A Review on Lignin Valorization for Sustainable Resource Recovery: Current Microbial and Enzymatic Methods and the Roles of Ionic Liquids and Deep Eutectic Solvents

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Lignin, as the most abundant renewable aromatic polymer on earth, holds immense potential as a feedstock for value-added products. However, its recalcitrant and heterogeneous structure presents significant challenges to efficient valorization. While microbial and enzymatic bioconversion offers a sustainable and specific route for lignin depolymerization, industrial implementation is often hindered by limitations such as low enzymatic efficiency, poor operational stability, and restricted substrate accessibility. This review systematically summarizes the current state of lignin bioconversion, focusing on the capabilities of various fungi and bacteria and the ligninolytic enzymes they produce, notably laccases and peroxidases. A key emphasis is placed on the emerging roles of "green" solvents, specifically ionic liquids (ILs) and deep eutectic solvents (DESs), in overcoming these limitations. These solvents not only enhance lignin solubility but also can activate and stabilize ligninolytic enzymes, thereby enabling more efficient depolymerization reactions. This review examines the mechanisms, advantages, and current challenges of integrating ILs and DESs into biomass lignin upgrading strategies. Finally, it discusses future research directions and potential application prospects.

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INTRODUCTION

Lignin, one of the three primary components of lignocellulosic biomass alongside cellulose and hemicellulose, is the second most abundant natural renewable polymer after cellulose (Li *et al.* 2025). This complex macromolecule is composed of phenylpropane units linked by C-C and C-O bonds. It imparts structural rigidity to plant cell walls and plays a crucial role in defense against microbial and insect attacks. However, this same structural complexity hinders the efficient conversion of lignocellulosic materials into pulp or biofuels (Vanholme *et al.* 2010). The pulp and paper industry alone is estimated to generate approximately 70 million tons of lignin annually (Nadányi *et al.* 2022). Additionally, the rapidly expanding bioenergy industry is projected to contribute an additional 62 million tons of lignin residues each year from global cellulosic ethanol production (Chen and Wan 2017). Despite this abundance, industrial lignin utilization remains below 5%, primarily due to its recalcitrant nature, which arises from complex inter-unit linkages, high degree of polymerization, and inherent heterogeneity (Dessie *et al*. 2023). Consequently, most lignin is currently burned as a low-value fuel, a practice that

contradicts the sustainability goals of "carbon peaking" and "carbon neutrality". Therefore, developing efficient valorization pathways is crucial for advancing the lignocellulosic biomass industry.

Lignin depolymerization is a key strategy for the valorization of this complex biopolymer (Zhou *et al*. 2023). This process involves cleaving the chemical bonds between structural units to reduce the molecular weight and heterogeneity of lignin, thereby producing relatively uniform low molecular weight products, such as monomers, dimers, and low molecular weight oligomers. For industrial lignin, depolymerization methods are primarily categorized as thermochemical methods (*e.g*., high-temperature pyrolysis, hydrogenolysis, catalytic oxidation, gasification) and biological methods (utilizing microorganisms and enzymes). Unlike thermochemical conversions, which typically require significant energy input, biological conversions generally occur under milder and more selective conditions. Biological degradation involves the mineralization of organic matter by microorganisms such as fungi, bacteria, and cyanobacteria. Under aerobic conditions, many microorganisms secrete specific enzymes, including laccases and peroxidases, which act on lignin polymers to promote their depolymerization and conversion (Chio *et al*. 2019; Polman *et al*. 2021), ultimately producing carbon dioxide and water.

Biodegradation presents a compelling alternative to thermochemical methods, operating under milder conditions with advantages in environmental sustainability, specificity, and cost-effectiveness. The use of enzymes as green biocatalysts further enhances this profile, offering a renewable and often less toxic alternative to conventional chemical catalysts. Nevertheless, the practical application of lignin biodegradation is hampered by significant challenges, such as low enzyme production yields, slow reaction kinetics, and the inherent instability of many ligninolytic enzymes. This review explores advanced microbial and enzymatic depolymerization strategies, with a particular focus on the emerging role of "green" solvents, specifically ionic liquids (ILs) and deep eutectic solvents (DESs), in enhancing lignin valorization, aiming to promote the development of this promising valorization method.

Lignin-Degrading Microorganisms

In nature, the degradation of wood is primarily driven by the combined action of bacteria, fungi, and other microbial communities. Certain microorganisms have the ability to modify and degrade lignin, although the extent of their action varies depending on the type of microorganism.

Bacteria

Bacteria are found in various environments, such as soil, compost fermentation products, and termite intestines, where they play an important role in lignin degradation. These microorganisms are characterized by short growth cycles, small size, and large populations. Lignin-degrading bacteria are primarily concentrated in the phyla Actinobacteria, Proteobacteria, and Firmicutes (Bugg *et al*. 2011, Ahmad *et al*. 2010). Among these, *Actinobacteria* exhibits the strongest and most stable lignin degradation capacity. Zhang *et al*. (2019) found that *Mycobacterium smegmatis*, a bacterium from the Actinobacteria phylum, can efficiently degrade lignin in lignocellulosic biomass, achieving a lignin degradation rate of 50% after 8 days of treatment while maintaining high levels of manganese peroxidase (MnP) activity.

Fungi

Fungi are generally more efficient lignin degraders than bacteria, primarily due to their robust secretory systems capable of producing a diverse array of extracellular oxidative enzymes that directly attack the recalcitrant lignin structure (Martínez *et al*. 2005). They interact with substrates through spores or mycelium, then release various enzymes to degrade the fiber cell wall. Based on the type of degradation they perform, wood decay fungi can be classified into three main categories: white-rot fungi (*e.g*., *Phanerochaete chrysosporium*, *Trametes versicolor*, *Pleurotus ostreatus*), brown-rot fungi (*e.g*., *Gloeophyllum trabeum*, *Rhodonia placenta*), and soft-rot fungi (*e.g*., *Chaetomium globosum*, *Aspergillus niger*). Different strains vary in their ability to degrade woody fibers, with white-rot and brown-rot fungi being the most prevalent and effective in biodegradation. Given the distinct advantages and limitations of different fungal types in lignin degradation, a comparative summary is provided in Table 1 to offer a clear overview of their degradation characteristics, facilitating the selection of appropriate strains for specific applications.

Table 1. Comparison of Lignin Degradation Characteristics by Different Wood-Decay Fungal Types

Fungal Type	Advantages in Lignin Degradation	Disadvantages in Lignin Degradation	Degradation Effects and Characteristics
White-Rot Fungi	1. The only fungus capable of completely degrading lignin to CO_2 and H_2O. 2. Most comprehensive enzymatic system. 3. Most widely used, with mature technology.	1. Most strains degrade non-selectively, leading to cellulose loss. 2. Longer growth and degradation cycles. 3. Sensitive to cultivation conditions.	Highly efficient degradation, but efficiency varies greatly depending on the strain and conditions
Brown-Rot Fungi	1. Rapidly degrades cellulose and hemicellulose. 2. Leaves modified lignin residues.	1. Cannot fully degrade lignin, only modifies and partially depolymerizes lignin through Fenton's reaction. 2. Residual lignin structure is complex and difficult to utilize.	Primarily acts on modification, with low degradation rates, aiming to obtain encapsulated polysaccharides.
Soft-Rot Fungi	1. Strong environmental adaptability, tolerant to high humidity. 2. Some strains produce lignin peroxidase and other oxidases.	1. Weakest degradation ability. 2. Very slow degradation rate. 3. Primarily targets polysaccharides.	Limited degradation, only slowly erodes phenolic components, and cannot effectively depolymerize lignin macromolecules.

White rot fungi can be further categorized based on their decay patterns into "selective" and "simultaneous" decay. Examples of selective white-rot fungi include *Ceriporiopsis subvermispora*, *Dichomitus squalens*, *Phanerochaete chrysosporium*, and *Phlebia radiata*. In selective decay, lignin and hemicellulose are preferentially removed, leaving cellulose relatively intact. In contrast, non-selective white-rot fungi, such as *Trametes versicolor* and *Fomes fomentarius*, exhibit simultaneous decay, where cellulose, hemicellulose, and lignin are degraded concurrently. Notably, the same white-rot species

may exhibit either selective or simultaneous decay depending on the substrate, and both patterns can even occur within a single substrate. Furthermore, white-rot fungi are the only organisms known to completely degrade lignin into water-soluble products and CO_2. In contrast, brown-rot fungi preferentially depolymerize cellulose and hemicellulose, generally lacking the ligninolytic oxidases characteristic of white-rot fungi. Nevertheless, studies indicate that brown-rot fungi can modify lignin while rapidly degrading the polysaccharide components (Qi *et al*. 2022).

Zheng *et al*. (2021) inoculated four species of white-rot fungi (*Phanerochaete chrysosporium*, *Pleurotus ostreatus*, *Irpex lacteus*, and *Phlebia acerina*) into raw oat straw and cultured them for 28 days to analyze the effects of different white-rot fungi on the chemical composition of the straw. The results showed that *P. acerina* exhibited the highest degree of lignin degradation (46.5%), followed closely by *P. chrysosporium* (44.2%), while *P. ostreatus* and *I. lacteus* demonstrated slightly lower lignin degradation efficiency compared to the other two species. Moreover, white-rot fungi exhibit substrate non-specificity, enabling them to degrade a wide range of organic materials. They achieve effective organic degradation by producing highly oxidative hydroxyl radicals, which provide advantages of low cost and high efficiency.

Although biological methods for lignin removal primarily rely on fungal degradation, fungi have a narrow tolerance range for environmental conditions. Under extreme conditions, such as high temperatures or strongly acidic or alkaline environments, their lignin degradation efficiency significantly decreases (Haq *et al*. 2020). In contrast, bacteria combine large population sizes, rapid reproduction rates, and high mutation rates, enabling them to expand their environmental tolerance through adaptive evolution. However, numerous studies indicate that the ligninolytic enzymes produced by bacteria often exhibit unstable activity, and their degradation efficiency is generally far lower than that of corresponding fungal enzymes (Bugg *et al*. 2011). Therefore, future research may focus on either strictly controlling the reaction conditions for fungi or engineering bacteria capable of producing high-yield, efficient enzymes.

Research has also shown that, in addition to bacteria and fungi, actinomycetes and cyanobacteria can degrade lignocellulosic materials (Kumar *et al*. 2020). Moreover, certain prokaryotes, including *Pseudomonas* and *Bacillus* species, can produce ligninolytic enzymes (Premnath *et al*. 2021). However, these enzymes are predominantly intracellular or membrane-bound, limiting their role in extracellular lignin biodegradation.

Ligninolytic Enzymes

Ligninolytic enzymes, predominantly produced by white-rot fungi and bacteria, are regarded as green biocatalysts due to their renewable nature and ability to operate under mild conditions, including near-neutral pH, room temperature, and atmospheric pressure, without the need for toxic solvents (Juneidi *et al*. 2018). The production of these enzymes varies significantly among different microbial strains and growth conditions, with many fungi or bacteria secreting multiple enzymes that act synergistically (Pollegioni *et al*. 2015). The most extensively studied ligninolytic enzymes include laccases (EC 1.10.3.2) and peroxidases, the latter of which can be further subdivided into lignin peroxidases (LiPs, EC 1.11.1.14), manganese peroxidases (MnPs, EC 1.11.1.13), and versatile peroxidases (VPs, EC 1.11.1.16). Additionally, aryl-alcohol oxidases (AAOs) and other auxiliary enzymes also participate in lignin degradation (Wang *et al*. 2020). Research indicates that the production capabilities of ligninolytic enzymes vary widely among microbial species. For example, white-rot fungi such as *Pleurotus ostreatus* and *Cerrena sp.* exhibit high laccase

activity, whereas *Phanerochaete chrysosporium* and *Trametes versicolor* are known for their high production of lignin peroxidase and manganese peroxidase (Suryadi *et al.* 2022).

Utilizing purified enzymes for the degradation of lignocellulosic materials, rather than whole fungi, presents significant advantages for industrial application. This approach mitigates challenges such as prolonged fungal cultivation cycles (often weeks), difficulties in meeting large-scale fermentation demands, and unwanted consumption of polysaccharides, which preserves the sugar yield for downstream processes. However, the industrial adoption of enzymatic delignification faces several hurdles. The high cost of enzyme production and purification remains a primary concern. While laccases are commercially available, their effective use often requires expensive redox mediators (*e.g.*, ABTS, HBT), which can be cost-prohibitive at scale. Peroxidases such as LiP and MnP require stoichiometric amounts of H_2O_2, whose cost and management (as an excess can inactivate the enzymes) add operational complexity. Furthermore, the inherent sensitivity of these enzymes to denaturing conditions (*e.g.*, shifts in pH and temperature, shear stress) and their limited recyclability pose significant challenges for continuous industrial processes (Woo *et al.* 2022). Recent advancements in enzyme immobilization technology aim to address these limitations by enhancing the stability, reusability, and operational performance of enzyme preparations, thus improving their cost-effectiveness and expanding their application scope.

The time required for effective delignification varies significantly across different enzymes and systems. Isolated enzyme treatments are generally faster than whole-fungi cultures but can still range from hours to days. In a study by Cui *et al.* (2024), the degradation of alkaline lignin was investigated using three laccase-mediator systems (LMS). Among these, the laccase/ABTS system achieved the highest degradation rate of 39% under optimized conditions (32 °C, pH 5, ABTS concentration of 2 mmol/L, reaction time 24 h). Peroxidase-based reactions, such as those involving LiP and MnP, typically operate within a timescale of several hours to a few days, depending on factors including enzyme loading, H_2O_2 feeding strategy, and lignin substrate characteristics. It is important to note that although enzymatic pretreatment is generally faster than fungal cultivation, it is typically a batch process. As such, the total time required for enzymatic treatment must also include the time needed for enzyme production.

The enzymatic degradation of lignin begins with the synergistic action of microbial extracellular depolymerases and H_2O_2, which target critical chemical bonds within the lignin macromolecule. This process involves the cleavage of inter-unit ether linkages, oxidative breakdown of C_α-C_β and alkyl-aryl bonds, as well as demethylation and the opening of aromatic rings. Through these concerted actions, the complex lignin structure is systematically depolymerized into low-molecular-weight aromatic fragments and other small molecules. Importantly, these enzymes demonstrate broad substrate specificity, enabling them to degrade not only lignin but also a variety of structurally related compounds, including various phenolic and non-phenolic compounds such as polycyclic aromatic hydrocarbons and halogenated aromatics.

Laccases

Laccases are a class of multicopper oxidase enzymes widely found in nature, consisting of monomeric, dimeric, or tetrameric glycoproteins with an average molecular weight of 60 to 80 kDa. Although most laccases are acidic proteins with a low isoelectric point, some exhibit a near-neutral or alkaline isoelectric point due to their distinctive amino acid composition and sequence. Each laccase molecule contains four copper ions: one type

1 (T1), one type 2 (T2), and a coupled binuclear type 3 copper pair (T3), which collectively form the enzyme's active site. Laccases are widely distributed in insects, plants, and microorganisms, utilizing one mole of atmospheric oxygen as an electron acceptor to oxidize four moles of phenolic compounds, producing two moles of water as a byproduct. This characteristic makes them ideal "green catalysts" and promising candidates for lignin-oxidizing enzymes (Deng *et al.* 2019). Currently, laccases are applied in various fields, including biological pulping, textile dye bleaching, and bioremediation.

However, laccases have a relatively low redox potential (<0.8 V), which limits their direct oxidation action mainly to phenolic compounds. Since up to 70% of non-phenolic lignin requires a higher redox potential (>1.2 V) for oxidation, this portion of lignin cannot be completely decomposed by laccases alone (Feng *et al*. 2019). Research has shown that laccases can oxidize non-phenolic lignin regions by promoting aromatic ring cleavage, C_α-C_β cleavage, C_α oxidation, and β-ether cleavage with the aid of small-molecule mediators (Canas and Camarero 2010), making these mediators crucial components in lignin degradation systems. To date, the most effective mediators identified include 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), N-hydroxybenzotriazole (HBT), 2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPO), and H_2O_2 (Weng *et al*. 2021; Suryadi *et al*. 2022). The choice of mediator significantly impacts the efficiency of lignin degradation. Cui *et al.* (2024) investigated the degradation of alkaline lignin using three laccases-mediator systems (LMS): laccases/H_2O_2, laccases/HBT, and laccases/ABTS. They found the laccases/ABTS system exhibited the highest degradation rate. Under optimized conditions (32 °C, pH 5, ABTS concentration 2 mmol/L, reaction time 24 h), the degradation rate reached 39%.

Given its efficacy, the use of laccases for the pretreatment of lignocellulosic biomass is gaining increasing attention. Laccase treatment can effectively degrade the lignin components of cell walls, enhancing the enzymatic hydrolysis efficiency of fiber materials. Deng *et al*. (2019) reported that laccase treatment of wheat straw reduced the non-productive adsorption of cellulase onto lignin by 28% and increased the yield of reducing sugars by 26%.

Moreover, recent studies show that laccase treatment can enhance the reactivity and antioxidant activity of lignin. Li *et al*. (2018) found that the antioxidant activity of laccase-degraded alkali lignin (IC_{50} = 56.75 μg/mL) was significantly higher than that of untreated lignin (IC_{50} = 396.2 μg/mL). Similarly, Sun *et al.* (2025) observed that laccase treatment significantly enhanced the antioxidant activity of waste hydrolysis lignin (WHL). Through experimental optimization, the optimal conditions for maximizing antioxidant activity were identified as pH 5.0, 25 °C, a reaction time of 6 h, and a laccase dosage of 1.2 mL/g. Under these parameters, the IC_{50} value of treated WHL decreased to 29.8 μg/mL, representing a 107.1% enhancement in antioxidant activity compared to untreated WHL.

Manganese peroxidases

MnPs are glycosylated extracellular proteins with a molecular weight of approximately 40 to 50 kDa per molecule. Their active site comprises a heme group, one Mn^{2+} ion, and two Ca^{2+} ions, with the Ca^{2+} ions primarily contributing to the structural integrity of the enzyme's three-dimensional fold. MnPs were first discovered in the white-rot fungus *Phanerochaete chrysosporium* (Paszczyński *et al.* 1988) and have since been identified in various other organisms. During lignin degradation, MnPs oxidize Mn^{2+} to Mn^{3+}.

Due to the instability of Mn^{3+} in aqueous environments, it forms diffusible chelates with organic acids such as oxalic acid. The primary role of these Mn^{3+} chelates is to interact with and oxidize phenolic lignin substructures, which constitute a significant portion of the polymer. Additionally, MnPs exhibit a secondary activity and are capable of directly degrading a limited range of non-phenolic substrates, including veratryl alcohol and *p*-anisidine, albeit often with lower efficiency compared to their action on phenolic units.

In the presence of H_2O_2 as an electron acceptor, MnPs oxidize Mn^{2+} to Mn^{3+}, which subsequently oxidizes phenolic compounds to generate phenoxy radicals. These radicals initiate C-O ether bond cleavage, ultimately yielding small molecule compounds such as protocatechuate and vanillin (Nayanashree and Thippeswamy 2015). Cui *et al*. (2020) found that the application of heterologously expressed MnPs to corn stover resulted in a lignin degradation rate of 35.8%.

Lignin peroxidases

LiPs, also known as diarylpropane oxidases, are heme-containing oxidoreductases with molecular masses ranging from approximately 40 to 68 kDa and acidic isoelectric points. Their active site contains an iron atom, which plays a crucial role in their enzymatic function. During lignin degradation, hydrogen peroxide (H_2O_2) acts as an electron acceptor, enabling LiPs to oxidize aromatic substrates and convert them into free radicals (Hofrichter *et al*. 2010; Aly *et al*. 2025). Additionally, LiPs can directly abstract an electron from non-phenolic lignin substructures to produce aryl cation radicals, which subsequently undergo a variety of chemical rearrangements and cleavages. Studies with lignin model compounds and synthetic lignin have confirmed that LiPs catalyze C-O and C-C bond cleavage, aromatic ring opening, and related oxidative transformations (Rashid and Bugg 2021; Singh *et al*. 2024).

The catalytic efficiency of LiPs is highly dependent on the reaction milieu, particularly the pH. LiPs exhibit optimal activity under slightly acidic conditions (typically pH 3-5). This is because the catalytic cycle relies on the heme group in its active site, which is protonated under acidic conditions to form the crucial catalytic intermediate. In contrast, under basic conditions, the enzyme's structure, particularly around the heme pocket, can be disrupted, leading to loss of activity. Moreover, the highly reactive radical species generated by LiPs, which facilitate lignin degradation, are unstable and readily quenched in alkaline environments, further reducing the degradation efficiency.

Studies indicate that the synergistic action between LiPs and other enzyme systems or small molecules can enhance the yield of reducing sugars (Feng *et al.* 2019). The combination of two or three ligninolytic enzymes has proven to be the most effective strategy for the biodelignification of lignocellulosic biomass, surpassing both microbial cell biodelignification and the use of individual enzymes. For instance, Zhang *et al*. (2020) found that supplementing LiPs with laccases and MnPs significantly increased the lignin degradation rate. When these three enzymes were combined, the degradation extent of alkaline lignin reached its highest percentage at 29.0%. Additionally, the combination of LiPs with laccases and malonic acid led to a nearly 22.5 mg/g increase in the reducing sugar content of lignocellulose fermentation products. Using alkali lignin as a substrate, Zhang *et al*. (2020) achieved 29.0% degradation through the combined action of laccases, LiPs, and MnPs, resulting in the production of degradation products such as 1,13-tridecanediol, carboxylic acids, and amides.

Versatile peroxidases

Versatile peroxidases (VPs) are heme-containing peroxidases produced by certain bacteria and various fungi (Kumar and Chandra 2020). Genome studies have revealed that VPs incorporate sequence elements characteristic of both the LiPs and MnPs families. This hybrid enzyme possesses the catalytic activity of both, enabling it to oxidize Mn^{2+} to Mn^{3+} and degrade typical substrates of LiPs and MnPs. Importantly, VPs not only are able to recognize substrates of LiPs and MnPs, but they also oxidize certain non-phenolic substrates that are resistant to these two enzymes, such as polycyclic aromatic hydrocarbons (Cui *et al*. 2020).

VPs are a relatively recent discovery among natural ligninolytic enzymes. However, their natural production levels are insufficient to meet industrial application demands. Consequently, molecular techniques such as homologous or heterologous expression are typically employed to enhance production, and several VP gene sequences have now been obtained. VPs demonstrate high catalytic activity in biological delignification and biomass transformation. For instance, they significantly improved the saccharification efficiency of corn stover, achieving a glucose yield of 349 mg/g and a cellulose hydrolysis rate of 91.5% after 28 days (Kong *et al.* 2017).

The complex process of lignin breakdown by these enzymes involves a series of oxidative reactions. Figure 1 illustrates the simplified catalytic pathways of laccase, manganese peroxidase, lignin peroxidase, and versatile peroxidase (Datta *et al.* 2017; Janusz *et al.* 2017; Kantharaj *et al.* 2017; Atiwesh *et al.* 2022; Alruwaili *et al.* 2023).

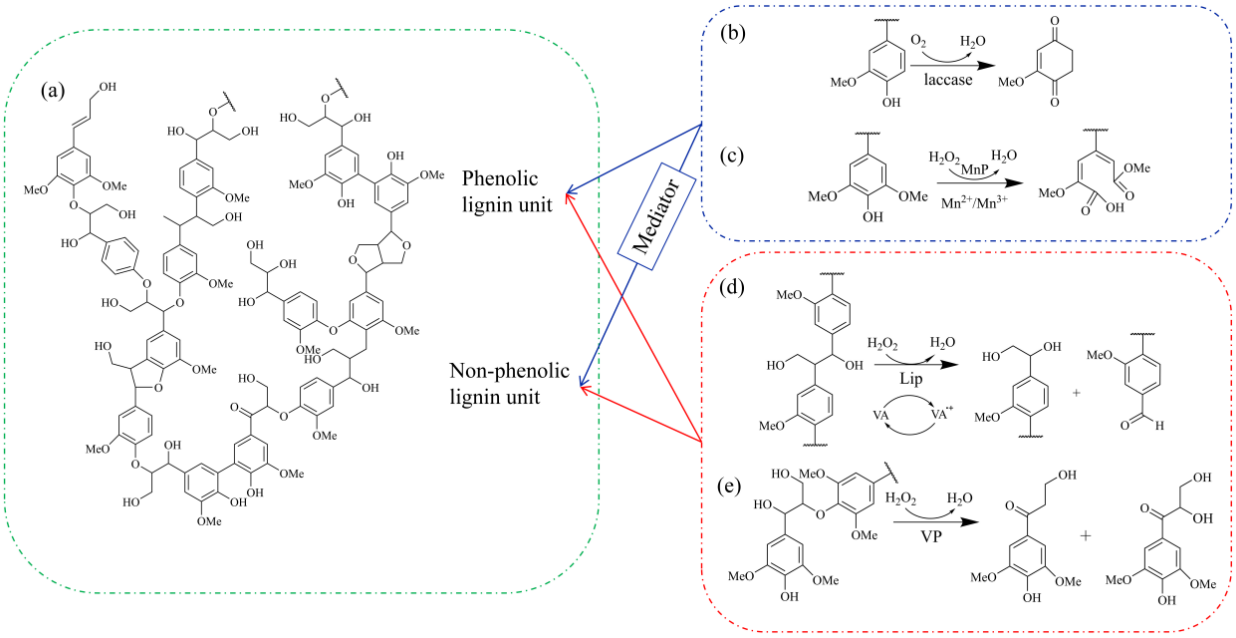

Fig. 1. Simplified pathways of lignin degradation by microbial enzymes. (a) Chemical structure of lignin, (b) Lignin degradation by laccase, (c) Lignin degradation by manganese peroxidase, (d) Lignin degradation by lignin peroxidase, (e) Lignin degradation by versatile peroxidase

To enable a systematic comparison of the key attributes of these lignin-degrading enzymes, Table 2 summarizes their respective advantages, main challenges, and typical reaction efficiencies, information that is crucial for evaluating their industrial applicability.

Table 2. Comparison of Key Lignin-Degrading Enzymes

Lignin-Degrading Enzymes	Advantages	Main Challenges	Reaction Efficiency
Laccase	Most environmentally friendly (requires only oxygen, byproduct is water); already commercialized.	Limited oxidation ability, requires expensive/toxic media for efficient degradation.	Slow, requiring hours to days (dependent on media).
Manganese peroxidase	Wide range of action, degrades through Mn^{3+} mediator penetration.	Complex system, requires Mn^{2+}, H_2O_2, and organic acids simultaneously.	Similar to Lignin peroxidase, limited by complex systems.
Lignin peroxidase	Strongest oxidation capacity, can directly attack the lignin core structure.	Relies on and is easily inactivated by H_2O_2; poor stability under industrial conditions.	Rapid action, but system instability.
Versatile peroxidase	Comprehensive functionality, combines advantages of LiP and MnP.	Extremely low natural yield, relies on genetic engineering, highest cost.	Efficient in the lab, but not yet scaled up for commercial use.

Enhancing Lignin Valorization Using Ionic Liquids and Deep Eutectic Solvents

Although enzymatic lignin degradation presents numerous advantages, it still faces significant challenges in industrial applications. Ligninolytic enzymes often lose activity rapidly under non-optimal conditions, such as non-neutral pH or temperatures outside the range of 30 to 40 °C. Recent studies suggest that "green solvents," such as ILs and DESs, may provide a solution, as they efficiently solubilize lignin while also potentially activating and stabilizing ligninolytic enzymes.

Ionic liquids

ILs are receiving increasing attention for biomass pretreatment and lignin valorization due to their powerful solvation capacity. Composed of specific cation-anion pairs, ILs are molten salts that are liquid below 100 °C and are capable of dissolving various organic and inorganic compounds. They exhibit tunable physicochemical properties, as well as significant chemical and thermal stability. Recent studies confirm their excellent capability to dissolve lignocellulosic biomass and its components, including lignin, cellulose, and hemicellulose (Zhang *et al.* 2025), with particularly high efficacy for lignin solubilization (Yu *et al.* 2022). For example, Pu *et al*. (2007) reported that the solubility of softwood kraft lignin in several ILs, including 1,3-dimethylimidazolium methyl sulfate ([MMIM][$MeSO_4$]), 1-hexyl-3-methylimidazolium trifluoromethanesulfonate ([HMIM][CF_3SO_3]), 1-butyl-3-methylimidazolium methyl sulfate ([BMIM][$MeSO_4$]), 1-butyl-3-methylimidazolium chloride ([BMIM][Cl]), 1-butyl-3-methylimidazolium bromide ([BMIM][Br]), and 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIM][BF_4]), ranged from 14.5 to 344 g/L. Dias *et al*. (2024) found that kraft lignin solubility reached 73.9 wt% in [BMIM][$MeSO_4$] and 77.71 wt% in pyrrolidinium octanoate ([Pyrr][Oc]). The efficacy of ILs in lignin dissolution is highly dependent on the structure of their constituent

cations and anions. Figure 2 presents the chemical structures of some ionic liquids commonly employed for lignin dissolution, extraction, and modification.

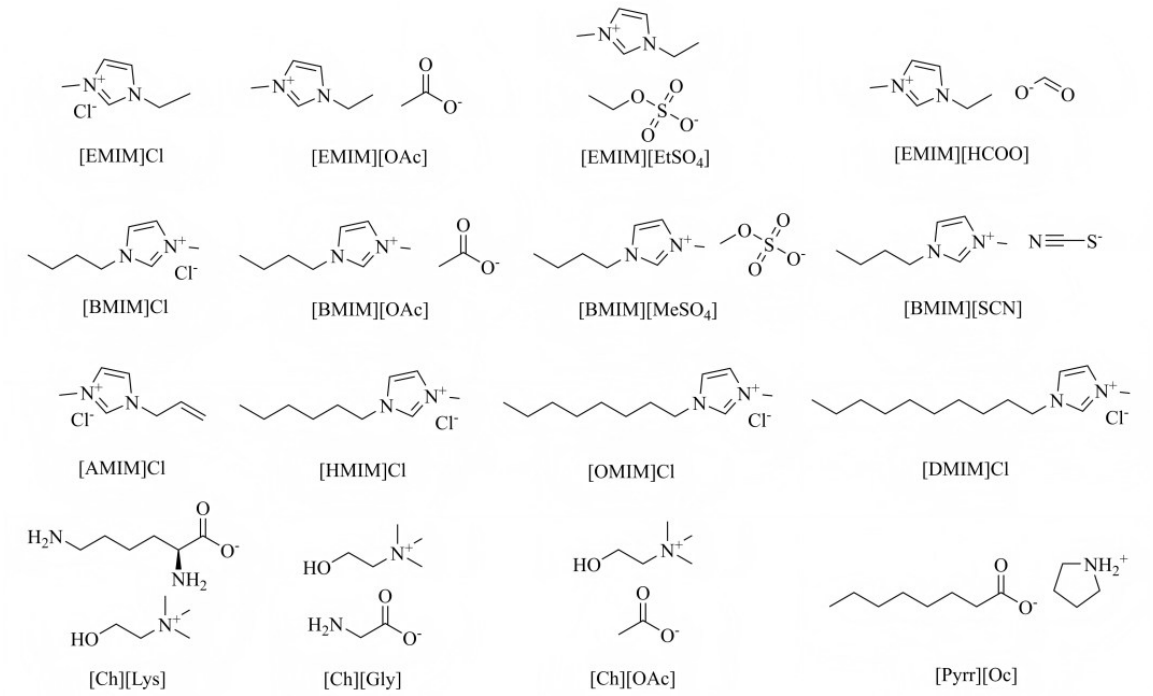

Fig. 2. Ionic liquids commonly used for lignin dissolution, extraction, and modification

The composition, concentration, and temperature of ILs significantly affect the activity of ligninolytic enzymes. Key properties that affect enzyme performance include the cationic alkyl chain length, the use of bio-derived anions and cations, lower hydrogen bond basicity and nucleophilicity, hydrophobic characteristics, and reduced viscosity and surface activity (Gao *et al*. 2015). Cations with longer alkyl chains are generally associated with enhanced enzyme stability, which is related to the surface activity exhibited by cations with long alkyl chains in aqueous solutions (Klahn *et al*. 2011; Yang *et al*. 2007). Rodríguez *et al*. (2011) investigated the activity and stability of laccase in [BMIM][Cl], 1-octyl-3-methyl imidazolium chloride ([OMIM][Cl]), and 1-decyl-3-methyl imidazolium chloride ([DMIM][Cl]). Their results indicated that, under pH 9.0 conditions, laccase retained its initial activity well at an IL concentration of 10%. Notably, the stability of laccase in [BMIM][Cl] was well maintained compared to the control group. The degree of laccase inactivation increased with the length of the alkyl chain in the IL, following the trend: [DMIM][Cl] > [OMIM][Cl] > [BMIM][Cl]. Similarly, Machado *et al*. (2014) investigated the effects of 1-ethyl-, 1-butyl-, and 1-hexyl-3-methylimidazole chloride on the catalytic activity, thermal stability, and deactivation kinetics of horseradish peroxidase. Their findings revealed that both 1-ethyl- and 1-butyl-ILs did not significantly affect enzyme activity at concentrations as high as 25% (w/v) at 25 °C, while 1-hexyl-IL led to a decrease in activity. This suggests that an increase in the alkyl chain length of ILs correlates with reduced enzyme activity and enhanced inactivation effects.

Bio-derived cations and anions—such as amino acids, glucose, and carboxylic acids—typically exhibit enhanced biocompatibility. For instance, biomass-derived ILs such as choline, glycine, and betaine demonstrate good biocompatibility (Chan *et al.* 2021). Yazdani *et al*. (2016) synthesized ten choline-amino acid-based ionic liquids (AAILs),

which featured cholinium cations paired with variable amino acid-derived anions. They assessed the microbial biocompatibility of these AAILs against Gram-positive (*Bacillus licheniformis* and *Staphylococcus aureus*) and Gram-negative (*Pseudomonas aeruginosa* and *Vibrio cholerae*) bacteria. The results showed that the EC_{50} values of all tested AAILs ranged from 160 to 1120 mg/L, which is considered "practically harmless" according to hazard rankings. Furthermore, a trend emerged showing that toxicity decreased with increasing molecular weight of the anions.

Additionally, the hydrogen bond basicity and nucleophilicity of IL anions influence enzyme performance. Lower hydrogen bond basicity and nucleophilicity can enhance interactions between charged sites and internal hydrogen bonds within the enzyme, stabilizing its structure and minimizing conformational changes, which helps maintain higher enzyme activity (Zhao 2010). Tavares *et al*. (2008) investigated the activity and stability of laccase in three different water-soluble ILs: 1-ethyl-3-methylimidazolium 2-(2-methoxyethoxy) ethyl sulfate ([EMIM][$MDEGSO_4$]), 1-ethyl-3-methylimidazolium ethyl sulfate ([EMIM][$EtSO_4$]), and 1-ethyl-3-methylimidazolium methanesulfonate ([EMIM][$MeSO_3$]). Their findings revealed that laccase experienced only a 10% loss in activity after being incubated for 7 days in a 25 wt% solution of [EMIM][$MDEGSO_4$].

Generally, increasing the concentration of ILs tends to diminish the activity of ligninolytic enzymes (Ventura *et al*. 2012). Dong *et al*. (2019) found that compared to acetic acid/sodium acetate buffer solution, Trametes KS-2 laccase retained over 90% of its activity after 1 h of incubation in a 2.5% (w/v) 1-ethyl-3-methylimidazole acetate ([EMIM][OAc]) solution, while its activity decreased to less than 40% in a 7.5% concentration of the IL. Hinckley *et al.* (2002) and Tavares *et al*. (2008) reported that laccase precipitated when the concentration of 4-methyl-N-butylpyridinium tetrafluoroborate ([4-MBP][BF_4]) exceeded 50% (v/v) or when 1-ethyl-3-methylimidazolium 2-(2-methoxyethoxy)ethyl sulfate ([EMIM][$MDEGSO_4$]) exceeded 75% (v/v).

Recent studies have indicated that certain ILs can enhance lignin degradation mediated by laccase. For example, Stevens *et al*. (2019) investigated the interactions between laccases and various ILs, including diethylamine hydrogen sulfate ([DEA][HSO_4]), choline lysinate ([Ch][Lys]), and 1-ethyl-3-methylimidazolium acetate ([EMIM][OAc]). Their findings revealed that the presence of 10% [DEA][HSO_4] resulted in minimal loss of laccase activity, leading to a lignin conversion of 21.7%, which was a significant increase compared to the mere 2.12% observed without ILs. The main degradation products identified were vanillin, acetosyringone, syringaldehyde, and acetovanillone.

Chauhan and Choudhury (2021) conducted a comparative analysis of LiP, MnP, and laccase in the presence of pyridine and lauroyl choline ionic liquid (CLIL). They found that enzyme stability was maintained in CLIL while decreasing in pyridine. Additionally, in a MnP catalytic system utilizing glutathione as a mediator, lignin degradation extents of 40.8% and 31.8% were achieved in 40% (v/v) pyridine (with 1.5 M NaCl) and 0.15 mM CLIL, respectively.

Liu *et al*. (2021) used corn stover as a feedstock, employing biocompatible ILs for the integrated extraction of lignin and enzymatic hydrolysis. They developed an enzyme system using AAO and LiP to facilitate lignin degradation. Aqueous solutions of [Ch][Lys] and choline glycinate ([Ch][Gly]) effectively removed approximately 50% of the lignin content from corn stover. Moreover, the application of AAO and LiP in these IL aqueous solutions reduced the molecular weight of lignin in both solid and liquid phases, converting it into valuable low-molecular-weight compounds.

Moniruzzaman and Ono (2012) pretreated wood samples with [EMIM][OAc] at 80 °C for 1 h to disrupt the hydrogen bonding between cellulose and lignin. After cooling the wood/IL mixture, they added an acetate buffer containing laccase and HBT to promote enzymatic delignification at 50 °C. Their findings indicated that the IL-mediated swelling of lignocellulosic biomass prior to enzymatic treatment is an effective strategy for lignin removal and the subsequent extraction of cellulose fibers.

Ninomiya *et al*. (2018) observed that IL pretreatment followed by enzymatic treatment increased the total yield of vanillin and syringaldehyde from eucalyptus lignin during alkaline nitrobenzene oxidation depolymerization by 11.4%, achieving a total yield of 48% compared to 36.6% from untreated samples.

Chang *et al*. (2021) investigated the effects of adding 1-allyl-3-methylimidazolium chloride ([AMIM]Cl) and the surfactant Triton X-100 to the treatment of rice straw with laccase. The addition of 750 mg/L [AMIM]Cl and 500 mg/L Triton X-100 resulted in lignin removal extents of 18.5% and 31.8%, respectively, significantly exceeding the 12.0% removal achieved by laccase alone. Both additives also enhanced the enzymatic hydrolysis yield of the fiber-rich residue.

Pena *et al*. (2023) found that the high solubility of alkali lignin in [EMIM][OAc] markedly improved laccase-mediated degradation, leading to increased yields of phenolic products such as catechin hydrate, gallic acid, and 4-hydroxybenzoic acid.

These studies indicate that certain ILs can enhance the activity and stability of ligninolytic enzymes, thereby increasing lignin degradation. However, the production costs of ILs are relatively high, their synthesis processes are complex, and there are concerns regarding their toxicity and potential ecotoxicological effects due to limited biodegradability (Hou *et al.* 2013; Pätzold *et al.* 2019).

Deep eutectic solvents

DESs represent a promising class of green solvents suitable for biomass pretreatment and lignin valorization. These solvents are formed by mixing hydrogen bond acceptors (HBAs), such as quaternary ammonium salts (*e.g*., choline chloride, ChCl), amino acids (*e.g*., alanine, glycine, proline, glutamic acid), betaine, and amides, with hydrogen bond donors (HBDs) that can include polyols, sugars, carboxylic acids, or amides in specific molar ratios. DESs typically exhibit melting points lower than those of their individual components. Notably, choline- and betaine-based DESs are currently the most widely researched types. Choline, an essential nutrient similar to B vitamins, is safe and non-toxic, while betaine is a naturally abundant food compound. Similar to traditional ILs, DESs offer several advantages, including low vapor pressure and good thermal stability. Moreover, DESs are easier to prepare, less expensive, and generally less toxic than ILs. For example, the synthesis of DESs can be achieved through simple mixing and heating, avoiding the more complex steps of cation formation and anion exchange required for ILs. Research by Hou *et al*. (2013) investigated the toxicity of 11 DESs and 2 ILs, and found that choline-based DESs are significantly less toxic than the imidazolium IL [BMIM][BF_4], with the difference being an order of magnitude. Consequently, DESs show considerable promise for applications in lignin valorization and other fields.

Numerous studies have demonstrated the high solubility of lignin in various DESs, primarily attributed to the formation of extensive hydrogen bond networks between the components of DESs and the functional groups of lignin, especially the phenolic hydroxyls. The solubility of lignin in DESs is influenced by various factors, including the composition and ratio of the DESs (*e.g*., mass fraction, molar ratio) and the types of lignin used. For

example, Francisco *et al*. (2012) reported that the solubility of alkali lignin in betaine-lactic acid (1: 2) was 12.03 wt%, while ChCl-lactic acid (1: 2) only dissolved 5.38 wt%. Soares *et al*. (2017) found that a 50% mass fraction of a propionic acid-urea (2: 1) solution could dissolve 25 wt% of organic solvent lignin, whereas the solubility of alkaline lignin reached as high as 78 wt%. Similar to ILs, the properties of DESs are determined by their HBA and HBD components. Figure 3 displays the structures of common hydrogen bond acceptors and donors used in DESs for lignin processing.

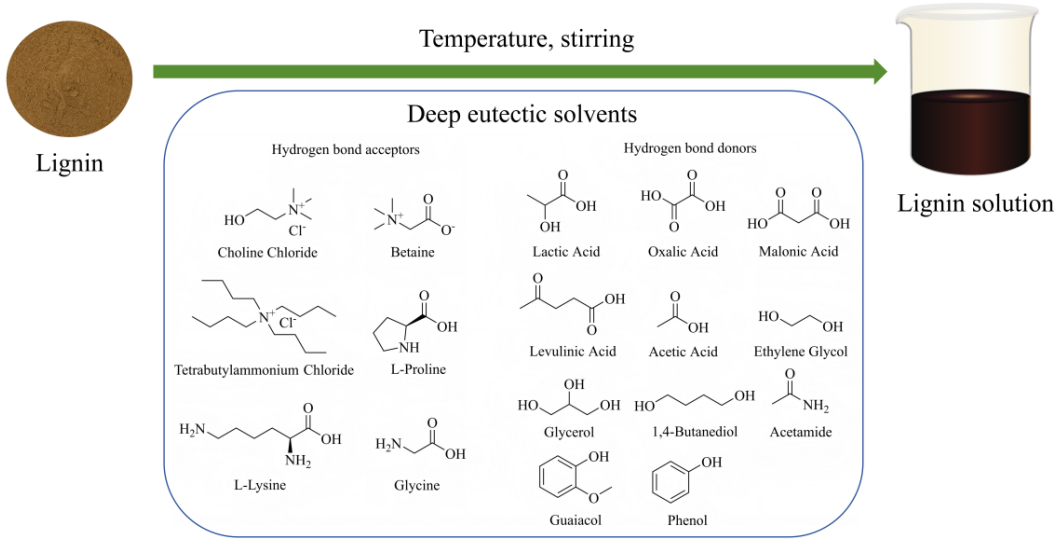

Fig. 3. Deep eutectic solvents commonly used for lignin dissolution, extraction, and modification

DESs also demonstrate significant potential in biocatalysis, particularly for stabilizing and activating ligninolytic enzymes such as laccase (Taklimi *et al*. 2023). Therefore, DESs have proven to be effective alternatives to traditional organic solvents and ILs in various biocatalytic processes (Xu *et al*. 2017).

The activity and stability of ligninolytic enzymes are influenced by the constituents, molar ratio, and concentration of the DESs used. Some DESs, particularly those based on ChCl, can inhibit enzyme activity. For instance, Choi *et al*. (2011) observed that laccase was inactive in ChCl-malic acid. Similarly, Khodaverdian *et al.* (2018) also found that laccase catalytic activity decreased in ChCl-based DESs, which is likely due to the presence of Cl^- ions disrupting the enzyme's intramolecular hydrogen bonds, leading to denaturation and reduced activity. In addition, recent studies suggest that DESs composed of ChCl and polyols exhibit a lower inhibitory effect on laccase activity compared to ChCl alone. This reduction in inhibition may be attributed to two factors: first, polyols can positively influence laccase activity, and second, the strong interactions between ChCl and polyols reduce the Cl^- concentration in the solution, preventing Cl^- from reaching the enzyme's active site (Yang *et al.* 2025).

Conversely, specific HBAs such as choline dihydrogen phosphate (ChDHP), choline dihydrogen citrate, betaine citrate, betaine, alanine, trehalose, and hydroxyl-rich HBDs including erythritol, xylitol, sorbitol, and glycerol are beneficial for enzyme stabilization. Compounds such as quaternary ammonium compounds with hydrophobic regions (*e.g*., betaine) and certain amino acids like glycine, alanine, and proline can serve as stabilizers (Zhao 2005), effectively preventing protein aggregation and denaturation under stress. Natural osmolytes such as glycerol, xylitol, and sorbitol also provide significant protein-stabilizing effects (Zhao *et al.* 2011).

Yang *et al*. (2025) conducted a systematic analysis of the activity and stability of laccase in 18 DESs. Their findings indicated that DESs composed of five HBAs—ChDHP, betaine citrate, betaine, proline, and trehalose—significantly enhanced enzyme activity and thermal stability. In contrast, DESs that included ChCl as the HBA inhibited laccase activity. Notably, the ChDHP-glycerol DES system improved laccase activity by 198% through hydrogen bond stabilization. This enhancement is primarily attributed to ChDHP, a choline salt, which stabilizes protein conformation, as demonstrated with cytochrome C. Furthermore, betaine-based DESs were found to significantly enhance the thermal stability of laccase. For instance, a 50% betaine-sorbitol solution allowed laccase to retain 92% of its activity after one hour at 70°C, which is 55 times higher than that in a buffer solution.

Toledo *et al*. (2019) evaluated the activity of laccase in 16 choline- and betaine-based DESs, finding that most of these solutions protected enzyme activity. DESs composed of betaine, choline dihydrogen phosphate, and choline dihydrogen citrate enhanced laccase activity in oxidation reactions, with a 25% mass fraction of the choline dihydrogen citrate-xylitol (2: 1) system yielding a remarkable activity level of 200%.

Khodaverdian *et al.* (2018) found that specific betaine-based DESs significantly improve both the activity and stability of laccase. For instance, in a 50% (v/v) betaine-glycerol (1: 2) solution, the laccase activity was enhanced 3-fold compared to the buffer control. This improvement is largely attributable to betaine's ability to stabilize proteins under dehydrating or osmotic stress conditions, thereby preventing aggregation and inactivation. Varriale *et al*. (2022) further demonstrated that betaine-based DESs improve laccase thermal stability, extend their half-life, and maintain residual activity following high-temperature incubation.

In addition, Toledo *et al*. (2019) found that DESs with an increased number of hydroxyl groups typically demonstrated superior performance in enhancing the activity and thermal stability of ligninolytic enzymes. Their research indicated that when ChDHC was used as the HBA in combination with different polyols at a 2:1 ratio, the enzyme activity at a 50 wt% DES mass fraction followed the order: ChDHC: ethylene glycol < ChDHC: glycerol < ChDHC: erythritol < ChDHC: xylitol.

The integration of DESs with enzymatic treatments significantly enhances the efficiency of biomass pretreatment for lignin removal and sugar yield improvement. Sawhney *et al*. (2023) employed a combined pretreatment technology for straw, integrating a chemical method (ChCl-formic acid), a physical method (microwave), and a biological method (laccase), achieving a sugar yield of 422 mg/g and a saccharification efficiency of 72.6%. This represents approximately a 1.67-fold increase in reducing sugar yield compared to untreated material. Similarly, Lin *et al.* (2025) pretreated corn straw using three DESs (betaine-glycerol, betaine-xylitol, betaine-sorbitol) combined with laccase. The DESs solutions not only did not inhibit laccase activity but also improved its thermal stability. The betaine-glycerol/laccase combination maximized lignin removal, yielding a 130% increase in fermentable sugar yield compared to the control group. Likewise, Yang *et al*. (2025) utilized betaine-sorbitol and laccase to pretreat distillers' grains (DG) and poplar wood (PW). Their results indicated significant increases in glucose and xylose conversion rates after pretreatment, particularly a 54% increase in glucose conversion and a 77% increase in xylose conversion rates in PW. The authors proposed that the betaine-sorbitol/laccase treatment disrupted the compact lignocellulosic structure, dissolved lignin and hemicellulose, and broke the covalent and non-covalent bonds in lignin-carbohydrate complexes, thereby enhancing the accessibility of cellulase.

The integrated approach of combining solvents with enzymatic treatment represents a promising direction. Table 3 provides a comparison of the advantages, disadvantages, mechanisms, and effects on lignin for ILs, DESs, and their combined systems with enzymes.

Table 3. Effects of Various Treatment Methods on Lignin Conversion Efficiency

Method	Advantages	Disadvantages & Limitations	Reaction Mechanism	Main Effects on Lignin
Ionic Liquids (ILs)	1. Strong lignin dissolution capability 2. High tunability with customizable structures 3. Low volatility, good thermal stability	1. High cost 2. Some ILs are difficult to recycle and reuse 3. Potential inhibition of enzyme activity 4. Inadequate environmental toxicity assessments	Cations and anions disrupt intermolecular forces such as hydrogen bonds and π-π stacking, leading to lignin dissolution.	Dissolves lignin, alters its structure, reduces heterogeneity, and enhances enzyme accessibility.
Deep Eutectic Solvents (DESs)	1. Low cost, biodegradable, and low toxicity 2. Easy to prepare, with readily available raw materials 3. Some DESs are biocompatible	1. Some DESs (*e.g.*, choline-based) may inhibit enzyme activity 2. High viscosity, potentially affecting mass transfer 3. Recycling and reuse technology not yet mature	Hydrogen bond donors/acceptors interact with functional groups (such as phenolic hydroxyls) of lignin, disrupting its hydrogen bond network and enabling dissolution.	Selective extraction/solubilization of lignin, partial depolymerization, while retaining cellulose.
IL/DES + Enzyme (Combination System)	1. Synergistic effect: enzyme digestion efficiency significantly enhanced after solvent pre-treatment 2. Increased reaction rate and final product yield 3. Mild conditions, with great potential for eco-friendly processes	1. Two-step process can be complex, increasing operational costs 2. Finding compatible enzyme-solvent pairs is a significant challenge 3. Residual solvents may poison enzymes or fermentation microorganisms	The solvent swells/dissolves biomass, disrupting the lignin-carbohydrate complex structure; enzymes can then more effectively access and break lignin chemical bonds.	Significant lignin removal, reduced lignin molecular weight, and disruption of lignin-carbohydrate linkage bonds.

CONCLUSIONS

1. Ligninolytic enzymes play a crucial role in the valorization of lignocellulosic materials, with applications in pulping, biofuel production, fine chemical synthesis, and the preparation of composite materials. However, several challenges hinder their commercialization, including maintaining enzyme stability, controlling reaction costs, and ensuring substrate accessibility. To overcome these limitations and enhance enzymatic lignin depolymerization, research should focus on two primary strategies: (1) developing cost-effective, high-yield enzyme production technologies *via* molecular modification through protein engineering and enzyme immobilization, and (2) optimizing enzymatic reaction systems to improve catalytic efficiency. In this regard, the integration of novel green solvents, particularly ILs and DESs, shows significant promise, as these solvents not only enhance lignin solubility but also can stabilize and activate ligninolytic enzymes.
2. Despite their potential, several key challenges remain in the utilization of ILs and DESs for lignin valorization. First, the interaction mechanisms between these solvents and ligninolytic enzymes are poorly understood. Systematic molecular-level studies are necessary to clarify how these solvents influence enzyme conformation, activity, and stability. Such insights are crucial for designing solvent systems that optimize enzyme performance. Second, establishing clear structure-activity relationships between solvent composition and enzyme activity/stability is essential for developing cost-effective, high-performance catalytic systems. Future research should focus on designing tailored enzyme-solvent pairs for lignin valorization in green solvent media, which will be pivotal for advancing a sustainable, circular bioeconomy. Success in these areas will facilitate the large-scale application of integrated lignin valorization technologies, transforming this abundant renewable resource into valuable chemicals, materials, and fuels.

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REFERENCES CITED

Ahmad, M., Taylor, C. R., Pink, D., Burton, K., Eastwood, D., Bending, G, D., and Bugg, T. D. H. (2010). "Development of novel assays for lignin degradation: Comparative analysis of bacterial and fungal lignin degraders," *Molecular Biosystems* 6(5), 815-821. https://doi.org/10.1039/b908966g

Alruwaili, A., Rashida, G. M. M., and Bugg, T. D. H. (2023). "Application of *Rhodococcus jostii* RHA1 glycolate oxidase as an efficient accessory enzyme for lignin conversion by bacterial Dyp peroxidase enzymes," *Green Chemistry* 25(9), 3549-3560. https://doi.org/10.1039/D3GC00475A

Aly, A. B. A., Shanmugaraj, B., and Ramalingam, S. (2025). "Industrial applications of *Phanerochaete chrysosporium* lignin-degrading enzymes: Current status, production

challenges, and future directions," *World Journal of Microbiology and Biotechnology* 41(5), article 171. https://doi.org/10.1007/s11274-025-04388-6

Atiwesh, G., Parrish, C. C., Banoub, J., and Le, T. A. T. (2022). "Lignin degradation by microorganisms: A review," *Biotechnology Progress* 38(2), article e3226. https://doi.org/10.1002/btpr.3226

Bugg, T. D. H., Ahmad, M., Hardiman, E. M., and Rahmanpour, R. (2011). "Pathways for degradation of lignin in bacteria and fungi," *Natural Product Reports* 28(12), 1883-1896. https://doi.org/10.1039/c1np00042j

Bugg, T. D. H., Ahmad, M., Hardiman, E. M., and Singh, R. (2011). "The emerging role for bacteria in lignin degradation and bio-product formation," *Current Opinion in Biotechnology* 22(3), 394-400. https://doi.org/10.1016/j.copbio.2010.10.009

Canas, A. I., and Camarero, S. (2010). "Laccases and their natural mediators: Biotechnological tools for sustainable eco-friendly processes," *Biotechnology Advances* 28(6), 694-705. https://doi.org/10.1016/j.biotechadv.2010.05.002

Chan, J. C., Zhang, B. X., Martinez, M., Kuruba, B., Brozik, J., Kang, C., and Zhang, X. (2021). "Structural studies of *Myceliophthora Thermophila* Laccase in the presence of deep eutectic solvents," *Enzyme and Microbial Technology* 150, article 109890. https://doi.org/10.1016/j.enzmictec.2021.109890

Chang, K. L., Liu, C. H., Phitsuwan, P., Ratanakhanokchai, K., Lin, Y. C., Dong, C. D., Lin, M. H., and Yang, G. C. C. (2021). "Enhancement of biological pretreatment on rice straw by an ionic liquid or surfactant," *Catalysts* 11, article 1274. https://doi.org/10.3390/catal11111274

Chauhan, A. K., and Choudhury, B. (2021). "Suitability of organic solvent and cholinium based ionic liquid activated novel lignolytic enzymes of *H. aswanensis* for enhanced Kalson lignin degradation," *International Journal of Biological Macromolecules* 165, 107-117. https://doi.org/10.1016/j.ijbiomac.2020.09.137

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

Chio, C. L., Sain, M., and Qin, W.S. (2019). "Lignin utilization: A review of lignin depolymerization from various aspects," *Renewable and Sustainable Energy Reviews* 107, 232-249. https://doi.org/10.1016/j.rser.2019.03.008

Choi, Y. H., van Spronsen, J., Dai, Y. T., Verberne, M., Hollmann, F., Arends, I.W.C. E., Witkamp, G. J., and Verpoorte, R. (2011). "Are natural deep eutectic solvents the missing link in understanding cellular metabolism and physiology?," *Plant Physiology* 156(4), 1701-1705. https://doi.org/10.1104/pp.111.178426

Cui, D. K., Yi, G. B., Ye, D. W., Zhu, G. Z., Xiao, Y., and Chen, Z. J. (2024). "Catalytic degradation of alkali lignin by laccase mediator system and its structural evolution studies," *Transactions of China Pulp and Paper* 39(2), 44-51. https://doi.org/10.11981/j.issn.1000-6842.2024.02.44

Cui, T. W., Yuan, B., Lian, C., Fang, B. R., Mao, X. Y., and Fei, Q. (2020). "Evaluation and analysis of activity assays of ligninolytic enzyme," *Chemical Industry and Engineering Progress* 39(12), 5189-5202. https://doi.org/10.16085/j.issn.1000-6613.2020-0248

Cui, Z. L., Wang, H. C., Wu, J. M., and Chen, H. Y. (2020). "Expression and enzymatic properties of a manganese peroxidase from *Irpex Iacteus*," *Journal of Biology* 37(6), 46-50. https://doi.org/10.3969/j.issn.2095-1736.2020.06.046

Datta, R., Kelkar, A., Baraniya, D., Molaei, A., Moulick, A., Meena, R. S., and

Formanek, P. (2017). "Enzymatic degradation of lignin in soil: A review," *Sustainability* 9(7), article 1163. https://doi.org/10.3390/su9071163

Deng, Z. C., Xia, A., Liao, Q., Zhu, X. Q., Huang, Y., and Fu, Q. (2019). "Laccase pretreatment of wheat straw: Effects of the physicochemical characteristics and the kinetics of enzymatic hydrolysis," *Biotechnology for Biofuels* 12, 1-12. https://doi.org/10.1186/s13068-019-1499-3

Dessie, W., Luo, X. F., He, F. L., Liao, Y. H., Duns, G. J., and Qin, Z. D. (2023). "Lignin valorization: A crucial step towards full utilization of biomass, zero waste and circular bioeconomy," *Biocatalysis and Agricultural Biotechnology* 51, 102777. https://doi.org/10.1016/j.bcab.2023.102777

Dias, R. M., Vilas-Boas, S. M., and da Costa, M. C. (2024). "Unveiling the ability of protic and aprotic ionic liquids to dissolve and modify kraft lignin," *Separation and Purification Technology* 350, article 127977. https://doi.org/10.1016/j.seppur.2024.127977

Dong, S. J., Zhang, B. X., Wang, F. L., Xin, L., Gao, Y. F., Ding, W., He, X. M., Liu, D., Hu, X. M. (2019). "Efficient lignin degradation of corn stalk by *Trametes* with high laccase activity and enzymatic stability in salt and ionic liquid," *BioResources* 14(3), 5339-5354. https://doi.org/10.15376/biores.14.3.5339-5354

Feng, N. J., Guo, L. F., Ren, H., Xie, Y. M., Jiang, Z. H., Ek, M., and Zhai, H. M. (2019). "Changes in chemical structures of wheat straw auto-hydrolysis lignin by 3-hydroxyanthranilic acid as a laccase mediator," *International Journal of Biological Macromolecules* 122, 210-215. https://doi.org/10.1016/j.ijbiomac.2018.10.153

Francisco, M., van den Bruinhorst, A., and Kroon, M. C. (2012). "New natural and renewable low transition temperature mixtures (LTTMs): Screening as solvents for lignocellulosic biomass processing," *Green Chemistry* 14(8), 2153-2157. https://doi.org/10.1039/c2gc35660k

Gao, W. W., Zhang, F. X., Zhang, G. X., and Zhou, C. H. (2015). "Key factors affecting the activity and stability of enzymes in ionic liquids and novel applications in biocatalysis," *Biochemical Engineering Journal* 99, 67-84. https://doi.org/10.1016/j.bej.2015.03.005

Haq, I., Mazumder, P., and Kalamdhad, A. S. (2020). "Recent advances in removal of lignin from paper industry wastewater and its industrial applications – A review," *Bioresource Technology* 312, article 123636. https://doi.org/10.1016/j.biortech.2020.123636

Hinckley, G., Mozhaev, V. V., Budde, C., and Khmelnitsky, Y. L. (2002). "Oxidative enzymes possess catalytic activity in systems with ionic liquids," *Biotechnology Letters* 24, 2083-2087. https://doi.org/10.1023/a:1021305229969

Hofrichter, M., Ullrich, R., Pecyna, M. J., Liers, C., and Lundell, T. (2010). "New and classic families of secreted fungal heme peroxidases," *Applied Microbiology and Biotechnology* 87(3), 871-897. https://doi.org/10.1007/s00253-010-2633-0

Hou, X. D., Liu, Q. P., Smith, T. J., Li, N., and Zong, M. H. (2013). "Evaluation of toxicity and biodegradability of cholinium amino acids ionic liquids," *PloS One* 8(3), article e59145. https://doi.org/10.1371/journal.pone.0059145

Janusz, G., Pawlik, A., Sulej, J., Swiderska-Burek, U., Jarosz-Wilkolazka, A., and Paszczynski, A. (2017). "Lignin degradation: Microorganisms, enzymes involved, genomes analysis and evolution," *Fems Microbiology Reviews* 41(6), 941-962. https://doi.org/10.1093/femsre/fux049

Juneidi, I., Hayyan, M., and Hashim, M. A. (2018). "Intensification of biotransformations

using deep eutectic solvents: Overview and outlook," *Process Biochemistry* 66, 33-60. https://doi.org/10.1016/j.procbio.2017.12.003

Kantharaj, P., Boobalan, B., Sooriamuthu, S., and Mani, R. (2017). "Lignocellulose degrading enzymes from fungi and their industrial applications," *International Journal of Current Research and Review* 9(21), 1-12. https://doi.org/10.7324/IJCRR.2017.9211

Khodaverdian, S., Dabirmanesh, B., Heydari, A., Dashtban-Moghadam, E., Khajeh, K., and Ghazi, F. (2018). "Activity, stability and structure of laccase in betaine based natural deep eutectic solvents," *International Journal of Biological Macromolecules* 107, 2574-2579. https://doi.org/10.1016/j.ijbiomac.2017.10.144

Klähn, M., Lim, G. S., Seduraman, A., and Wu, P. (2011). "On the different roles of anions and cations in the solvation of enzymes in ionic liquids," *Physical Chemistry Chemical Physics* 13(4), 1649-1662. https://doi.org/10.1039/c0cp01509a

Kong, W., Fu, X., Wang, L., Alhujaily, A., Zhang, J. L., Ma, F. Y., Zhang, X. Y., and Yu, H. B. (2007). "A novel and efficient fungal delignification strategy based on versatile peroxidase for lignocellulose bioconversion," *Biotechnology for Biofuels* 10(1), 1-15. https://doi.org/10.1186/s13068-017-0906-x

Kumar, A., and Chandra, R. (2020). "Ligninolytic enzymes and its mechanisms for degradation of lignocellulosic waste in environment," *Heliyon* 6(2), article e03170. https://doi.org/10.1016/j.heliyon.2020.e03170

Li, J. Y., Pi, C. Y., Zhang, J. T., Jiang, F. T., Bao, T. T., Gao, L., and Wu, X. (2025). "Fungal bioconversion of lignin-derived aromatics, pathways, enzymes, and biotechnological potential," *Biotechnology Advances* 83, article 108624. https://doi.org/10.1016/j.biotechadv.2025.108624

Li, Z. L., Zhang, J. B., Qn, L., and Ge, Y. Y. (2018). "Enhancing antioxidant performance of lignin by enzymatic treatment with laccase," *ACS Sustainable Chemistry & Engineering* 6(2), 2591-2595. https://doi.org/10.1021/acssuschemeng.7b04070

Lin, K. X., Zhang, W. T., Fan, X. Y., Li, X. Y., Wang, N. M., Yu, S. Y., and Lu, L. (2025). "Deep eutectic solvents assisted laccase pretreatment for improving enzymatic hydrolysis of corn stover," *Bioprocess and Biosystems Engineering* 48(2), 209-219. https://doi.org/10.1007/s00449-024-03102-4

Liu, E. S., Segato, F., Prade, R. A., and Wilkins, M. R. (2021). "Exploring lignin depolymerization by a bi-enzyme system containing aryl alcohol oxidase and lignin peroxidase in aqueous biocompatible ionic liquids," *Bioresource Technology* 338, article 125564. https://doi.org/10.1016/j.biortech.2021.125564

Martínez, Á. T., Speranza, M., Ruiz-Dueñas, F. J., Ferreira, P., Camarero, S., Guillen, F., Martinez, M. J., Gutierrez, A., and del Rio, J. C. (2005). "Biodegradation of lignocellulosics: Microbial, chemical, and enzymatic aspects of the fungal attack of lignin," *International Microbiology: The Official Journal of the Spanish Society for Microbiology* 8(3), 195-204. https://doi.org/10.2436/im.v8i3.9526

Moniruzzaman, M., and Ono, T. (2012). "Ionic liquid assisted enzymatic delignification of wood biomass: A new 'green' and efficient approach for isolating of cellulose fibers," *Biochemical Engineering Journal* 60, 156-160. https://doi.org/10.1016/j.bej.2011.11.001

Nadányi, R., Ház, A., Lisy, A., Jablonsky, M., Surina, I., Majová, V., and Baco, A. (2022). "Lignin modifications, applications, and possible market prices," *Energies* 15(18), article 6520. https://doi.org/10.3390/en15186520

Nayanashree, G., and Thippeswamy, B. (2015). "Biodegradation of natural rubber by laccase and manganese peroxidase enzyme of *Bacillus subtilis*," *Environmental Processes* 2, 761-772. https://doi.org/10.1007/s40710-015-0118-y

Ninomiya, K., Ochiai, K., Eguchi, M., Kuroda, K., Tsuge, Y., Ogino, C., Taima, T., and Takahashi, K. (2018). "Oxidative depolymerization potential of biorefinery lignin obtained by ionic liquid pretreatment and subsequent enzymatic saccharification of eucalyptus," *Industrial Crops and Products* 111, 457-461. https://doi.org/10.1016/j.indcrop.2017.10.056

Paszczyński, A., Crawford, R. L., and Huynh, V. B. (1988). "Manganese peroxidase of *Phanerochaete chrysosporium*: Purification," *Methods in Enzymology* 161, 264-270. https://doi.org/10.1016/0076-6879(88)61028-7

Pätzold, M., Siebenhaller, S., Kara, S., Liese, A., Syldatk, C., and Holtmann, D. (2019). "Deep eutectic solvents as efficient solvents in biocatalysis," *Trends in Biotechnology* 37(9), 943-959. https://doi.org/10.1016/j.tibtech.2019.03.007

Pena, C. A., Ballesteros, L. F., Rodríguez, H., Rodil, E., Teixeira, J. A., and Michelin, M. (2023). "Laccase-mediator system for the ionic liquid-assisted treatment of a technical lignin with partial dissolution," *Biomass and Bioenergy* 177, article 106928. https://doi.org/10.1016/j.biombioe.2023.106928

Pollegioni, L., Tonin, F., and Rosini, E. (2015). "Lignin-degrading enzymes," *The FEBS Journal* 282(7), 1190-1213. https://doi.org/10.1111/febs.13224

Polman, E. M. N., Gruter, G. J. M., Parsons, J. R., and Tietema, A. (2021). "Comparison of the aerobic biodegradation of biopolymers and the corresponding bioplastics: A review," *Science of the Total Environment* 753, article 141953. https://doi.org/10.1016/j.scitotenv.2020.141953

Premnath, N., Mohanrasu, K., Rao, R. G. R., Dinesh, G. H., Prakash, G. S., Ananthi,V., Ponnuchamy, K., Muthusamy, G., and Arun, A. (2021). "A crucial review on polycyclic aromatic Hydrocarbons-Environmental occurrence and strategies for microbial degradation," *Chemosphere* 280, article 130608. https://doi.org/10.1016/j.chemosphere.2021.130608

Pu, Y. Q., Jiang, N., and Ragauskas, A. J. (2007). "Ionic liquid as a green solvent for lignin," *Journal of Wood Chemistry and Technology* 27(1), 23-33. https://doi.org/10.1080/02773810701282330

Qi, J. Y., Jia, L., Liang, Y. J., Luo, B., Zhao, R. R., Zhang, C., Wen, J. L., Zhou, Y. H., Fan, M. Z., Xia, Y. (2022). "Fungi's selectivity in the biodegradation of Dendrocalamus sinicus decayed by white and brown rot fungi," *Industrial Crops and Products* 188(B), article 115726. https://doi.org/10.1016/j.indcrop.2022.115726

Rashid, G. M. M., and Bugg, T. D. H. (2021). "Enhanced biocatalytic degradation of lignin using combinations of lignin-degrading enzymes and accessory enzymes," *Catalysis Science & Technology* 11(10), 3568-3577. https://doi.org/10.1039/D1CY00431J

Sawhney, D., Vaid, S., Bangotra, R., Sharma, S., Dutt, H. C., Kapoor, N., Mahajan, R., and Bajaj, B. K. (2023). "Proficient bioconversion of rice straw biomass to bioethanol using a novel combinatorial pretreatment approach based on deep eutectic solvent, microwave irradiation and laccase," *Bioresource Technology* 375, article 128791. https://doi.org/10.1016/j.biortech.2023.128791

Singh, A. K., Fernandez-Lafuente, R., Schmidt, J. E., Boczkaj, G., Bilal, M. (2024). "Biocatalytic functionalities of lignin peroxidase-based systems in lignin depolymerization and pollutants removal from environmental matrices," *Current*

Pollution Reports 10(3), 345-361. https://doi.org/10.1007/s40726-024-00310-0

Soares, B., Tavares, D. J. P., Amaral, J. L., Silvestre, A. J. D., Freire, C. S. R., and Coutinho, J. A. P. (2017). "Enhanced solubility of lignin monomeric model compounds and technical lignins in aqueous solutions of deep eutectic solvents," *ACS Sustainable Chemistry & Engineering* 5(5), 4056-4065. https://doi.org/10.1021/acssuschemeng.7b00053

Stevens, J. C., Das, L., Mobley, J. K., Asare, S. O., Lynn, B. C., Rodgers, D. W., and Shi, J. (2019). "Understanding laccase-ionic liquid interactions toward biocatalytic lignin conversion in aqueous ionic liquids," *ACS Sustainable Chemistry & Engineering* 7, 15928-15938. https://doi.org/10.1021/acssuschemeng.9b02151

Sun, L.J., Xu, X.Y., Gu, X.Y., Hu, D.L., and Zhou, Z. (2025). "Enhanced antioxidant activity of lignin for improved asphalt binder performance and aging resistance in sustainable road construction," *Construction and Building Materials* 471, article 140364. https://doi.org/10.1016/j.conbuildmat.2025.140364

Suryadi, H., Judono, J. J., Putri, M. R., Eclessia, A. D., Ulhaq, J. M., Agustina, D. N., and Sumiati, T. (2022). "Biodelignification of lignocellulose using ligninolytic enzymes from white-rot fungi," *Heliyon* 8(2), article e08865. https://doi.org/10.1016/j.heliyon.2022.e08865

Taklimi, S. M., Divsalar, A., Ghalandari, B., Ding, X. T., Di Gioia, M. L., Omar, K. A., and Saboury, A. A. (2023). "Effects of deep eutectic solvents on the activity and stability of enzymes," *Journal of Molecular Liquids* 377, article 121562. https://doi.org/10.1016/j.heliyon.2022.e08865

Tavares, A. P. M., Rodriguez, O., and Macedo, E. A. (2008). "Ionic liquids as alternative co-solvents for laccase: Study of enzyme activity and stability," *Biotechnology and Bioengineering* 101(1), 201-207. https://doi.org/10.1002/bit.21866

Toledo, M. L., Pereira, M. M., Freire, M. G., Silva, J. P. A., Coutinho, J. A. P. and Tavares, A. P. M. (2019). "Laccase activation in deep eutectic solvents," *ACS Sustainable Chemistry & Engineering* 7(13), 11806-11814. https://doi.org/10.1021/acssuschemeng.9b02179

Vanholme, R., Demedts, B., Morreel, K., Ralph, J., and Boerjan, W. (2010). "Lignin biosynthesis and structure," *Plant Physiology* 153(3), 895-905. https://doi.org/10.1104/pp.110.155119

Varriale, S., Delorme, A. E., Andanson, J. M., Devemy, J., Malfreyt, P., Verney, V., and Pezzella, C. (2022). "Enhancing the thermostability of engineered laccases in aqueous betaine-based natural deep eutectic solvents," *ACS Sustainable Chemistry & Engineering* 10(1), 572-581. https://doi.org/10.1021/acssuschemeng.1c07104

Ventura, S. P. M., Santos, L. D. F., Saraiva, J. A., and Coutinho, J. A. P. (2012). "Concentration effect of hydrophilic ionic liquids on the enzymatic activity of *Candida antarctica* lipase B," *World Journal of Microbiology and Biotechnology* 28(6), 2303-2310. https://doi.org/10.1007/s11274-012-1037-y

Wang, J., Yan, K., Wang, W., and Zhou, Y. (2020). "A method for high-throughput screen-ing hydrolase of lignin β-aryl ether linkage from directed evolution by glutathione (GSH) assay," *Bioresources and Bioprocessing* 7, article 29. https://doi.org/10.1186/s40643-020-00317-7

Weng, C. H., Peng, X. W., and Han, Y. J. (2021). "Depolymerization and conversion of lignin to value-added bioproducts by microbial and enzymatic catalysis," *Biotechnology for Biofuels* 14(1), article 84. https://doi.org/10.1186/s13068-021-01934-w

Woo, W. X., Tan, J. W., Tan, J. P., Luthfi, A. A. I., Abdul, P. M., Manaf, S. F. A., Yeap, S. K. (2022). "An insight into enzymatic immobilization techniques on the saccharification of lignocellulosic biomass," *Industrial & Engineering Chemistry Research* 61(30), 10603-10615. https://doi.org/10.1021/acs.iecr.2c01154

Xu, P., Zheng, G. W., Zong, M. H., Li, N., and Lou, W. Y. (2017). "Recent progress on deep eutectic solvents in biocatalysis," *Bioresources and Bioprocessing* 4, article 34. https://doi.org/10.1186/s40643-017-0165-5

Yang, L., Chen, J., Xie, K. S., He, K. W., Sheng, W. Y., Zhou, M., Jiang, G. Y, and Tian, Y. Q. (2025). "Effects of deep eutectic solvent on laccase activity and thermal stability," *Bioresource Technology* 436, article 132992. https://doi.org/10.1016/j.biortech.2025.132992

Yang, Z., Deng, J., and Chen, L. F. (2007). "Effect of ionic and non-ionic surfactants on the activity and stability of mushroom tyrosinase," *Journal of Molecular Catalysis B: Enzymatic* 47(1-2), 79-85. https://doi.org/10.1016/j.molcatb.2007.03.001

Yazdani, A., Sivapragasam, M., Lévêque, J. M., and Moniruzzaman, M. (2016). "Microbial biocompatibility and biodegradability of choline-amino acid based ionic liquids," *Journal of Microbial & Biochemical Technology* 8(8), 415-421. https://doi.org/10.4172/1948-5948.1000318

Yu, K., Ding, W. L., Lu, Y. M., Wang, Y. L., Liu, Y. R., Liu, G. Y., Huo, F., and He, H. Y. (2022). "Ionic liquids screening for lignin dissolution: COSMO-RS simulations and experimental characterization," *Journal of Molecular Liquids* 348, article 118007. https://doi.org/10.1016/j.molliq.2021.118007

Zhang, K., Xu, R., Abomohra, A., Xie, S. X., Yu, Z. S., Guo, Q., Liu, P., Peng, L., and Li, X. K. (2019). "A sustainable approach for efficient conversion of lignin into biodiesel accompanied by biological pretreatment of corn straw," *Energy Conversion and Management* 199, article 111928. https://doi.org/10.1016/j.enconman.2019.111928

Zhang, L., Zhan, B. X., Zhang, S. Z., Ji, H. Y., Peng, S., Fan, M. H., and Yan, L. F. (2025). "Direct dissolution of lignocellulosic biomass by malonic acid-DBU protonic ionic liquid and preparation of high-performance all-biomass films," *Green Chemistry* 27(6), 1789-1805. https://doi.org/10.1039/d4gc05552g

Zhang, S. T., Xiao, J. L., Wang, G., and Chen, G. (2020). "Enzymatic hydrolysis of lignin by ligninolytic enzymes and analysis of the hydrolyzed lignin products," *Bioresource Technology* 304(1), article 122975. https://doi.org/10.1016/j.biortech.2020.122975

Zhao, H. (2005). "Effect of ions and other compatible solutes on enzyme activity, and its implication for biocatalysis using ionic liquids," *Journal of Molecular Catalysis B: Enzymatic* 37(1-6), 16-25. https://doi.org/10.1016/j.molcatb.2005.08.007

Zhao, H. (2010). "Methods for stabilizing and activating enzymes in ionic liquids-A review," *J. \Chem. Technol. Biotechnol.* 85(7), 891-907. https://doi.org/10.1002/jctb.2375

Zhao, H., Baker, G. A., and Holmes, S. (2011). "Protease activation in glycerol-based deep eutectic solvents," *Journal of Molecular Catalysis B: Enzymatic* 72(3-4), 163-167. https://doi.org/10.1016/j.molcatb.2011.05.015

Zheng, M. H., Zuo, S. S., Niu, D. Z., Jiang, D., Tao, Y., Xu, C. C. (2021). "Effect of four species of white rot fungi on the chemical composition and in vitro rumen degradability of naked oat straw," *Waste and Biomass Valorization* 12, 435-443. https://doi.org/10.1007/s12649-020-00991-w

Zhou, M., Fakayode, O. A., Ren, M. N., Li, H. X., Liang, J. K., Yagoub, A. E. A., Fan, Z. L., and Zhou, C. S. (2023). "Laccase-catalyzed lignin depolymerization in deep eutectic solvents: Challenges and prospects," *Bioresources and Bioprocessing* 10(1), 21. https://doi.org/10.1186/s40643-023-00640-9

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