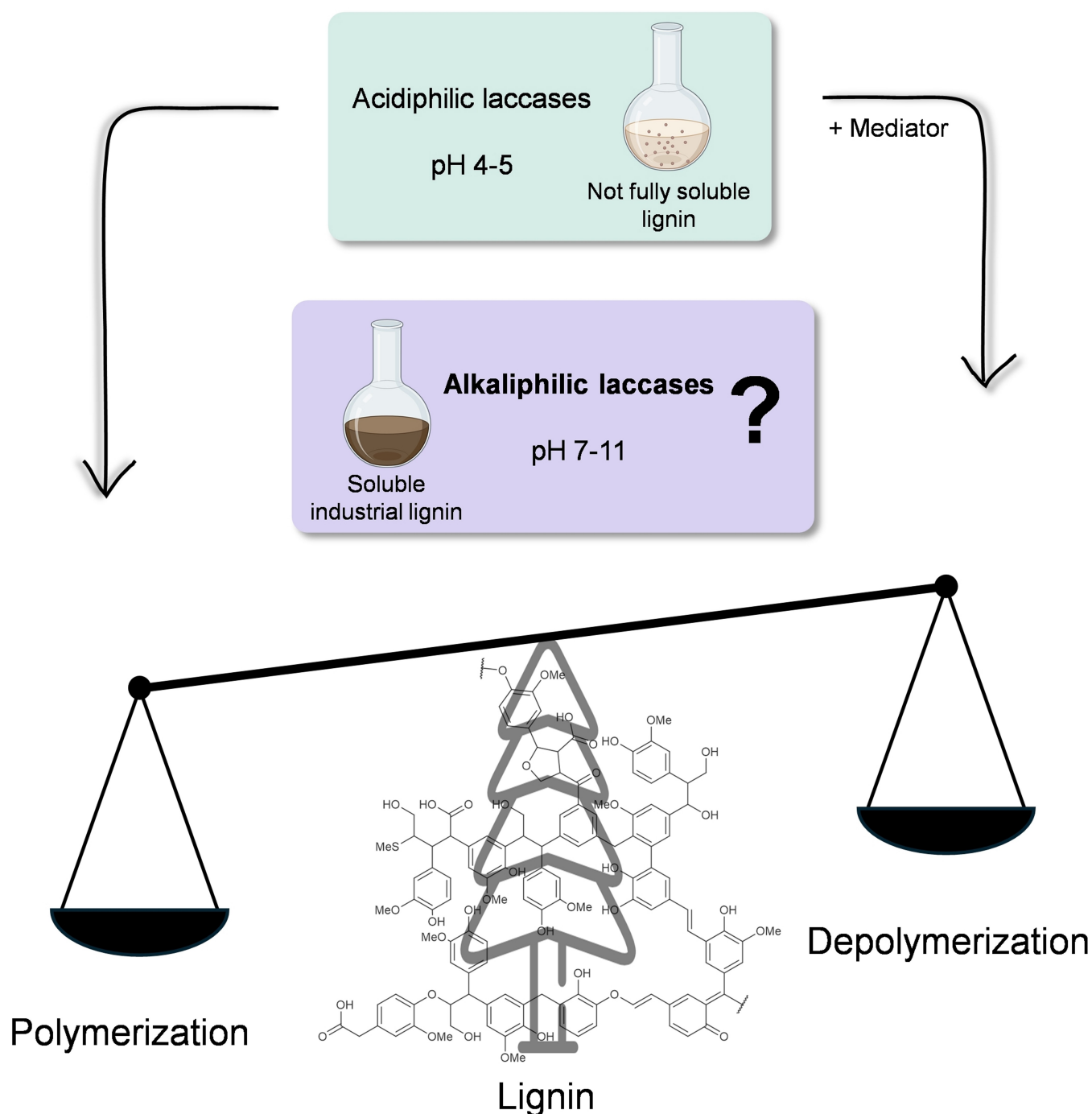# Enzymatic Treatment of Lignin in Alkaline Homogeneous Systems: A Review on Alkaliphilic Laccases

Lou Delugeau,[a] Stéphane Grelier,[a] and Frédéric Peruch*[a]

ChemSusChem

Review
doi.org/10.1002/cssc.202402377

Chemistry Europe
European Chemical
Societies Publishing

This short review explores the enzymatic treatment of lignin in alkaline homogeneous systems, focusing on alkaliphilic laccases. In acidic conditions, native laccases are known to promote lignin polymerization, while the addition of mediators enables depolymerization into valuable small molecules. Alkaliphilic laccases, which remain active in basic pH where the vast majority of industrial lignins are soluble, present an interesting alternative. However, the literature shows varied outcomes – polymerization, depolymerization, or both processes – making it difficult to draw clear trends. This review aims to summarize the current state of the art of the enzymatic treatment of lignin in alkaline conditions.

1. Introduction

Lignin is the most abundant aromatic biopolymer on Earth, found in plant cell walls, where it contributes to rigidity and hydrophobicity.[1] Lignin acts as a binding matrix that compatibilizes cellulose and hemicelluloses in the plant cell wall, forming a complex composite of these three biopolymers, which provides structural integrity and resistance to microbial degradation. Its structure varies widely among plant species and tissues, being composed of three main phenylpropanoid units derived from the oxidative coupling of *p*-coumaryl, coniferyl, and sinapyl alcohols (Figure 1a).[2] The lignin formation process is driven by a cocktail of ligninolytic enzymes present in the wood, which generate resonant radical forms. These radicals are highly reactive and unstable, allowing them to couple with each other, leading to a wide variety of chemical bonds and cross-linking either by stable carbon-carbon (β−β′, β-1, 5-5′, β-5) or more labile ether (β-O-4, 4-O-5, α-O-4) linkages, as shown in Figure 1b. These structural variations depend on plant type and growth conditions.

The extraction and separation processes of lignin from cellulose and hemicellulose alters further its structure, making its valorization challenging due to high heterogeneity and poor solubility.[3–4]

In the last decades, significant research has been devoted to lignin valorization, focusing on its use as a polymer for dispersion agents, additives, and metal adsorbents, as well as its depolymerization through thermal, reductive, and oxidative pathways to produce phenolic monomers, BTX platform chemicals, and compounds of interest like vanillin, respectively.[5–7] The enzymatic degradation of lignin, particularly using laccases, has garnered attention due to its mild and aqueous operating conditions, bypassing the need for high pressure or hazardous chemicals. Laccases (EC 1.10.3.2) belong to multi-copper oxidases, and catalyze phenolic substrate oxidation while reducing O_2 to water.[8] They are versatile enzymes widely distributed across fungi, bacteria, plants, and insects. Prospective and challenges of laccase application in biotechnology were recently reviewed.[9–11] Despite their potential, laccases typically exhibit optimal activity under mesophilic and acidic conditions, where technical lignins are often difficult to dissolve, limiting their efficiency in lignin processing.

To address this issue, several strategies have been developed to enhance lignin solubility and improve enzymatic action. One such approach is the use of water-soluble lignosulfonates, which are derived from lignin and can be processed more easily under acidic conditions.[12–22] In addition, ionic liquids, *i.e.* salts that possess a low melting point and are composed entirely of ions have been used to improve lignin solubility and to promote depolymerization in laccase-catalyzed reactions. Though these promising methods often require intensive workups and may not be environmentally friendly.[23–26] Fractionation techniques offer an alternative pathway to separate lignin into homogeneous fractions with defined chemical properties and solubility, potentially enhancing enzyme accessibility and reactivity. Fractionation can be achieved using methods such as pH variation, ultrafiltration, or organic solvent extraction.[27–29] These techniques produce lignin fractions derived from the same parent material, reducing structural heterogeneity. This process allows a control over the macromolecular structure of lignin, thereby enhancing its reactivity, solubility and accessibility for further enzymatic treatment and analytical studies.

Recently, researchers focused on a new class of enzymes: alkaliphilic laccases. Those enzymes have the capacity to be active at pH 10–12, where lignin is soluble, overcoming one on the main hurdle of lignin enzymatic valorization: its solubility. Indeed, the phenolic and carboxylic units of lignin are deprotonated under alkaline pH conditions (pKa range between 7.4 and 11.4), resulting in the efficient solubilization of the lignin substrates.[30] These enzymes can be identified either by isolating them from natural sources and testing their activity under alkaline conditions or through advanced genetic engineering techniques. Methods such as rational design or directed evolution have been employed to create laccase variants that maintain stability and activity in alkaline environments, offering new possibilities for lignin modification.[31–34]

This review aims to provide an overview of the treatment of soluble-in-alkali-water lignins with alkaliphilic enzymes. First, current knowledge on the impact of native laccases on lignin structural modifications will be summarized. Following this, this review will explore advancements and applications of alkaliphilic laccases, highlighting their potential in modifying lignin under basic conditions.

[a] *Univ. Bordeaux, CNRS, Bordeaux INP, LCPO, UMR 5629, Pessac, France*

Correspondence: *Frédéric Peruch, Univ. Bordeaux, CNRS, Bordeaux INP, LCPO, UMR 5629, 33600 Pessac, France.*
Email: frederic.peruch@enscbp.fr

ChemSusChem

Review
doi.org/10.1002/cssc.202402377

Chemistry Europe
European Chemical
Societies Publishing

2. Action of Native Laccases on Lignin and Challenges

2.1. Polymerization vs. Depolymerization in Acidic Conditions

Historically, laccases were used in biorefineries by inoculating wood with white-rot fungi, which acted on lignin to prepare it for industrial processing. This approach gained renewed interest as conventional bleaching agents like chlorine and chlorine dioxide are being banned or restricted, prompting the search for greener alternatives.[9,35–36] While fungal and enzymatic cocktails have been used for biomass preparation and pre-treatment, the focus has recently shifted significantly towards utilizing isolated laccases to treat industrial lignins, allowing researchers to study their effects on extracted and purified lignin more specifically.

Despite the solubility issues described earlier, laccases have been used for several years as a bio-catalyst to modify lignin. Two main behaviors can be observed: the lignin polymerization via oxidative coupling or the depolymerization via bond cleavage. The alteration in lignin structure begins when a phenylpropanoid subunit of lignin undergoes oxidation through the removal of a single electron.

The Figure 2 shows the different laccase-induced modifications that can occur on lignin. The first one consists in the cleavage of carbon–carbon and β-aryl bonds, leading to depolymerization.[37–38] A second pathway is a chemical modification, leading to oxidized or demethylated compounds.[39–46] Finaly, the radical species, which are stabilized by delocalization on the conjugated bonds, can react one with each other and create a new C–C or C–O–C bond, leading to the increase of the average molecular weight, i.e. the lignin polymerization.

The review of Munk et al. gives an overview of the possible products obtained from the enzymatic treatment of lignin with laccases.[47] Additionally, Agustin et al. reviewed the factors affecting laccase-catalyzed lignin polymerization, and West et al. gave an insight of the preferred conditions for laccase-mediated lignin polymerization.[43,48] Table 1 presents a summary of these findings.

2.2. Solubilization Discussion

In the enzymatic treatments described in Table 1, most studies used a co-solvent to solubilize lignin at pH levels compatible with laccase action. Maijala et al. used ethanol or propylene glycol up to 25%, and then added a 25 mM Na-citrate buffer at pH 3.5 or 5.5, depending on the enzyme employed.[49] Van de Pas et al. and Mattinen et al. employed a similar system with 25%, 50%, or 70% organic solvent (ethanol or propylene glycol) in 25 mM Na-citrate buffer at pH 5.5.[50–51] Dillies et al. used 1,4-dioxane (25% v/v), whereas Longe et al. used isopropanol to dissolve lignin and proceed to the enzymatic treatment.[52] Mattinen et al. pre-dissolved lignin in an aqueous sodium hydroxide solution (100 mM NaOH) and then add a 50 mM sodium citrate buffer at pH 5.[53]
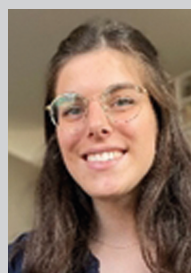
Some of the studies used a fractionation method in order to work with lignins that have narrowly distributed chain sizes, sometimes differing functionalities from native lignins, and increased solubility in a chosen solvent, which facilitates both analytical procedures and enzymatic treatments. Van de Pas fractionated isolated lignin samples by sequential solvent extraction with ether, followed by ether/acetone 4:1 (v:v), and finally acetone, according to Thring et al.[50,54] Gouveia followed the procedure of Gosselink et al., proceeding to a successive

Stéphane GRELIER received is PhD from the University of Bordeaux 1 in 1991 where he got an associate professor position. After an associate researcher position at the University of Michigan State University, he was appointed professor. After serving as adjunct director of Sciences du Bois et des Biopolymères Unit until 2011 he has joined the LCPO. His researches focus on the biorefinery of wood, cellulose and hemicelluloses chemistry, lignin chemistry and biobased phenolic synthons to develop biobased polymers through green process such as enzymatic catalysis.
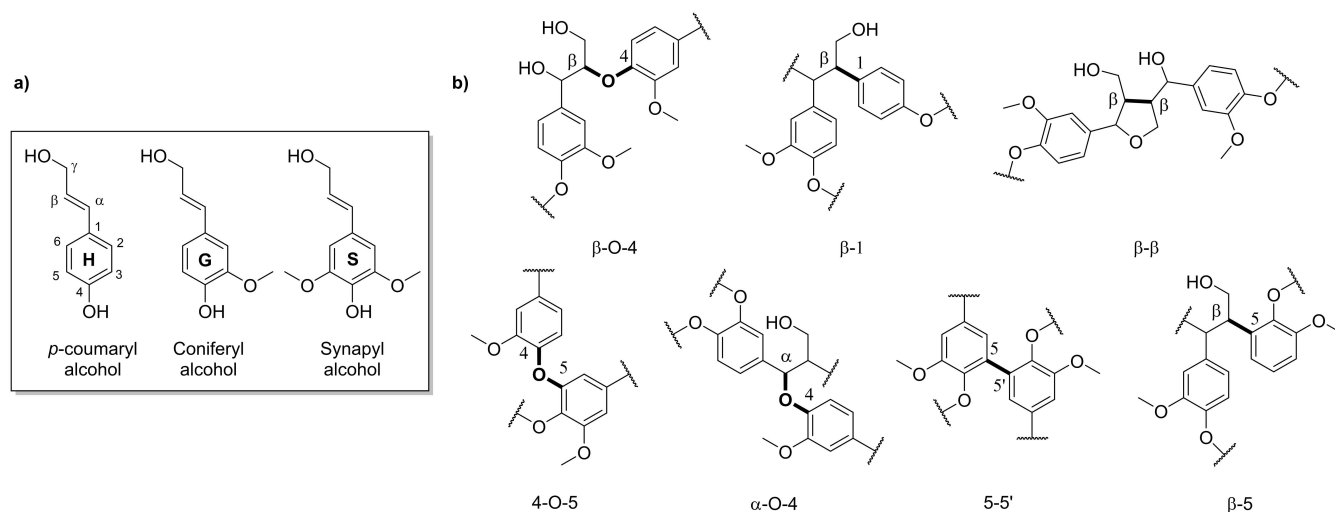
Frédéric Peruch received his engineering degree from the ENSCP Bordeaux in 1994. He obtained his PhD from Bordeaux University in 1998 in the field of coordination polymerization. After a postdoctoral position at University College of Dublin, he got a CNRS position at the Institut Charles Sadron in Strasbourg in 1999. He came back to Bordeaux in 2006 and became senior CNRS researcher in 2013. He is currently leading the team 'Polymerization Catalysis and Engineering' within LCPO. His current research interests are i) Ring-Opening polymerizations, ii) enzymatic catalysis applied to polymer science and iii) elastomeric materials.

Lou Delugeau received her master's degree from ENSCBP in 2021. She obtained her PhD at the end of 2024 after working in the Laboratory of Chemistry of Organic Polymers (LCPO, CNRS-University of Bordeaux) at the interface between the catalysis and biopolymers teams. Her PhD dealt with the enzymatic modification of lignin in alkaline conditions.

ChemSusChem

Review
doi.org/10.1002/cssc.202402377

Chemistry Europe
European Chemical
Societies Publishing

Figure 1. a) The three constitutive monomers of lignin: p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol; **b)** illustration of the principal bonds in lignin.
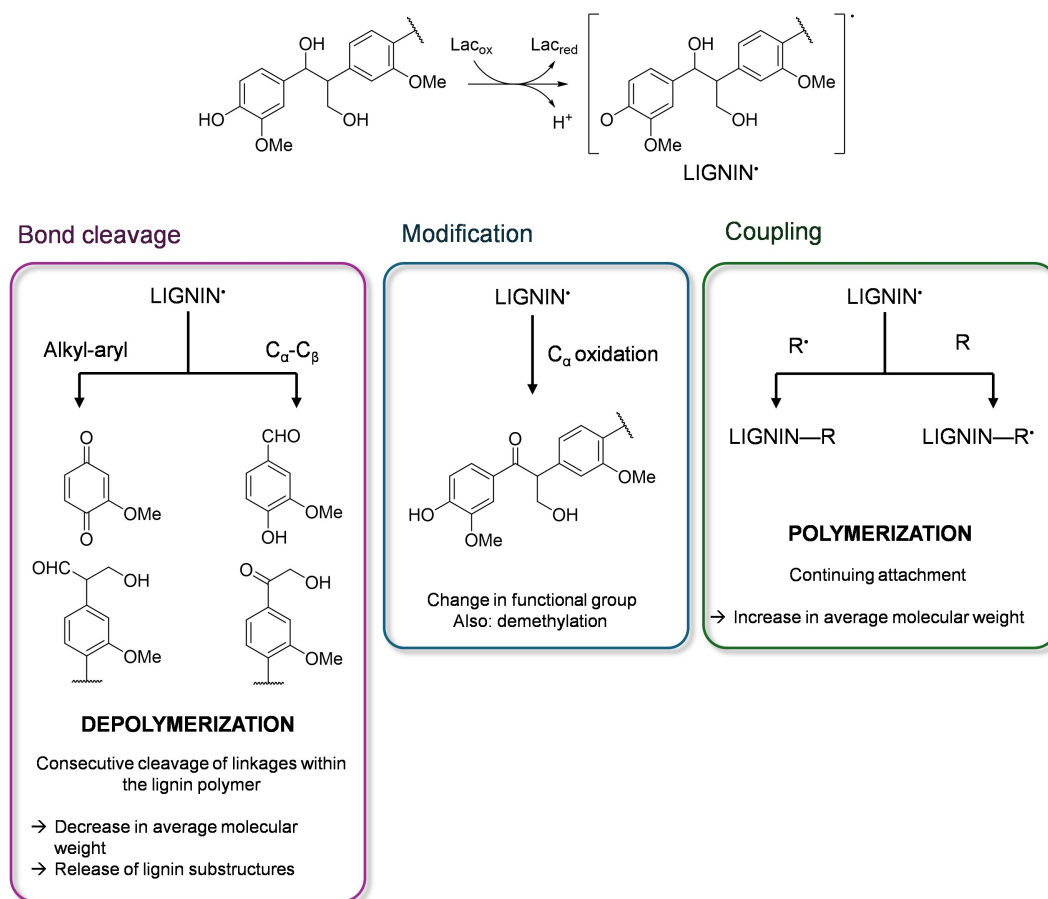
Figure 2. Proposal for plausible changes that may occur during action of laccase on lignin.

extraction of lignin with water and organic solvents (dichloromethane, propanol and methanol).[55–56]

Bourbonnais *et al.* are the only researchers to use the term "suspended" instead of "solubilized" when referring to the process of dissolving lignin. They suspended hardwood kraft pulp in sodium acetate buffer (0.05 M, pH 5.0), before adding

laccase and ABTS as the mediator. This distinction implies a physical state where lignin is not fully dissolved in solution, which is expected at such low pH values.[38]

A critical aspect that is often overlooked in the literature is the choice and nature of buffers used in experiments, both in terms of chemical composition and concentration. For example,

ChemSusChem

Review
doi.org/10.1002/cssc.202402377

Chemistry
Europe
European Chemical
Societies Publishing

Table 1. Overview of scientific studies concerning the effect of laccase from different sources on lignin-related substrates in various conditions.

Substrate	Laccase	Solvent	Reaction pH	Reaction conditions	Mediator	Result	Source
Spruce MWL	*Trametes hirsuta*	25 mM citric acid buffer	4.5	20 °C, 24 h	–	P	Grönqvist (2005)[57]
DHP (water-soluble fraction)	*Coriolus versicolor*	10 mM acetate buffer	4	30 °C, 24 h	–	P	Kondo (1990)[62]
DHP	*Trametes hirsuta, Melanocarpus albomyces, Thielavia arenaria*	25, 50 and 70 % (v/v/v) ethanol or propylene glycol; 25 mM Na-citrate buffer	5.5 or 3.5	RT, 24 h	–	P	Maijala (2012)[49]
Soda Lignin, Spruce enzymatic mild acidolysis lignin, Eucalyptus dioxane lignin	*Trametes hirsuta*	1) solubilization with 100 mM NaOH and 1 M HCl 2) addition of 50 mM sodium citrate buffer	5	RT, 24 h	–	P	Mattinen (2008)[53]
Birch and mixed HW OSL Pine steam exp. (fractionated)	*Trametes hirsuta, Melanocarpus albomyces, Thielavia arenaria*	1) solubilization with 0.1 M NaOH 2) addition of 1 M HCl and 25 mM succinate buffer	5.5	30 °C, nd	–	P	Van De Pas (2011)[50]
DHP	*Melanocarpus albomyces*	25, 50 or 70 % organic solvent (ethanol or propylene glycol) in 25 mM Na – citrate buffer	5.5	RT, 24 h	–	P	Mattinen (2011)[51]
Monomeric and dimeric lignin model compound	*Trametes hirsuta*	10 mM acetate buffer	4.5	25 °C, 200 min	–	P	Rittstieg (2002)[58]
OL, soda WL, SWKL, AL (acetone/water soluble fraction)	*Trametes versicolor*	acetone/air-saturated water 50 : 50 (v/v)	Nd	20 °C, 24 h	–	P	Fiţigău (1970)[63]
¹⁴C-labeled DHP	*Phanerochaete cinnabarius*	50 mM sodium acetate	4	RT, 4 d	3-HAA	Laccase alone: P LMS: D	Eggert (1996)[64]
HW KL	*Trametes versicolor*	50 mM sodium acetate	5	50 °C, 24 h	ABTS	Laccase alone: P LMS: D	Bourbonnais (1995)[38]
OSL, KL, AL. Soluble sulphate pine lignin	*Trametes hirsuta and Trametes ochracea*	0.1 M citrate−phosphate buffer	5	RT, 48 h	ABTS, HBT, SPP	Laccase alone: P LMS: D	Shleev (2006)[59]
OSL, KL	*Trametes versicolor*	Acetate buffer + 1,4 dioxane (25 %v/v)	4.6	30 °C, 5 d	ABTS, TEMPO	LMS: D	Dillies (2020)[52]
Eucalyptus KL (fractionated)	*Myceliophthora thermophila*	100 mM phosphate buffer	6	70 °C, 2 h	Aromatic lignin compounds*	Laccase alone: P LMS: D	Gouveia (2012)[55]
OS, AL, KL	*Cerrena unicolor*	300 mM citric acid 400 mM sodium phosphate dibasic and isopropanol	5	RT, 48 h	ABTS, VA, HBT, HAA, TTBP, TBDMP	LMS: D	Longe (2018)[65]
Agave KL	*Fusarium proliferatum*	NaOH	6	RT, 0–144 h	ABTS/HBT	Laccase alone: oxidation ABTS: D HBT: mild P	Hernández Fernaud (2006)[66]

P: polymerization; D: depolymerization. **3-HAA**: 3-hydroxyanthranilate; **ABTS**: 2,20-azinobis-(3-ethylbenzthiazoline)-6-sulfonate; **HAA**: 3-hydroxyanthranilic acid; **HBT**: 1-hydroxybenzotriazole; **SPP**: 1-(3′-sulphophenyl)-3-methylpyrazolone-5; **TEMPO**: (2,2,6,6-tetramethylpiperidin-1-yl)oxyl; **TBDMP**: 4-tert-butyl-2,6-dimethylphenol; **TTBP**: 2,4,6-tri-tert-butylphenol; **VA**: violuric acid. *aromatic compounds for Gouveia's paper: violuric acid, syringaldehyde pyrocatechol, guaiacol, vanillin. **AL**: Alkali Lignin; **DHP**: dehydrogenation polymer; **KL**: Kraft Lignin; **MWL**: Milled Wood Lignin; **OSL**: Organosolv Lignin; **SW** and **HW**: soft wood and hard wood; **WS**: wheat straw.

with the same laccase *Trametes hirsuta*, Grönqvist *et al.* used a 25 mM citric acid buffer at pH 4,[57] while Mattinen *et al.* opted for a 50 mM sodium citrate buffer at pH 5.5.[53] Rittsieg *et al.* employed a 10 mM acetate buffer at pH 4.5,[58] and Shleev *et al.* used a 0.1 M citrate–phosphate buffer at pH 5.[59] These variations may impact experimental outcomes and are barely discussed.

2.3. Action of The Laccases on Lignins in Acidic Conditions

A clear trend emerges: when a laccase alone is applied to lignins, whether they are alkali, organosolv, kraft, or even DHP or dimeric models, polymerization occurs. This is typically detected via size exclusion chromatography, where an increase in molecular weight is observed. However, when a mediator is introduced into the system, the opposite effect seems to happen, namely depolymerization.

This tendency has been widely reported and admitted in literature.[60] Laccase alone can only oxidize phenolic subunits, as non-phenolic subunits have a higher redox potential (> 1.5 V *vs.* NHE). However, the combination of redox mediators with laccases expands the catalytic capabilities of laccases by enabling the oxidation of non-phenolic substrates.[61]

In the Laccase Mediator System (LMS), laccase oxidizes a mediator, which then diffuses and oxidizes the target substrate, including those that are otherwise inaccessible to laccase (Figure 3). Mediators, either natural (e.g., syringaldehyde) or synthetic (e.g., ABTS), possess higher redox potentials than laccase. These small, recyclable molecules should be eco-friendly and cost-effective.[67]

Nevertheless, the effect of the Laccase Mediator System (LMS) on lignin polymerization or depolymerization is ambiguous, with outcomes depending on factors like reaction conditions and the type of mediator used. Longe *et al.* came to the conclusion that the laccase-mediated oxidation of different lignins is mediator dependent : a decrease of molecular weight up to 73% could be obtained on organosolv lignin with laccase–VA systems, but only 49%, 43%, and 39% when laccase was used with ABTS, TBDMP, and 1-HBT, respectively, leading to the identification of different products.[65] This trend can also be seen in Hernández Fernaud *et al.* study, that shows a polymerization occurring with the use of HBT as a mediator.[66] Studies by Crestini *et al.* among others suggest a competition between depolymerization and repolymerization, influenced by the radical species created during the process.[68–71] Crestini and Argyropoulos showed that using the laccase-mediator system allows the reaction pathway to shift towards side-chain oxidation and oxygen addition, while suppressing the formation of condensed structures. HBT, as a mediator, primarily targets free phenolic groups in lignin, promoting side-chain oxidation via a benzylic hydrogen-abstraction mechanism, leading to oxygen addition and reduced coupling reactions such as 5-5′ and 4-O-5′.[68]

Finally, Longe *et al.* observed a fast decrease of the lignins molecular weight in the first minutes of the reaction, followed by a slow polymerization over time, reaching even higher molecular weight than the original sample for reaction time over 48 h.[65] This point shows the importance of the reaction time, where both polymerization and depolymerization could happen simultaneously, the radical coupling being favored by long time reactions.

This competition between reaction pathways explains the variability in results, highlighting the crucial role of mediator identity, though the exact conditions favoring one reaction over the other remain unclear.

2.4. Lignosulfonates and Laccases

Different trends are observed for the case of the laccase treatment of lignosulfonates. These latter are obtained from the bisulfite process, which is derived from the paper industry. Discovered in the late 19th century, this process involves treating wood in an acidic environment with hydrogen sulfite (HSO_3^-) and sulfite (SO_3^-) ions at temperatures around 120–140 °C. In an acidic medium, an oxonium ion is formed and reacts with bisulfite ions to produce lignosulfonates. The introduction of this new functional group into the lignin structure imparts new and valuable properties, such as solubility in water at acidic-mild pH, where industrially available laccases are active. Several studies summarized in Table 2 have explored these laccase-induced modifications.

Interestingly, laccases tend to promote polymerization when applied to lignosulfonates, with or without mediator, likely due to the full solubility of these lignins in aqueous media. This solubility ensures that the radicals formed during the reaction can effectively couple with each other, facilitating a higher degree of polymerization compared to less soluble lignins. An exception to the general trend was observed in the study by Leonowicz *et al.*, where either polymerization or depolymerization occurs depending on the initial molecular weight of the substrate.[22] However, their results indicate that the depolymerized fraction was significantly smaller than the polymerized product, suggesting that extensive depolymerization is limited, as low-molecular-weight products are quickly repolymerized. Those behaviors highlight the importance of solubility in driving the enzymatic action of laccases and confirms that the reaction conditions play a key role in determining the outcome.

This limited solubility of lignin at acidic pH allowed laccases to be effective in treatments under such conditions, where lignin remains only partially dissolved. However, the challenges posed by incomplete solubilization have driven researchers to explore alternatives. The increasing interest in alkaliphilic laccases, capable of operating under neutral to alkaline
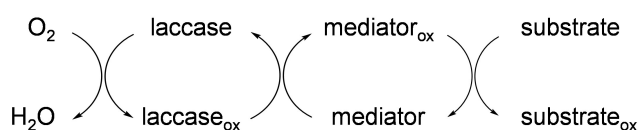
O_2 — laccase — mediator$_{ox}$ — substrate

H_2O — laccase$_{ox}$ — mediator — substrate$_{ox}$

Figure 3. Laccase Mediator System.

ChemSusChem

Review
doi.org/10.1002/cssc.202402377

Chemistry Europe
European Chemical
Societies Publishing

Table 2. Overview of scientific studies concerning the effect of laccase from different sources on lignosulfonates in various conditions.

Laccase	pH	Mediator	Result	Source
Trametes villosa, Trametes hirsuta	4.5	–	use of mediator improved P	Nugroho Prasetyo (2010)[14]
Myceliophthora thermophila	7	–	[enzyme] ↗: P ↗	Huber (2016)[17]
Trametes villosa, Myceliophthora thermophila (MtL)	5	–	initial [lignin] ↗ and MtL activity: P ↗	Areskogh (2010)[21]
Trametes hirsuta	3–8	–	pH 3,4 and 8: low P; pH 6,7: P; T ↗: oxidation rate ↗	Legras-Lecarpentier (2019)[16]
Myceliophthora thermophila	7	–	P	Mayr (2023)[12]
Myceliophthora thermophila	4.3	–	[enzyme] ↗: P ↗	Magina (2020)[15]
Trametes hirsuta	5–6	HBT	Low Mw: P, high Mw: DP	Leonowicz (1985)[22]
Myceliophthora thermophila	7	HBT, TEMPO, ABTS, vanillin	P favored with continuous O2 supply over use of mediators	Huber (2016)[13]
Trametes villosa, Aspergillus oryzae	6	DMP or guaiacol	P improved with the use of mediators	Euring (2022)[19]

P: Polymerization, **DP**: Depolymerization. **ABTS**: 2,20-azinobis-(3-ethylbenzthiazoline)-6-sulfonate; **DMP**: 2,6-dimethoxyphenol; **HBT**: 1-hydroxybenzotriazole; **TEMPO**: (2,2,6,6-tetramethylpiperidin-1-yl)oxyl.

conditions where lignin is more fully soluble, opens new possibilities for homogeneous lignin treatment. As a result, several studies now focus on harnessing the potential of these enzymes for more efficient lignin modification.

3. Application of Alkaliphilic Laccases on Lignin

Alkaliphilic laccases are enzymes of considerable interest due to their activity in alkaline pH conditions. This characteristic is particularly advantageous as industrial lignins are soluble at such alkaline pH levels. The ability of alkaliphilic laccases to function efficiently in these conditions enables effective enzymatic treatment of lignins, making them a valuable tool in industrial processes and biotechnological applications. To date, there have been approximately twenty research articles exploring the enzymatic treatment of various lignins using these alkaliphilic laccases, mainly since 2020.

3.1. Polymerization

The polymerization of lignin catalyzed by alkaliphilic laccases has been widely studied as a strategy to enhance lignin's molar mass and structural complexity. Table 3 presents an overview of the polymerization results obtained under alkaline or slightly alkaline conditions, highlighting the influence of reaction

Table 3. Overview of scientific studies concerning the alkaliphilic-laccase-induced polymerization of different sources of lignins, or lignin models, in various conditions.

Substrate	Laccase	Reaction pH	Source
GGGE dimer	CtLac (Caldalkalibacillus thermarum strain TA2.A1) (b)	8	Ghatge (2018)[72]
Aromatic compounds*	Laccase-like Oxidases from Ascomycete Curvularia geniculata VKM F-3561 (f)	7.2	Renfeld (2023)[73]
SW and HW KL	M. albomyces (MaL) (f) S. ipomoea (SilA) (b)	7-10	Moya (2011)[74]
HW KL	Myceliophthora thermophila from Novozymes (b)	7.3	Gouveia (2013)[75]
KL/fractionated KL	CotA (b)	9	Mayr (2021)[76]
AL	Bacillus ligniniphilus L1 (b)	7.9	Morales (2020)[77]
AL	Ms-Lac (f)	10	Qiu (2008)[78]
Steam-exploded WS AL	MSLac (Mycelia Sterilia YY-5) (f)	10	Weihua (2008)[79]
Fractioned AL	MetZyme® from MetGen (b)	10	Wang (2021)[80]
Fractioned AL	MetZyme® from MetGen (b)	10.5	Lu (2024)[81]

(f) fungal and (b) bacterial; **AL**: Alkali Lignin; **KL**: Kraft Lignin; **MWL**: Milled Wood Lignin; **OSL**: Organosolv Lignin; **GGGE**: guaiacylglycerol-β-guaiacyl ether; **SW** and **HW**: soft wood and hard wood; **WS**: wheat straw. *Aromatic compounds in Renfeld's study : ferulic acid, coniferyl alcohol, caffeic acid, guaiacol, 3-methoxycathecol.

ChemSusChem

Review
doi.org/10.1002/cssc.202402377

Chemistry
Europe
European Chemical
Societies Publishing

parameters such as pH, enzyme concentration, and reaction time on lignin modification.

Ghatge *et al.* examined the action of an alkaliphilic bacterial laccase, CtLac, derived from *Caldalkalibacillus thermarum* strain TA2.A1 on the dimeric lignin model compound guaiacylglycerol-β-guaiacyl ether (GGGE). When dissolved in citrate phosphate buffer (pH 8; 1 mM CuCl$_2$) at 70 °C for 12 h, the primary outcome was the formation of a 5-5' coupling product, indicating CtLac's potential for oxidative coupling in alkaline, high-temperature conditions.[72] Renfeld *et al.* explored the action of a new fungal laccase that is able to be active at pH 6.8–7.5, which is considered as slightly alkaline by the authors. They showed that ferulic acid, caffeic acid, guaiacol and 3-methoxycathecol formed bi-, tri-, tetramers, or even polymers in the case of the coniferyl alcohol.[73]

Moya *et al.* explored the polymerization of softwood (Indulin AT) and hardwood kraft lignins with two laccases: fungal MaL and bacterial SilA. Reactions were conducted in phosphate buffer (pH 7–8) and glycine-NaOH buffer (pH 9–10) at 30–50 °C. Under these conditions, polymerization was confirmed by an increase in molar masses, determined via mass spectrometry. Both laccases displayed distinct reactivity based on substrate, pH, and temperature. MaL achieved the highest polymerization with softwood kraft lignin (from 3700 to 9400 g/mol at pH 8), while SilA showed the greatest molecular weight increase with spruce lignin (3100 g/mol), gaining more than 2300 g/mol across all pH values. The results suggest MaL is broadly effective across pH ranges, while SilA, particularly in the presence of mediators, warrants further investigation.[74]

Gouveia *et al.* and Mayr *et al.* investigated kraft lignin modification under different bacterial laccase treatments. Gouveia *et al.* used *Myceliophthora thermophila* laccase from the company Novozyme (Bagsvaerd, Denmark) to polymerize hardwood kraft lignin in phosphate buffer (pH 6–8) at 60–80 °C for 2–6 h, resulting in three polymers and a 69-fold increase in molecular weight.[75] Conversely, Mayr *et al.* employed CotA laccase under alkaline conditions (NaOH, pH 9, overnight) on pure and fractionated kraft lignin, resulting in a 95% reduction in fluorescence, up to 65% phenol decrease, and significant increases in viscosity and molecular weight, including a 20-fold Mw increase for hardwood lignin and 6-fold for softwood lignin.[12]

Morales *et al.* demonstrated that treating alkali lignin with bacterial laccase *Bacillus ligniniphilus* in MM63 buffer at pH 7.9 led to a 6.75-fold increase in molecular weight, indicating significant repolymerization compared to acidic methods.[77] Similarly, Qiu *et al.* and Weihua *et al.* both used fungal laccase MS-Lac on steam-exploded wheat straw alkali lignin at pH 10, leading to increased molecular weight and reduced polydispersity, confirming the enzyme's efficacy in promoting lignin polymerization.[78–79]

Wang *et al.* and Lu *et al.* utilized MetGen® bacterial enzyme to modify fractionated alkali lignins. Wang's study involved fractionating lignin with isopropanol, ethanol, and methanol, followed by polymerization with alkaliphilic laccase in NaOH (pH 10). This treatment resulted in a 13.1-fold molecular weight increase and decreased S/G ratio after 6 h, with lower initial

molecular weights showing more extensive coupling.[80] Lu's study focused on laccase-catalyzed polymerization of birch alkaline lignin fractionated in isopropanol, in NaOH (pH 10.5) at 40 °C for 3 h. The copolymerization with tannins was also investigated. This method formed C—C and C—O—C bonds between lignin and tannins, demonstrating the versatility of laccase in producing complex polymeric structures.[81]

Size exclusion chromatography (SEC) was employed to show an increase of the Mw and a decrease of the dispersity. Some of the studies used NMR spectroscopy to elucidate the structure of the lignin polymerization: ^{31}P NMR shows the drop in phenolic-OH groups, and 2D-HSQC shows lignin-lignin condensation in C2, C5 and C6 positions. FTIR can also be used to show the apparition of C—O—C stretching in the lignin-modified samples.

3.2. Depolymerization

While the modification of lignin by alkaliphilic laccases has primarily focused on polymerization, several studies in the literature also highlight examples of lignin depolymerization under alkaline conditions. Those studies are presented in Table 4.

Buzzo *et al.* showed that 52% of the starting material was degraded within 32 h, thanks to UV-vis absorbance. In addition, they estimated the degradation thanks to SEM, showing that untreated lignin morphology was modified after treatment, showing a drastic alteration of the surface, without confirming any structural changes at the polymer level.[83] Sondhi *et al.* evaluated the bleaching capacities of the SN4 laccase on lignin at pH 9. They observed a decrease of the kappa number and an increase of the brightness, confirming the bleaching capacity of the system.[85] Xu *et al.* treated alkali lignin with a bacterial laccase at pH 11 for 48 h at 30 °C. The cleavage of aromatic cycles was observed by 2D-NMR along with the decrease of the lignin particle size by DLS, in aqueous conditions at pH 11.[84] Recently, Towle *et al.* developed a new analysis method: Fourier transform ion cyclotron resonance mass spectrometry (ESI-FT-ICR MS). In their study, they treated a METNIN macro lignin supplied by Metgen with MetZyme® in a 200 mM ammonium acetate solution at pH 9.5, for 24 h at 25 °C. The 2D-NMR showed a 50% loss of the signals related to β-β linkages. The developed ESI-FT-ICR MS showed that syringaresinol is targeted by the MetZyme® laccase, with a 95% decrease.[86]

Zhu, Yang, Levy-Booth, Singh, and Kontro *et al.* showed that the enzymatic treatment of lignin with their bacterial (except for Kontro who used a fungal laccase from *C. cinerea*) alkaliphilic laccases produced aromatic compounds. The use of SEC or GC-MS allowed them to detect the production of valuable lignin monomers such as vanillin, p-hydroxybenzaldehyde or syringaldehyde. The obtained products are summarized in Figure 4.[82,87–91]

Zhu *et al.* identified 15 compounds via GC-MS, reporting the percentages in Figure 5, and observed 39% lignin degradation by absorbance decrease, though no overall yield for depolymerization products was provided.[82] Similarly, Yang *et al.* (2019,

ChemSusChem

Review

doi.org/10.1002/cssc.202402377

Chemistry
Europe

European Chemical
Societies Publishing

Table 4. Overview of scientific studies concerning the alkaliphilic-laccase-induced depolymerization of different sources of lignins in various conditions.

| Substrate	Laccase	Reaction pH	Source
AL	*Bacillus ligniniphilus L1* (b)	9	Zhu (2017)[82]
AL	*Chitinophaga sp.* CB10 : Lac_CB10 (b)	10.5	Buzzo (2024)[83]
AL	*Sutcliffiella sp.* NC1 (b)	11	Xu (2023)[84]
SW sulfite pulp	SN4 laccase (b)	9	Sondhi (2015)[85]
METNIN macro (from Metgen Oy)	MetZyme® LIGNO™ (b)	9.5	Towle (2024)[86]
OSL and KL	CtLac (from *Caldalkalibacillus thermarum* strain TA2.A1) (b)	8	Yang (2019)[87]
Rice straw	CtLac (from *Caldalkalibacillus thermarum* strain TA2.A1) (b)	8	Yang (2023)[88]
13 C-DHP lignin	LacOST51 (b)	8	Levy-Booth (2022)[89]
Steam- and OS-pretreated corn stover, poplar, and lodgepole pine	sLac (from *Myxomatosis sp.* 75iv3) (b)	8	Singh (2017)[90]
Biorefinery HW lignin (ethanol fractionation)	*C. cinerea* (rCcLcc9) (f)	7	Kontro (2021)[91]

(f) fungal and (b) bacterial; **AL:** Alkali Lignin; **KL:** Kraft Lignin; **MWL:** Milled Wood Lignin; **OSL:** Organosolv Lignin; **SW** and **HW:** soft wood and hard wood; **WS:** wheat straw.
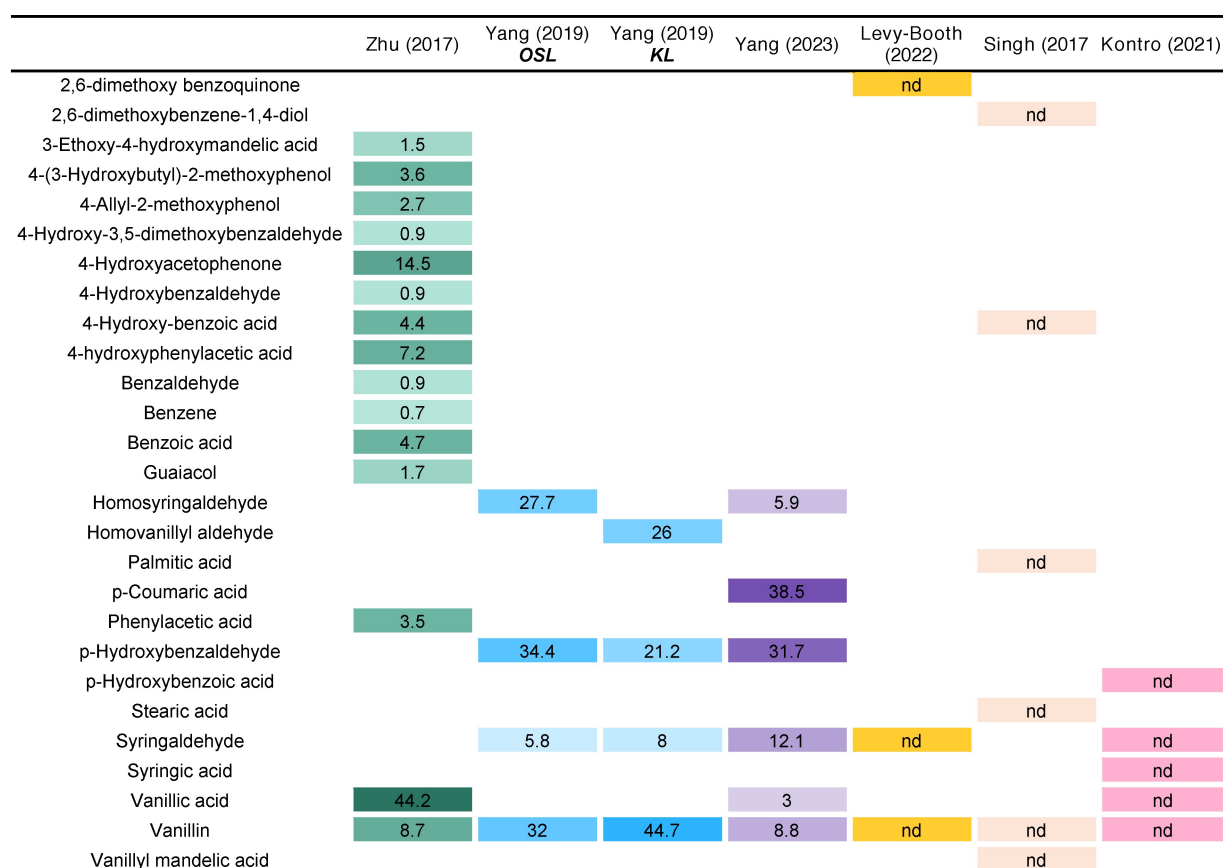
Figure 4. Overview of valuable lignin monomers from the alkaliphilic-laccase-induced depolymerization. The numbers represent a proportion (%) of the identified products. No global yields were reported.[82,87–91]

2023) did not report yields, presenting changes as % EIC (extracted ion chromatograms) area from HPLC-MS, relative to untreated samples.[87–88] For this review, the produced product percentages were standardized to total 100%. Levy-Booth and Singh detected products via HPLC without yield indication, while Kontro found a 3:1 syringyl-to-guaiacyl ratio using HPLC.[89–90]

Based on the results summarized in the table, vanillin and p-hydroxybenzaldehyde are the most frequently detected small phenolic compounds, particularly in studies using bacterial
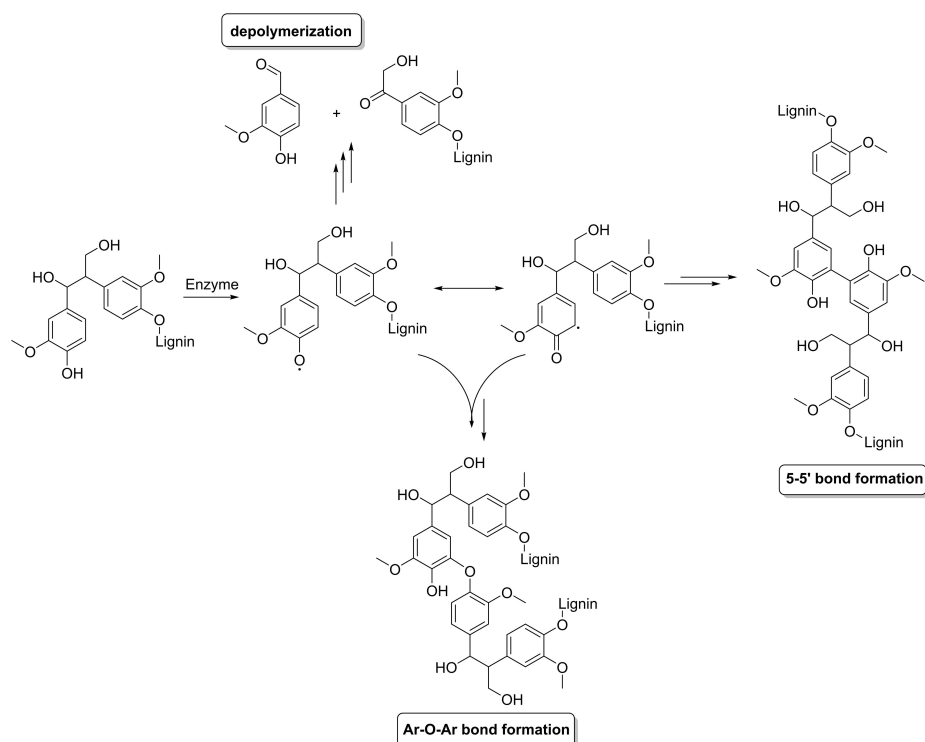
Figure 5. Proposed mechanisms for polymerization and depolymerization of lignin.

laccases under alkaline conditions. The variety of substrates and lignin types led to differing proportions of these phenolic compounds, but vanillin consistently appeared across most studies. One can wonder if the laccases used present a selectivity capacity. Levy-Booth's study only identified three degradation products, but it's important to consider that the starting material was DHP, which resembles homolignin and has a simpler structure compared to more complex industrial lignins.[89] This could explain the limited diversity of products observed.

Finally, some studies showed that both polymerization and depolymerization occurred during the lignin treatment with alkaliphilic laccases.

3.3. Both Polymerization and Depolymerization

The enzymatic treatment of various lignins with alkaliphilic laccases demonstrate that alkaliphilic-laccase-catalyzed reactions can lead to both polymerization and depolymerization processes, depending on the reaction conditions and lignin substrates. The overview of those studies is summarized in Table 5.

Two key studies focus on the enzymatic treatment of eucalyptus kraft lignin using fungal alkaliphilic laccases, each offering distinct methodologies and outcomes. Rodríguez-Escribano et al. conducted in 2022 a comprehensive study on eucalyptus kraft lignin in a B&R buffer (20 mM, pH 9–10), at 30 °C for periods ranging from 2 to 24 h. Initial treatment at 2 h showed an increase in free phenolic groups, indicative of depolymerization. However, after 24 h, a decrease in phenolic hydroxyl content coupled with an increase in molecular weight suggested a shift toward repolymerization. This study further demonstrated the potential of custom-engineered extremophilic laccases for varied industrial applications, including kraft lignin depolymerization, kraft pulp delignification and bleaching, and fiberboard production. The authors also proposed that employing alternative conditions, particularly through the use

Table 5. Overview of scientific studies concerning the alkaliphilic-laccase-induced both polymerization and depolymerization of different sources of lignins in various conditions.

Substrate	Laccase	Reaction pH	Source
Eucalyptus KL	Li10, Li11 and C-LeB (f)	9–10	Rodríguez-Escribano (2022)[92]
Eucalyptus KL	Li10, Li11, and C-LeB (f)	9–10	Rodríguez-Escribano (2023)[93]
HW lignin (alkali soluble fraction)	MetZyme® lignO™ (b)	10.5	Hämäläinen (2018)[94]
WS or OSL (acetone soluble fractions)	MetZyme® (b)	10.6	Pajer (2024)[95]
(f) fungal and (b) bacterial; **KL**: Kraft Lignin; **OSL**: Organosolv Lignin; **HW**: hard wood; **WS**: wheat straw.			

of bacterial laccases in conjunction with membrane operations, could prevent repolymerization, favoring continuous depolymerization.[92] In a subsequent study by Rodríguez-Escribano (2023), eucalyptus kraft lignin was similarly treated in a B&R buffer at pH 9–10, 30 °C for 24 h. At pH 9, a decrease in phenolic content accompanied by a rise in carbonyl content led to a significant increase in molecular weight. At pH 10, both phenolic and carbonyl contents showed slight increases, with SEC revealing new low molecular weight signals. By integrating a membrane separation system, the study successfully mitigated repolymerization, highlighting that oxidation and demethylation were predominant, but the condensation of oxidized products remained the dominant reaction mechanism.[93]

Pajer et al. explored solvent-based fractionation followed by oxidative treatment utilizing MetZyme® laccase. This approach enabled the selective isolation of valuable monomeric compounds and polymerized materials with significantly enhanced molecular weight and hydrophobicity. The oxidative treatment was performed on acetone-soluble fractions of sulfuric kraft lignin (SKL) and wheat straw organosolv lignin (WSL) in a sodium glycinate buffer, at pH 10.6, at 40 °C for 2 h. The study revealed that the balance between polymerization and depolymerization is influenced by several critical factors, including enzyme concentration, substrate type, and reaction time. At lower laccase concentrations, depolymerization is favored due to the enzyme's ability to cleave phenolic bonds, while higher concentrations promote repolymerization of the resulting fragments. Substrate characteristics also play a role, with terminal phenolic units being more susceptible to depolymerization, whereas smaller lignin fragments tend to undergo repolymerization. Moreover, extended reaction times increase the likelihood of repolymerization following initial depolymerization.[95]

Interestingly, the parameters established in Payer study do not align with those observed in previously presented bacterial laccase treatments. Both Morales et al. and Zhu et al. discussed the treatment of alkali lignin with the bacterial laccase Bacillus ligniniphilus, under slightly alkaline conditions, at pH 7.9 and 9, respectively.[77,82] Their findings, however, reveal opposite effects: Morales observed a significant polymerization, with a 6.75-fold increase in the weight-average molecular weight, while Zhu reported depolymerization, producing 15 distinct aromatic compounds. This highlights the ambivalence of alkaliphilic laccases on the same substrate and underscores the complexity of predicting clear trends in their enzymatic action.

Identifying clear trends in the alkaline enzymatic treatment of lignin is challenging due to the complex interplay of factors that influence polymerization and depolymerization. In this review, we have compiled, to the best of our knowledge, all relevant articles on this topic and attempted to identify key trends and draw conclusive insights. The comparison between studies leading to polymerization and depolymerization of lignin reveals distinct trends based on substrate type, enzyme source, and reaction conditions. Polymerization studies largely involve kraft lignin (KL), fractionated alkali lignin (AL), and softwood/hardwood lignins, with reactions favoring alkaline pH values (7–10.5) and temperatures ranging from 30 °C to 70 °C. Both bacterial and fungal laccases are employed, but bacterial

enzymes such as Bacillus ligniniphilus and MetZyme® play a key role, alongside fungal enzymes like Myceliophthora thermophila. These conditions typically promote oxidative coupling, leading to an increase in molecular weight. In contrast, depolymerization studies predominantly use lower molecular weight substrates like sulfite pulp and steam-exploded lignins, often treated with bacterial laccases under more extreme alkaline conditions (pH 9–11). Here, the focus shifts to breaking down lignin structures into smaller aromatic compounds, facilitated by bacterial laccases such as Chitinophaga sp. and CtLac. The use of untreated or steam-pretreated lignins in depolymerization, versus the fractionated lignins used in polymerization, further emphasizes the role of substrate pre-treatment in determining the enzymatic outcome. These findings highlight the complex balance between polymerization and depolymerization pathways, driven by enzyme type, pH, and substrate specificity.

3.4. Thoughts on the Mechanism

Pajer et al. proposed a radical-laccase-catalyzed degradation pattern of guaiacyl-glycerol-β-guaiacyl structures yielding 3 different monomeric phenols, including vanillin and vanillic acid, even though the reaction takes place at pH 10.6, where the phenols are deprotonated.[95]

Morales et al. proposed that the reaction mechanism involved phenyl radical activation within aromatic structures, leading to substitution and repolymerization reactions, which contributed to an increase in overall molar mass.[77] Rosado et al. thoroughly examined the oxidation mechanisms of fungal and bacterial laccases, highlighting a key difference: fungal laccases typically oxidize phenolic substrates in their protonated state, whereas bacterial laccases show optimal activity on substrates in their phenolate form, following deprotonation of the phenolic group.[96]

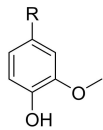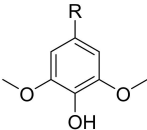Some examples of (de)polymerization reactions are shown on Figure 5.

The bacterial laccases-induced modifications are then dependent on the species pKa values. Ragnar et al. reported and determined the pKa values of a number of guaiacyl, syringyl and other phenols related to lignin.[30]

The pKa values reported in Table 6 highlight the critical pH range for lignin reactivity. In the case of alkaliphilic enzymes, the question arises whether solubilized lignin, likely deprotonated, could undergo radical coupling or depolymerization cascades. When treated with laccases at pH 9 or 10.6, the protonation state differs, which could affect reactivity. This factor, though significant, is not discussed in most studies, which assume radical-based mechanisms at any pH.

4. Summary and Outlook

In conclusion, the enzymatic treatment of lignin in alkaline homogeneous media using alkaliphilic enzymes presents diverse outcomes, including polymerization, depolymerization,

Table 6. pKa values of lignin-related phenols.[30]

R=H	9.93	9.98
R=CH₃	10.27	10.01
R=CH₂OH	9.78	9.87
R=CHO	7.40	7.34
R=COOH	9.39	9.49

or both simultaneously. While numerous studies exist on lignin modification by native laccases, it is well-established that laccases alone typically promote polymerization, with depolymerization occurring only when mediators are added. However, the mechanisms underlying these processes remain unclear and vary based on substrate and mediator choice. This area of research is highly promising and will undoubtedly continue to evolve in future studies. A key area for further investigation is the understanding of critical parameters such as buffer composition, pH, and the resulting protonation state of lignin. These factors could offer deeper insights into the interplay between lignin solubilization and its radical-mediated reactions, ultimately contributing to more precise control of polymerization and depolymerization phenomena.

Conflict of Interests

The authors declare no conflict of interest.

Keywords: lignin · alkaliphilic laccases · polymerization · depolymerization

[1] Q. Liu, L. Luo, L. Zheng, *Int. J. Mol. Sci.* **2018**, *19*, 335.
[2] J. Ralph, K. Lundquist, G. Brunow, F. Lu, H. Kim, P. F. Schatz, J. M. Marita, R. D. Hatfield, S. A. Ralph, J. H. Christensen, W. Boerjan, *Phytochem. Rev.* **2004**, *3*, 29–60.
[3] A. Eraghi Kazzaz, P. Fatehi, *Ind. Crops Prod.* **2020**, *154*, 112732.
[4] A. Tribot, G. Amer, M. Abdou Alio, H. De Baynast, C. Delattre, A. Pons, J.-D. Mathias, J.-M. Callois, C. Vial, P. Michaud, C.-G. Dussap, *Eur. Polym. J.* **2019**, *112*, 228–240.
[5] M. P. Pandey, C. S. Kim, *Chem. Eng. Technol.* **2011**, *34*, 29–41.
[6] Z. Sun, B. Fridrich, A. de Santi, S. Elangovan, K. Barta, *Chem. Rev.* **2018**, *118*, 614–678.
[7] C. Chio, M. Sain, W. Qin, *Renewable Sustainable Energy Rev.* **2019**, *107*, 232–249.
[8] A. Messerschmidt, R. Huber, *Eur. J. Biochem.* **1990**, *187*, 341–352.
[9] S. Roth, A. C. Spiess, *Bioprocess Biosyst. Eng.* **2015**, *38*, 2285–2313.
[10] G. Atiwesh, C. C. Parrish, J. Banoub, T.-A. T. Le, *Biotechnol. Prog.* **2022**, *38*, e3226.
[11] D. M. Mate, M. Alcalde, *Microb. Biotechnol.* **2017**, *10*, 1457–1467.
[12] S. A. Mayr, S. Wagner, R. Nagl, A. Pellis, M. Gigli, C. Crestini, M. Horvat, T. Zeiner, G. M. Guebitz, R. Weiss, N. Schwaiger, *ACS Sustainable Chem. Eng.* **2023**, *11*, 17739–17751.
[13] D. Huber, A. Ortner, A. Daxbacher, G. S. Nyanhongo, W. Bauer, G. M. Guebitz, *ACS Sustainable Chem. Eng.* **2016**, *4*, 5303–5310.
[14] E. Nugroho Prasetyo, T. Kudanga, L. Østergaard, J. Rencoret, A. Gutiérrez, J. C. Del Río, J. Ignacio Santos, L. Nieto, J. Jiménez-Barbero, A. T. Martínez, *Bioresour. Technol.* **2010**, *101*, 5054–5062.
[15] S. Magina, A. Barros-Timmons, D. V. Evtuguin, *Holzforschung* **2020**, *74*, 589–596.
[16] D. Legras-Lecarpentier, K. Stadler, R. Weiss, G. M. Guebitz, G. S. Nyanhongo, *ACS Sustainable Chem. Eng.* **2019**, *7*, 12621–12628.
[17] D. Huber, A. Pellis, A. Daxbacher, G. S. Nyanhongo, G. M. Guebitz, *Polymer* **2016**, *8*, 280.
[18] D. Huber, A. Ortner, A. Daxbacher, G. S. Nyanhongo, W. Bauer, G. M. Guebitz, *ACS Sustainable Chem. Eng.* **2016**, *4*, 5303–5310.
[19] M. Euring, K. Ostendorf, M. Rühl, U. Kües, *Front. Biomed. Biotechnol.* **2022**, *9*, 788622.
[20] E. Nugroho Prasetyo, T. Kudanga, L. Østergaard, J. Rencoret, A. Gutiérrez, J. C. Del Río, J. Ignacio Santos, L. Nieto, J. Jiménez-Barbero, A. T. Martínez, *Bioresour. Technol.* **2010**, *101*, 5054–5062.
[21] D. Areskogh, J. Li, G. Gellerstedt, G. Henriksson, *Biomacromolecules* **2010**, *11*, 904–910.
[22] A. Leonowicz, G. Szklarz, M. Wojtaś-Wasilewska, *Phytochemistry* **1985**, *24*, 393–396.
[23] V. Geniselli Da Silva, *Bioresour. Technol.* **2021**, *16*, 100824.
[24] J. C. Stevens, L. Das, J. K. Mobley, S. O. Asare, B. C. Lynn, D. W. Rodgers, J. Shi, *ACS Sustainable Chem. Eng.* **2019**, *7*, 15928–15938.
[25] C. A. Pena, L. F. Ballesteros, H. Rodríguez, E. Rodil, J. A. Teixeira, M. Michelin, *Biomass Bioenergy* **2023**, *177*, 106928.
[26] T. Itoh, Y. Takagi, *ACS Sustainable Chem. Eng.* **2021**, *9*, 1443–1458.
[27] T. Pang, G. Wang, H. Sun, W. Sui, C. Si, *Ind. Crops Prod.* **2021**, *165*, 113442.
[28] M. Gigli, C. Crestini, *Green Chem.* **2020**, *22*, 4722–4746.
[29] J. S. Rodrigues, V. Lima, L. C. P. Araújo, V. R. Botaro, *Ind. Eng. Chem. Res.* **2021**, *60*, 10863–10881.
[30] M. Ragnar, C. T. Lindgren, N.-O. Nilvebrant, *J. Wood Chem. Technol.* **2000**, *20*, 277–305.
[31] M. G. Fernández-López, R. A. Batista-García, E. T. Aréchiga-Carvajal, *J. Fungi* **2023**, *9*, 652.
[32] C. Novoa, G. V. Dhoke, D. M. Mate, R. Martínez, T. Haarmann, M. Schreiter, J. Eidner, R. Schwerdtfeger, P. Lorenz, M. D. Davari, F. Jakob, U. Schwaneberg, *ChemBioChem* **2019**, *20*, 1458–1466.
[33] A. P. Dubnovitsky, E. G. Kapetaniou, A. C. Papageorgiou, *Protein Sci.* **2005**, *14*, 97–110.
[34] M. Kolomytseva, N. Myasoedova, A. Samoilova, E. Podieiablonskaia, A. Chernykh, T. Classen, J. Pietruszka, L. Golovleva, *Process Biochem.* **2017**, *62*, 174–183.
[35] H. P. Call, I. Mücke, *J. Biotechnol.* **1997**, *53*, 163–202.
[36] K. Birikh, A. Michine, M. Heikkilä, P. Ihalainen, In *Borefineries*, Krzysztof Biernat, Ed.; IntechOpen, Rijeka, **2021**, p Ch. 3. https://doi.org/10.5772/intechopen.99415.
[37] S. Kawai, M. Asukai, N. Ohya, K. Okita, T. Ito, H. Ohashi, *FEMS Microbiol. Lett.* **1999**, *170*, 51–57.
[38] R. Bourbonnais, M. G. Paice, I. D. Reid, P. Lanthier, M. Yaguchi, *Appl. Environ. Microbiol.* **1995**, *61*, 1876–1880.
[39] P. Figueiredo, D. Morais de Carvalho, M. H. Lahtinen, K. S. Hilden, K. S. Mikkonen, *Sustain. Mater. Technol.* **2023**, *37*, 10.1016/j.susmat.2023.e00677.
[40] M. Wang, Y. Zhao, J. Li, *Holzforschung* **2018**, *72*, 357–365.
[41] Y. Shi, K. Zhu, Y. Dai, C. Zhang, H. Jia, *Chemosphere* **2020**, *248*, 10.1016/j.chemosphere.2020.125931.
[42] A. Ortner, D. Huber, O. Haske-Cornelius, H. K. Weber, K. Hofer, W. Bauer, G. S. Nyanhongo, G. M. Guebitz, *Process Biochem.* **2015**, *50*, 1277–1283.
[43] M. A. West, A. C. Hickson, M.-L. Mattinen, G. Lloyd-Jones, *BioResources* **2014**, *9*, 2782–2796.
[44] A. Dong, X. Fan, Q. Wang, Y. Yu, P. Wang, J. Yuan, A. Cavaco-Paulo, *J. Nat. Fibers* **2018**, *15*, 384–395.
[45] D. Areskogh, J. Li, P. Nousiainen, G. Gellerstedt, J. Sipilä, G. Henriksson, *Holzforschung* **2010**, *64*, 21–34.
[46] D.-J. Yang, H.-F. Zhou, S.-Q. Xie, X.-L. Wu, X.-Q. Qiu, *Acta Polym.* **2013**, 232–240.
[47] L. Munk, A. K. Sitarz, D. C. Kalyani, J. D. Mikkelsen, A. S. Meyer, *Biotechnol. Adv.* **2015**, *33*, 13–24.
[48] M. B. Agustin, D. M. de Carvalho, M. H. Lahtinen, K. Hilden, T. Lundell, K. S. Mikkonen, *ChemSusChem* **2021**, *14*, 4615–4635.
[49] P. Maijala, M.-L. Mattinen, P. Nousiainen, J. Kontro, J. Asikkala, J. Sipilä, L. Viikari, *J. Mol. Catal. B* **2012**, *76*, 59–67.
[50] D. Van De Pas, A. Hickson, L. Donaldson, G. Lloyd-Jones, T. Tamminen, A. Fernyhough, M.-L. Mattinen, *BioResources* **2011**, *6*, 1105–1121.

[51] M.-L. Mattinen, P. Maijala, P. Nousiainen, A. Smeds, J. Kontro, J. Sipilä, T. Tamminen, S. Willför, L. Viikari, *J. Mol. Catal. B* **2011**, *72*, 122–129.

[52] J. Dillies, C. Vivien, M. Chevalier, A. Rulence, G. Châtaigné, C. Flahaut, V. Senez, R. Froidevaux, *Biotechnol. Appl. Biochem.* **2020**, *67*, 774–782.

[53] M. L. Mattinen, T. Suortti, R. J. A. Gosselink, D. S. Argyropoulos, D. Evtuguin, A. Suurnäkki, E. de Jong, T. Tamminen, *BioResources* **2008**, *3*, 549–565.

[54] R. W. Thring, M. N. Vanderlaan, S. L. Griffin, *J. Wood Chem. Technol.* **1996**, *16*, 139–154.

[55] S. Gouveia, C. Fernández-Costas, M. A. Sanromán, D. Moldes, *Bioresour. Technol.* **2012**, *121*, 131–138.

[56] R. J. A. Gosselink, J. E. G. van Dam, E. de Jong, E. L. Scott, J. P. M. Sanders, J. Li, G. Gellerstedt, *Holzforschung* **2010**, *64*, 193–200

[57] S. Grönqvist, L. Viikari, M.-L. Niku-Paavola, M. Orlandi, C. Canevali, J. Buchert, *Appl. Microbiol. Biotechnol.* **2005**, *67*, 489–494.

[58] K. Rittstieg, A. Suurnakki, T. Suortti, K. Kruus, G. Guebitz, J. Buchert, *Enzyme Microb. Technol.* **2002**, *31*, 403–410.

[59] S. Shleev, P. Persson, G. Shumakovich, Y. Mazhugo, A. Yaropolov, T. Ruzgas, L. Gorton, *Enzyme Microb. Technol.* **2006**, *39*, 841–847.

[60] L. P. Christopher, B. Yao, Y. Ji, *Front. Energy Res.* **2014**, *2*, 10.3389/fenrg.2014.00012.

[61] D. Rochefort, D. Leech, R. Bourbonnais, *Green Chem.* **2004**, *6*, 14–24.

[62] R. Kondo, T. Iimori, H. Imamura, T. Nishida, *J. Biotechnol.* **1990**, *13*, 181–188.

[63] I. F. Fiţigău, F. Peter, C. G. Boeriu, *Acta Biochim. Pol.* **1970**, *60*, 10.18388/abp.2013_2065.

[64] C. Eggert, U. Temp, J. F. D. Dean, K.-E. L. Eriksson, *FEBS Lett.* **1996**, *391*, 144–148.

[65] L. F. Longe, J. Couvreur, M. Leriche Grandchamp, G. Garnier, F. Allais, K. Saito, *ACS Sustainable Chem. Eng.* **2018**, *6*, 10097–10107.

[66] J. R. Hernández Fernaud, A. Carnicero, F. Perestelo, M. Hernández Cutuli, E. Arias, M. A. Falcón, *Enzyme Microb. Technol.* **2006**, *38*, 40–48.

[67] K. Li, F. Xu, K.-E. L. Eriksson, *Appl. Environ. Microbiol.* **1999**, *65*, 2654–2660.

[68] C. Crestini, L. Jurasek, D. S. Argyropoulos, *Chem. Eur. J.* **2003**, *9*, 5371–5378.

[69] J. Li, G. Henriksson, G. Gellerstedt, *Bioresour. Technol.* **2007**, *98*, 3061–3068.

[70] A. G. Barneto, E. Aracri, G. Andreu, T. Vidal, *Bioresour. Technol.* **2012**, *112*, 327–335.

[71] D. Moldes, M. Díaz, T. Tzanov, T. Vidal, *Bioresour. Technol.* **2008**, *99*, 7959–7965.

[72] S. Ghatge, Y. Yang, W.-Y. Song, T.-Y. Kim, H.-G. Hur, *Appl. Microbiol. Biotechnol.* **2018**, *102*, 4075–4086.

[73] Z. V. Renfeld, A. M. Chernykh, B. P. Baskunov, A. S. Gaidina, N. M. Myasoedova, A. D. Egorova, O. V. Moiseeva, S. Y. Gorina, M. P. Kolomytseva, *Microorganisms* **2023**, *11*, 2698.

[74] R. Moya, P. Saastamoinen, M. Hernández, A. Suurnäkki, E. Arias, M.-L. Mattinen, *Bioresour. Technol.* **2011**, *102*, 10006–10012.

[75] S. Gouveia, C. Fernández-Costas, M. A. Sanromán, D. Moldes, *Bioresour. Technol.* **2013**, *131*, 288–294.

[76] S. A. Mayr, R. Subagia, R. Weiss, N. Schwaiger, H. K. Weber, J. Leitner, D. Ribitsch, G. S. Nyanhongo, G. M. Guebitz, *Int. J. Mol. Sci.* **2021**, *22*, 13161.

[77] G. M. Morales, S. S. Ali, H. Si, W. Zhang, R. Zhang, K. Hosseini, J. Sun, D. Zhu, *Front. Biomed. Biotechnol.* **2020**, *8*, 671.

[78] W. Qiu, H. Chen, *Bioresour. Technol.* **2008**, *99*, 5480–5484.

[79] Q. Weihua, C. Hongzhang, *Bioresour. Technol.* **2008**, *99*, 5480–5484.

[80] L. Wang, L. Tan, L. Hu, X. Wang, R. Koppolu, T. Tirri, B. van Bochove, P. Ihalainen, L. S. Seleenmary Sobhanadhas, J. V. Seppälä, S. Willför, M. Toivakka, C. Xu, *ACS Sustainable Chem. Eng.* **2021**, *9*, 8770–8782.

[81] Z. Lu, J. Tienaho, P. Kilpeläinen, L. Wang, H. Zhang, L. Hu, R. Liu, H. Brännström, E. Halmemies, X. Wang, M. Toivakka, C. Xu, *ACS Sustainable Chem. Eng.* **2024**, *12*, 8765–8779.

[82] D. Zhu, P. Zhang, C. Xie, W. Zhang, J. Sun, W.-J. Qian, B. Yang, *Biotechnology Biofuels* **2017**, *10*, 44.

[83] B. B. Buzzo, N. S. M. Lima, P. A. M. Pereira, E. S. Gomes-Pepe, C. C. F. Sartini, E. G. D. M. Lemos, *Microbiol. Spectr.* **2024**, *12*, e04013–23.

[84] Z. Xu, J. Li, P. Li, C. Cai, S. Chen, B. Ding, S. Liu, M. Ge, M. Jin, *J. Biores. Bioprod.* **2023**, 10.1016/j.jobab.2023.09.004.

[85] S. Sondhi, P. Sharma, N. George, P. S. Chauhan, N. Puri, N. Gupta, *3 Biotech* **2015**, *5*, 175–185.

[86] Z. Towle, F. Cruickshank, C. L. Mackay, D. J. Clarke, L. E. Horsfall, *Analyst* **2024**, *149*, 2399–2411.

[87] Y. Yang, W.-Y. Song, H.-G. Hur, T.-Y. Kim, S. Ghatge, *Int. J. Biol. Macromol.* **2019**, *124*, 200–208.

[88] Y. Yang, S. Ghatge, H.-G. Hur, *Appl. Microbiol. Biotechnol.* **2023**, *107*, 273–286.

[89] D. J. Levy-Booth, L. E. Navas, M. M. Fetherolf, L.-Y. Liu, T. Dalhuisen, S. Renneckar, L. D. Eltis, W. W. Mohn, *ISME J.* **2022**, *16*, 1944–1956.

[90] R. Singh, J. Hu, M. R. Regner, J. W. Round, J. Ralph, J. N. Saddler, L. D. Eltis, *Sci. Rep.* **2017**, *7*, 42121.

[91] J. Kontro, C. Lyra, M. Koponen, J. Kuuskeri, M. A. Kähkönen, J. Wallenius, X. Wan, J. Sipilä, M. R. Mäkelä, P. Nousiainen, K. Hildén, *Front. Bioeng. Biotechnol.* **2021**, *9*, 10.3389/fbioe.2021.767139.

[92] D. Rodríguez-Escribano, R. Pliego-Magán, F. de Salas, P. Aza, P. Gentili, P. Ihalainen, T. Levée, V. Meyer, M. Petit-Conil, S. Tapin-Lingua, M. Lecourt, S. Camarero, *Biotech. Biofuels Bioprod.* **2022**, *15*, 149.

[93] D. Rodríguez-Escribano, F. de Salas, R. Pliego, G. Marques, T. Levée, A. Suonpää, A. Gutiérrez, Á. T. Martínez, P. Ihalainen, J. Rencoret, S. Camarero, *Polymers* **2023**, *15*, 4433.

[94] V. Hämäläinen, T. Grönroos, A. Suonpää, M. W. Heikkilä, B. Romein, P. Ihalainen, S. Malandra, K. R. Birikh, *Front. Bioeng. Biotechnol.* **2018**, *6*, 10.3389/fbioe.2018.00020.

[95] N. Pajer, M. Gigli, C. Crestini, *ChemSusChem* **2024**, *17*, e202301646.

[96] T. Rosado, P. Bernardo, K. Koci, A. V. Coelho, M. P. Robalo, L. O. Martins, *Bioresour. Technol.* **2012**, *124*, 371–378.