemical treatments. This heterogeneous distribution of linkage structures has been elucidated using a variety of analytical techniques. For example, immunolabeling with monoclonal antibodies has enabled the localization of specific interunit linkages within the cell wall¹⁷, while autoradiographic approaches using labeled monolignols have provided insights into spatially resolved lignin formation and linkage distribution¹⁸. These methodological advances have contributed significantly to our understanding of the relationship between lignin structure and spatial localization.

In addition, in hardwoods, the distribution of aromatic units in lignin also varies depending on the cell wall region. Advanced analytical techniques, such as time-of-flight secondary ion mass spectrometry (TOF-SIMS), have been employed to visualize the distribution of syringyl and guaiacyl

units within cell wall regions¹⁹). The secondary wall of fibers is typically rich in syringyl units, whereas vessel elements and the CML tend to have higher proportions of guaiacyl units^{14,19,20}). These differences in lignin composition are thought to influence the distribution of linkage structure and lignin reactivity.

Moreover, in angiosperms, particularly monocotyledonous plants, lignin is often chemically modified by the incorporation of pendent groups such as *p*-coumaric acid and *p*-hydroxybenzoic acid^{21–24}). These modifications may affect interactions with solvents and solvolytic reactions. While UV-based methods provide information related to chromophoric structures, Raman spectroscopy enables the mapping of specific vibrational modes associated with lignin and related compounds.

The concentration of lignin also exhibits pronounced heterogeneity. In general, the CML contains approximately twice the lignin concentration of the secondary wall, with particularly high concentrations found in the cell corner regions^{13,14}). Furthermore, differences in lignin concentration are observed among cell types in hardwoods, including vessel elements, fibers, and parenchyma cells, and similar trends have been reported in monocotyledonous plants as well²⁵).

Overall, lignin exhibits heterogeneity within the cell wall in terms of spatial distribution, linkage structure, aromatic unit composition, chemical modifications, and concentration. These factors are considered to collectively influence both chemical reactivity and mass transport within the cell wall. Therefore, the topochemistry of delignification can be understood as a phenomenon that emerges from these structural heterogeneities.

Topochemistry of Delignification by thermochemical treatment

As described in the previous section, lignin exhibits heterogeneity in both structure and concentration within the cell wall. Such heterogeneity is reflected in delignification behavior during thermochemical treatments and cannot be adequately described as a simple homogeneous reaction. Rather, delignification proceeds as a coupled process involving chemical reactions (cleavage of linkages and recondensation) and the diffusion and solubilization of reagents and degradation products. As a result, the reaction progresses in a spatially heterogeneous manner within the cell wall. As will be shown in the following sections, delignification often proceeds from the secondary wall toward the CML, and can be interpreted in terms of a reaction front. In this section, the topochemistry of delignification in conventional chemical and mechanical pulping processes is first summarized. Subsequently, thermochemical treatments, particularly sub- and supercritical solvent systems designed to isolate solvent effects, are discussed with emphasis on differences among solvent types. Finally, recent developments in the topochemistry of delignification under other thermochemical treatments are outlined.

Chemical and mechanical pulping processes

The heterogeneous distribution of lignin within the cell wall also contributes to delignification behavior during chemical pulping processes. In conventional pulping methods, such as soda pulping, sulfite pulping, and kraft pulping, preferential delignification from the secondary wall (SW) relative to the middle lamella (ML) has been widely observed^{3–5}). In particular, studies based on UV microspectroscopic analyses have indicated that, in the initial stages of chemical pulping, preferential delignification from the SW proceeds in the order of kraft > acidic sulfite > neutral sulfite processes⁴). However, more recent studies on acid sulfite pulping have suggested that the penetration of cooking liquor and sulfonation reactions are not restricted to the SW and may also facilitate delignification in lignin-rich ML regions, leading to more complex topochemical behavior than previously recognized^{26, 27}). However, direct comparison between these studies should be made with caution because the investigated species differ, and species-dependent differences in lignin structure may also contribute to the observed variations.

In the case of organosolv pulping, delignification behavior varies significantly depending not only on the solvent species but also on the type of catalyst, similar to trends observed in sub- and supercritical solvent treatments discussed later. For example, when Norway spruce is treated in methanol in the presence of Mg(NO₃)₂ and CaCl₂ catalysts, delignification initially proceeds in the ML,

including the cell corner region (MLcc), while lignin in the SW dissolves more gradually²⁸⁾. In contrast, when methanol containing NaOH is used, preferential delignification from the SW is observed, similar to other chemical pulping processes²⁹⁾.

In mechanical pulping, delignification is negligible compared with chemical pulping, however, the behavior of cell wall fragmentation and fiber separation depends strongly on the processing conditions. For instance, refiner mechanical pulp (RMP) treatment often results in random cell separation and internal fragmentation within the cell wall owing to intensive mechanical action during refining. In contrast, thermomechanical pulp (TMP) and chemi-thermomechanical pulp (CTMP) processes utilize steam and/or chemical pretreatment to soften wood tissues prior to refining, thereby promoting fiber separation while reducing extensive cell wall damage³⁰⁾.

Furthermore, the fracture and delamination behavior of the cell wall also depends on the intrinsic properties of different morphological regions, which vary with the wood species. For example, in pine, fiber separation predominantly occurs at the interface between the SW and ML, whereas in spruce, delamination has been reported to occur within the SW, particularly between the S₁ and S₂ layers. These differences in fracture behavior significantly affect the physical and chemical properties of pulp surfaces³¹⁾. In addition, the thermoplastic behavior of lignin and its spatial distribution influence subsequent processing steps, making these effects important not only for pulp quality but also from the perspective of energy efficiency and processing costs³²⁾. More recently, mechanical pretreatment methods have been increasingly employed to enhance enzymatic saccharification of cellulose substrates. It has been demonstrated that differences in lignin distribution, depending on wood species and mechanical processing conditions, significantly affect enzymatic hydrolysis efficiency³³⁾.

Hydrothermal treatment

Among thermochemical treatments, we first describe the topochemistry of delignification during hydrothermal treatment using sub- and supercritical water. Depending on temperature and pressure, substances can exist in various phases, including solid, liquid, and gas. When the critical temperature (T_c) and critical pressure (P_c) are exceeded, the substance enters a supercritical state, forming a high-density, non-condensable fluid. For water, the critical point is T_c = 374 °C and P_c = 22.1 MPa; water above these conditions is defined as supercritical water, whereas subcritical water refers to water under conditions near but below the critical point. Sub- and supercritical water allow the tuning of important parameters of the reaction field, such as dielectric constant and ionic product, through changes in temperature and pressure. As a result, solvent properties can be continuously and significantly modulated, enabling water alone to span a wide range of characteristics from aqueous to non-aqueous-like environments. In particular, under supercritical conditions, the ionic product of water can vary significantly depending on temperature and pressure, particularly under subcritical and near-critical conditions, enhancing its hydrolytic capability³⁴⁾. In this section, we focus on studies using pure water without additional catalysts, and limit the discussion to examples employing two types of reactor systems: semi-flow and batch systems. In the batch system, reactants and solvent are sealed in a reaction vessel and subjected to the reaction, whereas in the semi-flow system, the sample is fixed in a reaction cell composed of sintered mesh and exposed to a continuous flow of solvent, allowing solubilized products to be rapidly removed from the system. Because the physical form of the sample significantly influences reactivity, the discussion is limited to cases using extractive-free sapwood flour unless otherwise noted.

In hydrothermal treatment, polysaccharides are hydrolyzed along with lignin, and therefore lignin is not selectively removed. At lower temperatures, hemicelluloses and part of the amorphous cellulose are decomposed, while at higher temperatures, crystalline cellulose is also degraded³⁵⁾. For example, when Japanese cedar and beech are subjected to semi-flow hydrothermal treatment at 230 °C/10 MPa/15 min, nearly all hemicelluloses are decomposed and solubilized, and lignin is partially removed (44% for cedar and 49% for beech). Subsequent treatment at higher temperature (270 °C/10 MPa/15 min) results in an additional 21% and 28% of lignin were removed from cedar and beech, respectively³⁶⁾. UV microspectroscopic observations of the treated samples (Figure 1) reveal preferential delignification from the SW in both species³⁵⁾. Transmission electron microscopic (TEM) observations after staining with 1% KMnO₄ indicate that the SW is largely removed, whereas the ML,

including the ML_{cc}, remains as a highly resistant structure. Although condensation reactions of lignin during hydrothermal treatment cannot be excluded, these results clearly demonstrate the topochemical differences in delignification behavior between these regions. SEM observations of insoluble residues after low-temperature treatment (230 °C) revealed the formation of numerous micrometer-scale voids within the cell wall. It is considered that lignin dissolved from the SW is transported through these voids and released into the lumen. Furthermore, analysis of

group content in the residual lignin shows a decrease in methoxy content per lignin unit as the reaction proceeds ³⁶⁾. Because methoxy groups in lignin are highly stable under thermochemical conditions and no catechol-type products derived from demethoxylation were identified in the soluble fraction, this change is more reasonably attributed to the preferential removal of guaiacyl units in softwoods and syringyl units in hardwoods rather than to demethoxylation. This interpretation is consistent with preferential delignification from the SW because syringyl-rich lignin is predominantly distributed in the secondary wall, whereas guaiacyl-rich lignin is relatively enriched in the ML and ML_{cc}.

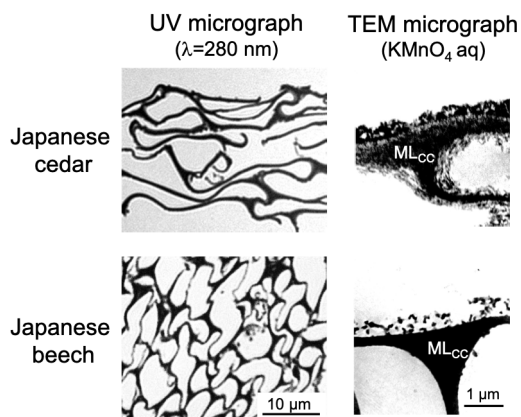


Fig.1. UV and TEM micrographs of insoluble residues from Japanese cedar and beech as treated by semi-flow hot-compressed water. ML_{cc}: middle lamella at cell corner region ³⁵⁾.

Among lignin structural factors, differences in linkage types significantly influence reactivity during hydrothermal treatment. Although model compound studies are difficult to perform in semi-flow systems due to reactor design, batch-type reactor studies using dimeric model compounds have shown that β -O-4 linkages are readily cleaved under sub- and supercritical conditions, producing monomeric products, whereas 5-5 linkages remain intact and exhibit strong resistance to hydrothermal treatment ³⁷⁾. Consistently, alkaline nitrobenzene oxidation analysis of lignin residues obtained from semi-flow hydrothermal treatment of cedar and beech shows a decrease in product yield as treatment proceeds, indicating preferential cleavage of ether linkages and the persistence of condensed structures ³⁸⁾. These results suggest that lignin in the ML is enriched in condensed carbon-carbon linkages, such as β -5 and 5-5 linkages, and therefore remains as an insoluble residue due to its resistance to degradation. However, because condensation reactions may also occur during hydrothermal treatment, the contribution of newly formed condensed structures in the residual lignin cannot be completely excluded. Therefore, these observations should be interpreted in conjunction with the previously reported topochemical distribution of lignin structures within the cell wall.

In monocotyledonous biomass, such as corn cob, semi-flow hydrothermal treatment (230 °C/10 MPa) results in more efficient delignification compared to woody biomass ³⁹⁾. Microscopic analysis of insoluble residues shows preferential delignification from the SW and strong resistance of ML lignin. The alkaline nitrobenzene oxidation yields are also low, suggesting that ML lignin is similarly enriched in condensed structures and resistant to hydrothermal degradation. Terminal *p*-coumaric acid residues are readily decomposed and solubilized, and are recovered as monomeric products in the aqueous fraction ³⁹⁾, with little remaining in the insoluble residue. Correspondingly, UV absorption spectra of

the residue do not show the characteristic long-wavelength absorption associated with cinnamic acid derivatives.

In batch-type hydrothermal treatment, similar topochemical behavior is observed as in semi-flow systems, with preferential delignification from the SW and strong resistance of ML lignin in both softwoods and hardwoods (Figure 2a). A notable difference is that frequent separation of cells is observed in batch systems, which is attributed to physical agitation within the reaction vessel.

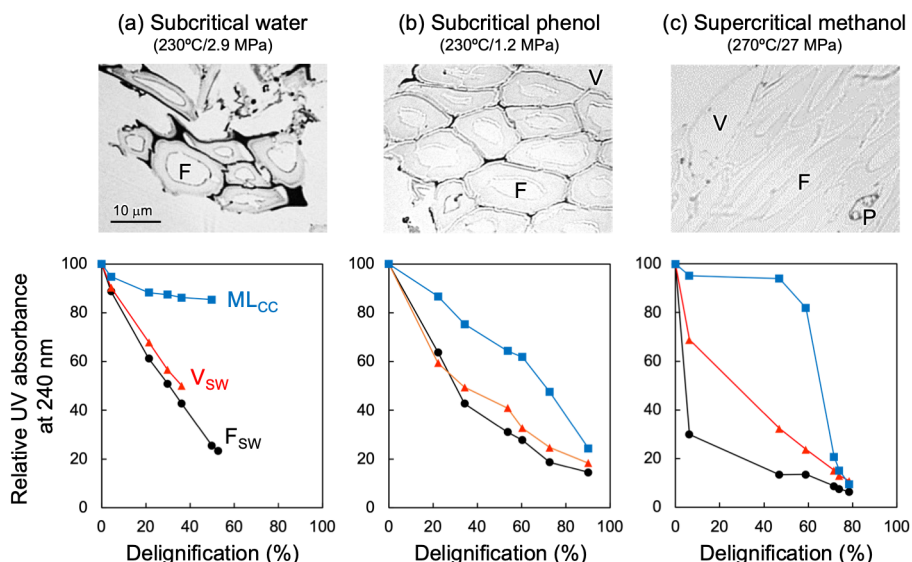


Fig. 2. UV micrographs of insoluble residues from Japanese beech treated by (a) subcritical water (230°C/2.9 MPa/10 min), (b) subcritical phenol (230°C/1.2 MPa/30 min), (c) supercritical methanol (270°C/ 27 MPa/30 min) in batch systems, and the relative change in UV absorbance (bottom). SW: secondary wall, ML_{CC}: middle lamella at cell corner, F: fiber, V: vessel, P: parenchyma.

Subcritical phenol treatment

The decomposition of lignin in sub- and supercritical fluids using solvents other than water has also been extensively studied. In this section, the behavior of lignin during treatment with phenolic solvents is first described. The critical point of phenol is $T_c = 421\text{ }^{\circ}\text{C}$ and $P_c = 6.1\text{ MPa}$; however, conditions used for lignin removal are generally milder than these values. Therefore, the discussion here focuses on lignin degradation under subcritical phenolic conditions⁴⁰⁾. A notable feature of subcritical phenol treatment is that lignin can be selectively decomposed and solubilized, while polysaccharide components are largely preserved^{41, 42)}. Regarding lignin degradation behavior, alkaline nitrobenzene oxidation analyses have shown that, although less effective than hydrothermal treatment, preferential cleavage of ether linkages still occurs⁴³⁾. Furthermore, analysis of the amount of reactive phenol incorporated into the soluble fraction, together with methoxy group analysis of the isolated phenol-soluble lignin, has demonstrated that lignin undergoes phenolysis and is solubilized as phenolic derivatives⁴²⁾. Similar trends have been observed in subcritical treatments using catechol, 2,4-dimethylphenol, anisole, and m-cresol, where preferential lignin decomposition is also promoted⁴⁴⁾. Among these solvents, catechol results in the lowest yield of insoluble residues and the highest degree of solubilization, likely due to the presence of two highly reactive phenolic hydroxyl groups. In contrast, anisole, which lacks phenolic hydroxyl groups, shows limited decomposition.

The topochemistry of delignification during subcritical phenol treatment (230 °C/1.2 MPa) has also been investigated. Figure 2 compares UV micrographs of insoluble residues obtained from beech treated with subcritical water (230 °C/2.7 MPa) and subcritical phenol, along with changes in relative UV absorbance in each morphological region. Similar to hydrothermal treatment, preferential

delignification from the SW is observed. However, in contrast to hydrothermal treatment, the MLcc, which exhibits strong resistance in water, is ultimately delignified under phenolic conditions (Figure 2b). This difference can be attributed to the phenolysis-induced modification of lignin. Not only SW lignin, which is rich in ether linkages, but also ML lignin, enriched in condensed structures, becomes phenolated, resulting in increased affinity for the solvent and enhanced solubilization. These results clearly demonstrate that the topochemistry of delignification is strongly dependent on the nature of the solvent.

Supercritical alcohol treatment

Lignin decomposition also proceeds during thermochemical treatments using sub- and supercritical alcohols. The critical point of methanol, which is the most extensively investigated alcohol solvent for lignin conversion, is approximately $T_c = 239\text{ }^{\circ}\text{C}$ and $P_c = 8.1\text{ MPa}$. Since lignin removal under catalyst-free conditions is generally achieved under supercritical conditions, the following discussion focuses on lignin degradation behavior in supercritical alcohol systems.

Minami et al. reported that, under supercritical methanol treatment ($270\text{ }^{\circ}\text{C}/27\text{ MPa}$), lignin in both Japanese cedar and beech is partially decomposed and solubilized⁴⁵⁾. Similar to hydrothermal and subcritical phenol treatments, a decrease in ether-type linkages in the residual lignin has been observed as the treatment proceeds. Consistent with this, model compound studies have demonstrated that ether linkages are readily cleaved, whereas condensed carbon–carbon linkages, such as β –5 and 5–5 linkages, remain relatively stable under these conditions⁴⁶⁾. This reaction behavior has also been observed for various monohydric linear alcohols, such as ethanol, 1-propanol, 1-butanol, and 1-octanol⁴⁷⁾. In particular, longer-chain alcohols have been shown to dissolve higher-molecular-weight lignin-derived products more effectively, likely due to enhanced solubility resulting from the incorporation of larger alkyl groups into the degradation products.

The topochemistry of delignification during supercritical methanol treatment of beech ($270\text{ }^{\circ}\text{C}/27\text{ MPa}$) is shown in Figure 2c⁴⁸⁾. As observed in subcritical phenol treatment, preferential delignification from the SW occurs initially, followed by delignification from the ML at later stages. This behavior in the ML can be attributed to solvolytic reactions (alcohololysis), which promote cleavage and modification of lignin, distinguishing it from hydrothermal conditions.

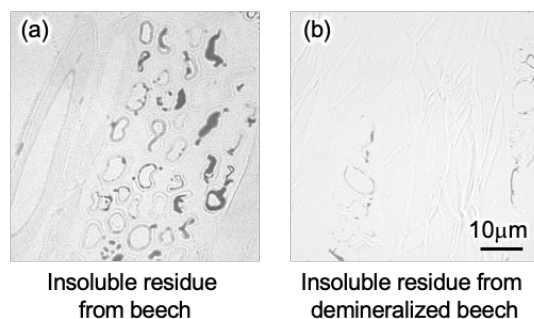


Fig. 3. UV micrographs of parenchyma region in the insoluble residue by supercritical methanol treatment ($270\text{ }^{\circ}\text{C}/27\text{ MPa}/30\text{ min}$) from (a) beech wood and (b) demineralized beech wood⁴⁷⁾.

In addition, localized formation of secondary products, likely derived from lignin and/or carbohydrates, has been observed in parenchyma cell lumina and vessel pits (Figure 3a). These regions are known to function as pathways for mass transport, where inorganic species and proteins may be present. To investigate their influence on delignification behavior, demineralization pretreatment was conducted prior to supercritical methanol treatment. As a result, the formation of UV-absorbing secondary products was suppressed (Figure 3b), and delignification efficiency was improved, suggesting that locally distributed inorganic species influence the formation of secondary products during supercritical methanol treatment. Similar localized formation of colored products within parenchyma lumina has also been reported in the pyrolysis of poplar⁴⁹⁾. Furthermore, model compound

studies examining the influence of proteins have shown that reactions between polysaccharides and proteins suppress the formation of furanic compounds and carbonaceous products, while also decreasing the selectivity toward lignin-derived monomers⁵⁰). These findings indicate that coexisting components can significantly influence lignin decomposition behavior. Although there are currently no detailed studies on the topochemistry of delignification in alcohols other than methanol, the influence of alkyl chain length on lignin degradation behavior suggests that the topochemical behavior of delignification is also likely to vary among different alcohol systems.

Other chemical treatments

In addition to sub- and supercritical fluid treatments, the topochemistry of delignification has also been investigated in a variety of thermochemical processes, including steam treatment (steam explosion), alkaline and acid treatments, ionic liquid processing, and deep eutectic solvent (DES) treatments^{7-9, 33, 51-58}). It should be noted that the form of the biomass used in these studies varies, including wood chips, sections, and powders, which can significantly influence the observed reactivity and structural changes. Steam treatment involves the use of water in the vapor phase at temperatures above its boiling point under pressurized conditions. In particular, rapid pressure release following such treatment is referred to as steam explosion, which induces significant disruption of the cell wall structure. During this process, changes in the distribution of lignin within the cell wall have been reported, and even under catalyst-free conditions, the resulting tissue structure differs from that obtained via hydrothermal treatment⁵²).

For example, TEM observations of softwood TMP after steam treatment (160 °C/10 min) have revealed the formation of voids within the SW, indicating degradation and solubilization of SW lignin. In contrast, SEM observations have shown the formation of droplets on cell surfaces (Figure 4). These droplets are considered to originate from lignin that has migrated from the secondary wall^{7, 8}). This phenomenon, often referred to as lignin migration, varies depending on biomass type, with differences in the extent of occurrence and droplet size between softwoods and monocotyledonous plants, suggesting a strong dependence on lignin structure⁵³). Such changes in lignin distribution during steam treatment enhance the accessibility of cellulose substrates to enzymes, and therefore this process is widely used as a pretreatment to improve enzymatic saccharification. Furthermore, additional improvements in saccharification efficiency have been reported when steam treatment is combined with catalysts such as alkali or acids. For instance, Wu et al. demonstrated that alkaline sulfonation treatment of softwood results in minimal change in the ML, while delamination occurs within the SW, significantly enhancing enzymatic saccharification efficiency³³).

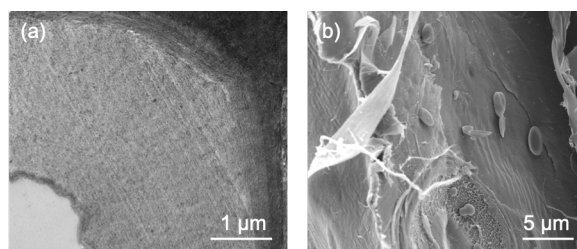


Fig. 4. (a) TEM and (b) SEM micrographs of steam treated softwood TMP (160°C/10 min). For TEM, lignin was stained with 1%KMnO₄ aq.

In treatments using ionic liquids, such as 1-ethyl-3-methylimidazolium acetate ([C2mim][OAc]), penetration of the solvent into poplar cell walls induces swelling of the structure, leading to lignin dissolution from the SW, while the ML remains relatively resistant^{54, 55}). Similar delignification behavior has been observed in other ionic liquid systems, such as 1-ethylpyridinium bromide ([EtPy][Br])⁵⁶), as well as in deep eutectic solvents treatments using choline chloride-based systems (e.g., choline chloride/lactic acid and choline chloride/vanillin)^{57, 58}).

These studies collectively demonstrate that the topochemistry of delignification plays a crucial role across a wide range of thermochemical treatments, particularly those involving solvent-based processes. The analytical techniques introduced above were originally developed for native lignin in cell walls, and therefore their application to thermochemically modified lignin structures requires caution. In particular, degradation, condensation, migration, and re-deposition of lignin during treatment may alter both the chemical structure and spatial distribution of lignin within the cell wall. Furthermore, UV-absorbing secondary products derived from lignin and/or carbohydrates may be formed during thermochemical processing. Consequently, microscopy images obtained after treatment do not always directly reflect the original distribution or structure of native lignin, and their interpretation should be supported by complementary chemical analyses.

Based on the findings discussed above, the topochemistry of delignification can be understood as being governed by the integrated effects of (i) structural heterogeneity among cell wall regions, (ii) reactivity differences associated with linkage structures, (iii) solvent characteristics, and (iv) the interplay between diffusion and migration of reaction products.

CONCLUSIONS

1. Lignin within lignocellulosic cell walls exhibits heterogeneity in spatial distribution, chemical structure, and concentration depending on cell type and its morphological region. This intrinsic heterogeneity governs the topochemical behavior observed during delignification, leading to preferential removal of lignin from the secondary wall and the persistence of condensed lignin in the compound middle lamella.
2. The topochemistry of delignification is strongly dependent on solvent characteristics. While hydrothermal treatment results in selective removal of lignin from more accessible regions, phenolic and alcoholic solvent systems promote solvolytic reactions, enabling delignification to proceed even in structurally recalcitrant regions and altering lignin redistribution behavior.
3. Overall, delignification should be understood as a coupled reaction–diffusion process governed by the interplay of structural heterogeneity, linkage-dependent reactivity, solvent properties, and mass transport phenomena, including diffusion and migration of degradation products. This comprehensive framework provides a basis for the rational design of efficient lignin valorization strategies.

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