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Feature Article

Oxidative upgrade of lignin – Recent routes reviewed

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ABSTRACT

Lignin is the second most abundant natural polymer. Its use and targeted functionalisation within biomass refinery processes, however, still needs to be further explored and developed. The oxidative functionalisation, and thus valorisation of lignin, is a very promising way to go, since it holds the possibilities to yield highly functionalised, monomeric or oligomeric products that can serve as starting materials for other valorisation processes in the chemical and pharmaceutical industries. Gaining a profound knowledge about the structure of lignin, being able to analyse structural features, and understanding the mechanisms that guide the reactions leading to the oxidative derivatisation, depolymerisation and functionalisation of lignin samples from different renewable sources are key requirements for developing successful valorisation protocols for lignin. In this review, we wish to revisit, and set into context, some important achievements in the field of oxidatively upgrading lignin. We will focus on organometal catalyses (MTO, salen complexes, POMs), biomimetic catalyses (porphyrins), and enzymatic catalyses (laccase, peroxidase) for upgrading lignin and lignin model compounds. Details of mechanistic implications and means of potential manipulations of reaction outcomes are discussed.

Contents

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1. Introduction

The development and exploitation of renewable, non-fossil-based resources has become increasingly important, since the use of fossil-based resources is no longer justifiable for practical, ecological, and socio-economic reasons. Many technologies have been developed and successfully implemented in order to end fossil-dependent energy production; many of these technologies are based on the use of biomass [1,2]. Biomass represents a readily available and renewable, and thus versatile alternative resource, and research focused on exploring the possibilities to exploit this resource is gaining momentum in light of the dwindling of our fossil-based resources. Fuels obtained from biomass-refinery processes are already replacing fossil-based fuels in everyday life; the use of biomass components and derivatives thereof, as substrates for the chemical industry that produces higher value applications, ranging from building materials to pharmaceutical applications, is, however, still in fledging stages [3].

Forest biomass comprises a rather complex mixture of carbohydrates, aromatics, lipids, proteins, and a wide range of smaller molecules such as vitamins, colourants and odorants. New mechanical and chemical processes are needed to obtain analytically pure and defined substances out of – or from – this mixture [4], allowing the use of these substances to be used in downstream industrial chemical transformations. Ideally, the biomass-derived substances should be readily usable in already established chemical processes that utilise other, already commercially available substances for further derivatisation [5]. Today, biorefinery processes aiming at the valorisation of the lignocellulosic part of the biomass, which consists of cellulose, lignin, and hemicellulose, produce, in analogy to petroleum refinery processes, several products including fuel for energy production, and chemicals [1–4]. From the viewpoint that an economically viable biorefinery program comprises the use of all components of the biomass in parallel processes that aim at the production of both, fuels and fine chemicals [6], the lignin component is currently still under-utilised [7].

Lignin, that is currently mostly obtained as "waste" in paper and biofuel productions, but that could also be isolated by more tailor-made processes with respect to the specificities of further transformations within the biorefinery cascades [3,8], is the second-most abundant renewable polymer: it contributes as much as 30% of the weight, and as much as 40% of the energy content of lignocellulosic biomass [9]. Lignocellulosic biorefinery thus receives enormous amounts of lignin, and the development of truly sustainable and efficient biorefinery processes should aim at the valorisation of lignin not only as energy, but also as a resource for starting materials for the chemical industries [10,11]. Noteworthy, lignin represents the only renewable source of aromatic fine chemicals [12–14], and direct and efficient conversion of lignin to discrete molecules or classes of lower-molecular weight aromatic, monomeric building blocks for polymer productions is a very interesting future opportunity. The controlled breaking of carbon–carbon and carbon–oxygen bonds in lignin represents a very selective depolymerisation that could produce a whole series of monomeric, aromatic species [14]. Technologies that rely on selective bond cleavages in lignin also have the potential to yield new types of building blocks for block-polymers [4,7]. Selective modifications of the polymer lignin itself are suitable to transform it into a structural base for complex co-polymers with various potential applications [15]. In the medicinal and pharmaceutical areas, potential applications of lignin-derived substances could comprise the use as building blocks for the fabrication of microcapsules, or the exploition of the antioxidant features of the polyphenolic structural features of lignin [16]. Existing and potential applications of lignin are summarised in Fig. 1.

Several reviews have been written to cover and present research on lignin, and on processes aiming at its valorisation [1,2,7,8]. The methods used for the valorisation of lignin range from classical chemical approaches such as pyrolysis (thermolysis) [17–20], hydrolysis [21,22], reduction (hydrogenolysis) [23–25], or oxidation [26,27], to newer biotechnological approaches [22]. In this review, we wish to focus on methods and technologies aiming at the oxidative upgrade of lignin *via* radical pathways, since the native structure of lignin comprises several distinct functional groups that can – in principle – be selectively further functionalised *via* oxidation [28]. After briefly revisiting the most important structural features of lignin, the most important methods to isolate lignin, and the tools used characterise lignin and the products obtained upon (oxidative) functionalisation, we will summarise important work in the field of

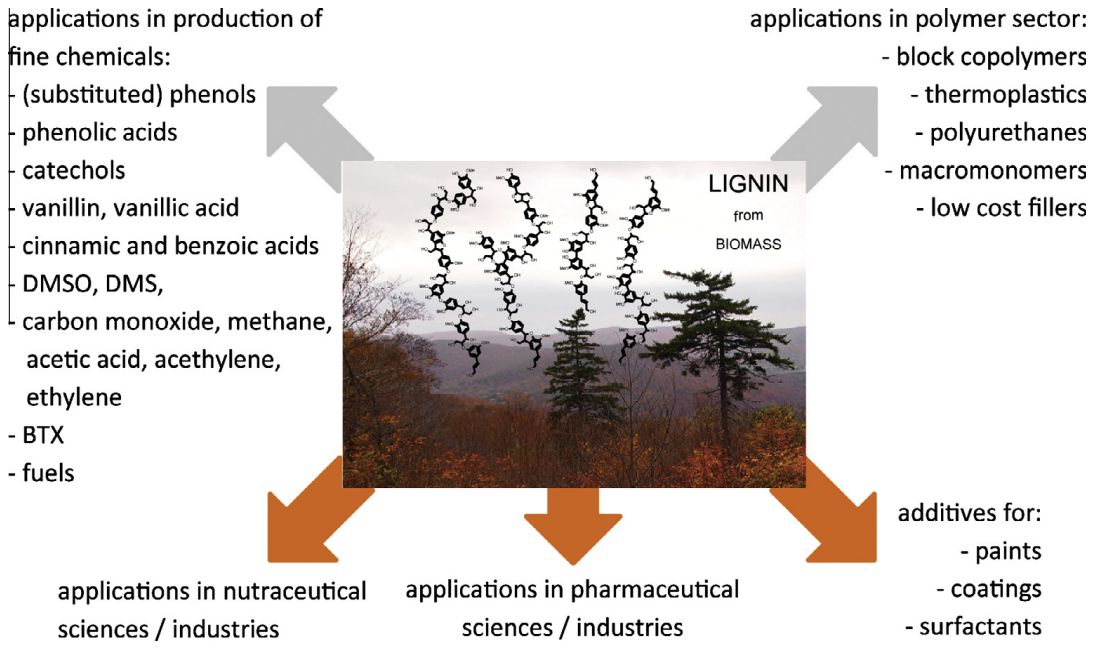


Fig. 1. Existing and potential applications of lignin as renewable resource from biomass.

oxidative lignin transformation using organometal-catalysed, biomimetic, and enzymatic processes.

2. Structural aspects of lignin

Before revisiting (partially structure modifying) processes and techniques for isolating and characterising lignin, we wish to briefly summarise its most important structural features, since this will ease further discussions. Lignin, described by Payen in 1838, and chemically defined by Schulze in 1865, is a hydrophilic substance present in plant cell walls. It chemically, and physically links the other matrix components of the cell walls, cellulose and hemicellulose [29]. This linking results in increased impermeability, mechanical strength, and rigidity of the plant cell walls; it also gives the cells a greater resistance to microbial attacks.

The distribution of lignin within the cell walls is, however, not uniform; the concentration of lignin in the middle lamella and the primary wall is higher than the concentration in the secondary wall [30]. Nonetheless, the majority of the total amount of lignin present in the plant, 75–85%, is located in the secondary wall, due to its considerably larger volume. The amount of lignin present in the plant varies from species to species [3,7], ranging from 20 ± 4% in hardwoods, to 28 ± 3% in softwoods and herbaceous angiosperms; monocots are less lignified (15 ± 4%).

Chemically, lignin (**1**) is seen as a highly complex phenolic polymer, which generally shows plant-specific compositions and linkage motifs [31,32]. Newer findings on milled wood lignin samples suggest, however, that lignin exists as linear oligomers (Fig. 2A), that presumably strongly interact in such a way, that traditional analyses of the molecular weights are biased and thus suggest higher molecular weight polymeric units [33]. To the best of the current knowledge, these lignin oligomers lack a defined primary structure, but rather represent random phenyl-propanoid (C9) polyphenols, which are mainly linked by arylglycerol ether bonds between phenolic *para*-coumaryl alcohol (**2**) (H-type), coniferyl alcohol (**3**) (G-type), and sinapyl alcohol (**4**) (S-type) units [34,35]. Depending on the plant-type, one of the different lignin-types dominants. Lignin of gymnosperms consists almost entirely of G-type lignin (G-lignin); dicotyledonous angiosperms produce a mixture of G- and S-type lignins (GS-lignin). All three types of lignin can be found in quantities in monocotyledonous lignin (GSH-lignin). Incomplete or modified monolignols accompany these three main lignin types in woody materials [36].

The comparable richness in binding types of lignin is the result of an interesting biosynthetic pathway (Scheme 1), in which monolignol radicals are formed initially [37,38].

Coupling of two radicals in form of a recombination reaction then forms a dehydrodimer that functions as a new monomer. Lignin formation is thus not an organised living radical polymerisation, but rather a series of polymerisation termination reactions involving ever-growing oligomers, resulting in a polydisperse polymer with no extended sequences of regularly repeating units. The composition of this polymer is generally characterised by the relative abundance of the H/G/S units, and by the distribution of different motifs of interunit linkages, which result from the various coupling events. Eight different motifs for interunit linkages are generally found (Fig. 2C); however, not all three lignin monomer units can undergo all coupling modes. Coupling is generally favoured at the β-position of the monolignol species, resulting in the formation of arylglycerol-β-aryl ethers (β-O-4′ motif, **1f**), phenylcoumarans (β-5′ motif, **1g**), pinoresinols (β-β′ motif, **1h**), diphenylethane dimers (β-1′ motif, **1i**), and spirodienones (SD motif, **1j**). Dilignols and higher oligomers preferentially couple at positions 4 and 5, yielding diaryl ethers (4-O-5′ motif, **1k**) and biphenyls (5–5′ motif, **1m**). Further

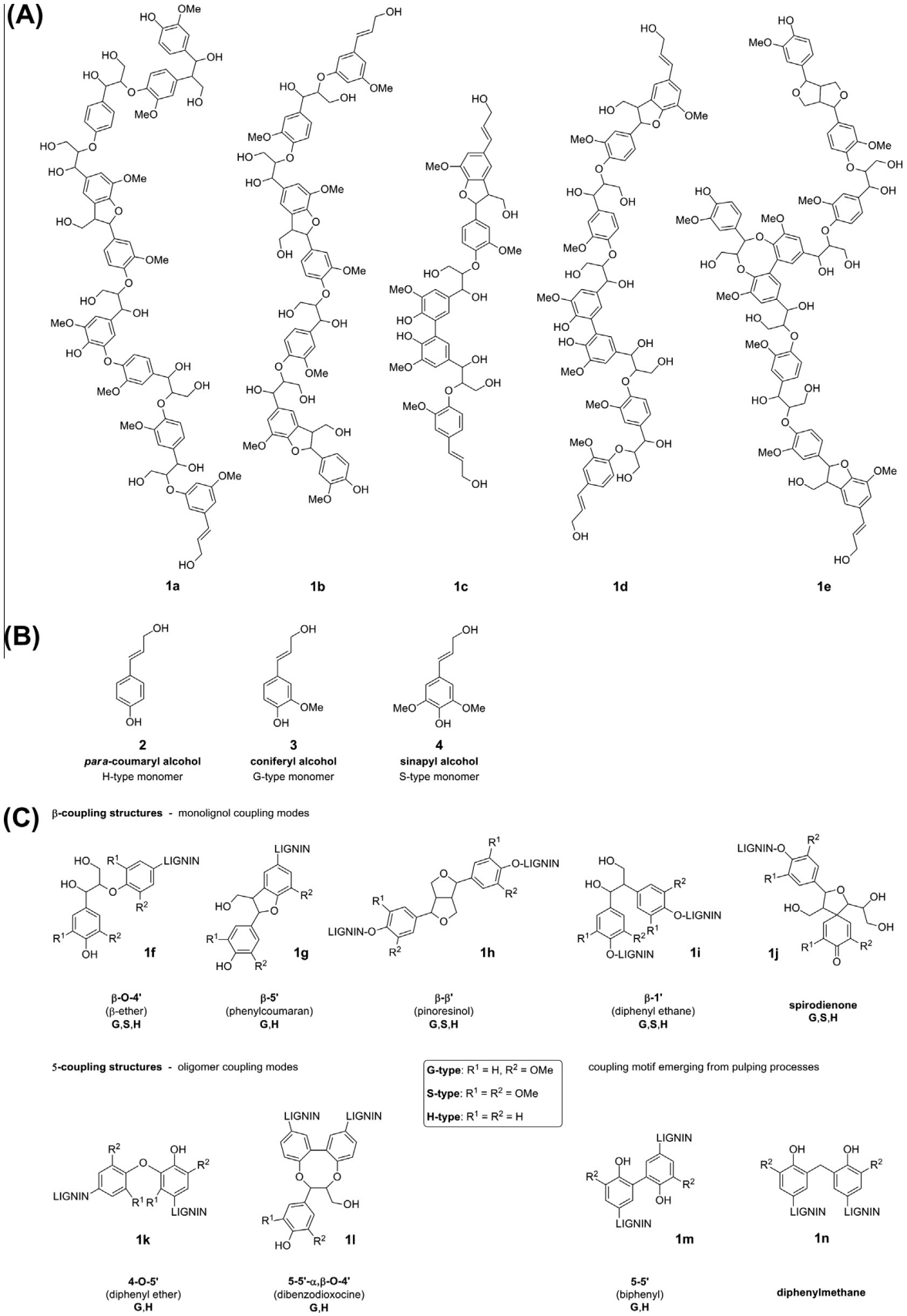
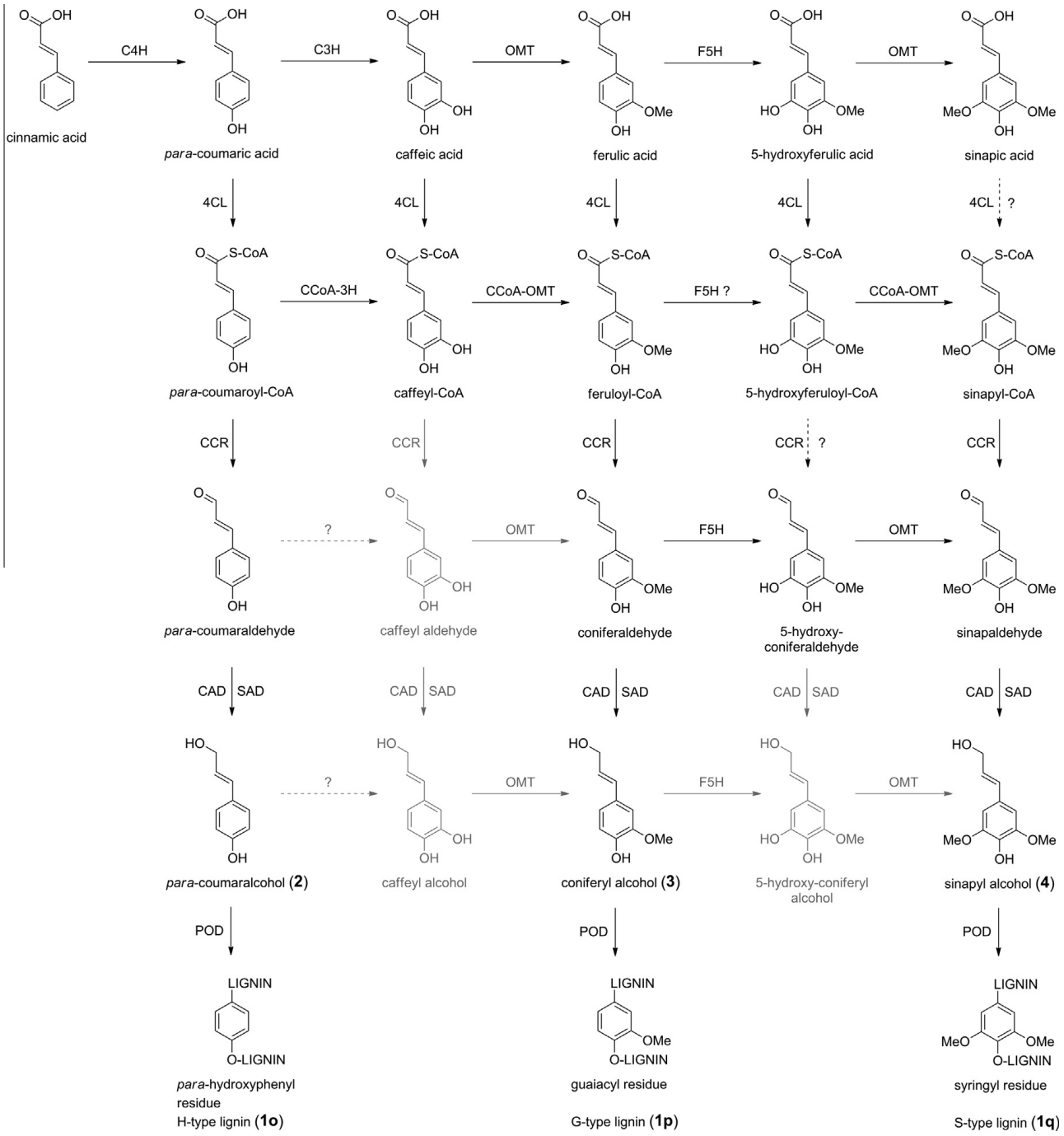


Fig. 2. (A) Representative structures of lignin biopolymers, (B) specific lignin types, and (C) main linkage motifs found in lignin and lignin extracts.

coupling of these oligomers is possible under formation of dibenzodioxocine units (5,5′-α,β-O-4′ (DBDO) motif, **1l**). Both the DBDO motif and the 4-O-5′ motif could constitute branching points in the lignin polymer [37–40]. However,



Scheme 1. Proposed biosynthetic pathway of lignin formation as described in Ref. 38 (C4H – cinnemate-4-hydroxylase; C3H – cinnemate-3-hydroxylase; OMT – *O*-methyltransferase; F5H – ferulate-5-hydroxylase; 4CL – CoA-ligase; CCR - cinnamoyl co-enzyme A reductase; CCoA-3H – coumaroyl-co-enzyme A 3 hydroxylase; CCoA-OMT – coumaroyl-co-enzyme *O*-methyl transferase; CAD – cinnamyl alcohol dehydrogenase; SAD – short-chain alcohol dehydrogenase; POD - peroxidase.

in light of the new structural findings that indicate a linear oligomeric structure rather a branched polymeric one, the roles of those motifs will have to be revisited.

In order to study reactions and processes that would allow a defined depolymerisation of lignin, model compounds are used that represent the aforementioned binding motifs. The most common ones are shown in Fig. 3.

3. Methods for isolating lignin

Raw plant biomass has to be treated in order to separate the valuable components of interest. A few rather general treatments yield differently composed feed streams for downstream processing facilities, and the processes run in these facilities have to be adopted to local specificities

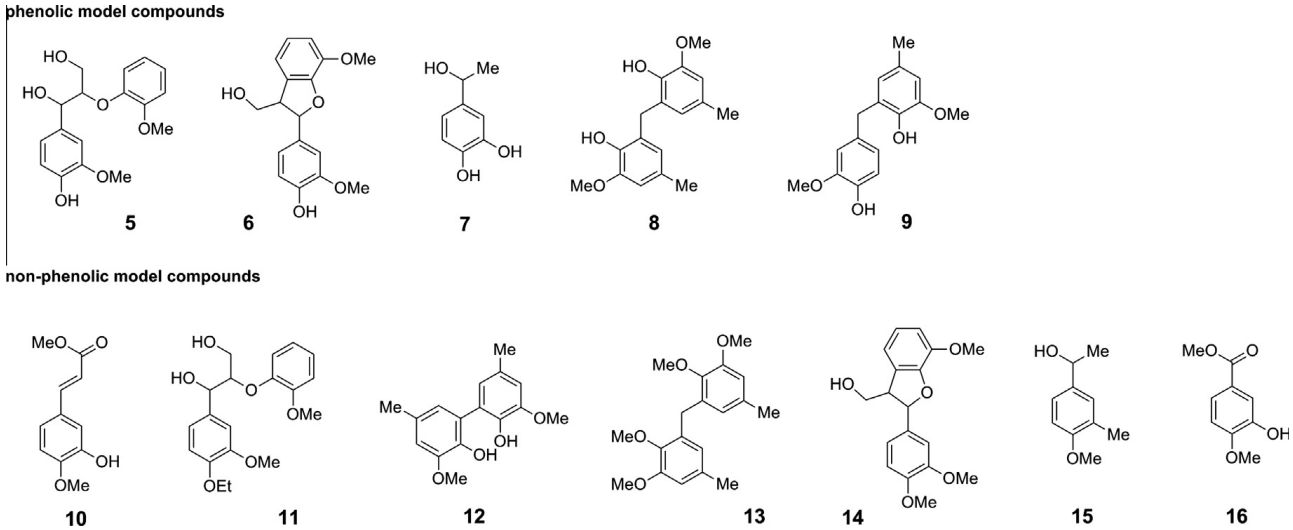


Fig. 3. Typical model compounds mimicking different structural motifs in lignin. See main text for references.

such as the source of the biomass [3]. Since a more uniform composition of the feed streams would be desirable from a process point of view, treatment processes for biomass should ideally account for, or be independent of the specificities of the biomass sources. Especially the inhomogeneous structural complexity of lignin represents a veritable challenge in this respect.

A gold standard procedure for lignin isolation does not exist. We thus wish to briefly present selected isolation processes that are currently used in research and industry, and outline the characteristics of the obtained lignin extracts. Knowing about the isolation method, and especially knowing how it might affect the structure of the lignin in the raw material, is crucial since isolated lignin represents the only source for obtaining chemical information about it, and for investigating the basic mechanisms of its microbiological degradation [35]. Table 1 contains an overview of the characteristics of lignins obtained by selected isolation techniques. The selected lignin types are the most widely used ones.

3.1. Milled wood lignin (MWL)

This lignin is obtained by Björkman's procedure [49]. Finely milled wood is extracted with a neutral organic solvent (e.g. 1,4-dioxane) to remove extraneous components. Only minor changes are supposed to occur in the structure of lignin during this procedure, and the lignin obtained is thus considered to be most representative of the lignin of the milled sample. MWL, however, is not considered to be representative of the lignin in the wood before the milling process.

3.2. Acidolysis lignin [50]

Lignin is extracted from plant tissues by a mild acid hydrolysis (0.2 M HCl in aqueous 1,4-dioxane, room

Table 1
Overview comparing the (average) characteristics of various lignin extracts discussed in the main text.

Lignin type	C9 molecular formula	Monomer molecular weight [Da]	Number-average molecular weight (M_n) [Da]	Poly-dispersity
Milled wood lignin[a]	$C_9H_{7.80}O_{2.41}(OCH_3)_{0.95}$	198	2800–14200	3.7–12.9
Cellulolytic enzyme lignin[b]	$C_9H_{8.02}O_{2.82}(OCH_3)_{0.90}$	187	~1900	5.7–6.7
Enzymatic mild acidolysis lignin (EMAL)[b]	$C_9H_{8.02}O_{2.82}(OCH_3)_{0.90}$	187	~2000	~3
Kraft lignin[c]	$C_9H_{8.5}O_{2.1}S_{0.1}(OCH_3)_{0.8}(CO_2H)_{0.2}$	180	1000–3000	2–4
Lignosulfonated lignin (softwood)[d]	$C_9H_{8.5}O_{2.5}(OCH_3)_{0.85}$ $(SO_3H)_{0.4}$	215–254	36000–61000	4–9
Lignosulfonated lignin (hardwood)[d]	$C_9H_{7.5}O_{2.5}(OCH_3)_{1.39}$ $(SO_3H)_{0.6}$	188	5700–12000	4–9
Organosolv lignin[e]	$C_9H_{8.53}O_{2.45}(OCH_3)_{1.04}$	188	>1000	2.4–6.4
Pyrolysis lignin[f]	$C_9H_{6.3-7.3}O_{0.6-1.4}(OCH_3)_{0.3-0.8}(OH)_{1-1.2}$	n.d.	300–600	2.0–2.2
Steam explosion lignin[g]	$C_9H_{8.53}O_{2.45}(OCH_3)_{1.04}$	188	1100–2300	1.5–2.8

[a] Norway spruce wood [41].
[b] Isolated from milled Norway spruce wood [41].
[c] Norway spruce wood [35].
[d] Norway spruce wood and aspen wood as softwood samples, eucalyptus wood as hardwood sample [42,43].
[e] Norway spruce wood [44,45].
[f] Beech wood [46,47].
[g] Japanese white birch wood and larch wood [48].

temperature). This lignin is reported to have only few carbohydrate impurities, while exhibiting a distribution of bond motifs that is supposed to be minimally affected by the isolation procedure and thus close to the natural one. Modification to the original protocol have been reported in which an alkaline treatment is used to isolate lignin extracts, termed mild acidolysis lignin [51]. Thioacidolysis represents another modification, in which ethanethiol is used instead of water. Higher yields, and less complex monomer mixtures are obtained [52].

3.3. Cellulolytic enzyme lignin (CEL)

This lignin is obtained from MWL after treatment with a commercially available cellulase–hemicellulase mixture to remove carbohydrate impurities. However, the enzyme mixture typically removes only up to 85–88% of the polysaccharides. Low number-average molecular weights (M_n) of around 1900 Da are observed, together with a polydispersity around 6, and an estimated average monomer molecular weight of 187 Da. Structurally, this lignin is similar to the one present in the original samples.

3.4. Enzymatic mild acidolysis lignin (EMAL) [53]

EMAL is obtained from refined CEL processes, cleaving lignincarbohydrate linkages using a mild acidolysis, while leaving ether bonds within the lignin structure intact. After an initial enzymatic hydrolysis, the solid remains are washed with acidified water before being treated with a dioxane/water mixture, containing 0.01 M hydrochloric acid under an inert atmosphere. EMAL is characterised by number-average molecular weights (M_n) similar to those obtained for CEL (ca. 2000 Da), while exhibiting an improved polydispersity (ca. 3). The estimated average monomer molecular weight lies by 187 Da.

3.5. Kraft lignin [54,55]

Kraft lignin (**1r**, Fig. 4A) is readily available since it represents the residues of chemical pulping processes in paper production. This lignin is precipitated from the "black liquor", by pH controlled precipitation. Kraft lignin is structurally highly modified, as approximately 70–75% of the hydroxyl groups become sulfonated during standard kraft pulping procedures. Degradation results in low number-average molecular weight (M_n) of about 1000–3000 Da, with a polydispersity between 2 and 4, and an estimated average monomer molecular weight of 180 Da. Kraft lignin is soluble in alkali and in basic solution and in highly polar organic solvents.

3.6. Sulfite lignin (lignosulfonate)

Lignosulfonate (**1s**, Fig. 4B) is the sulfonated lignin that is removed from wood by sulfite pulping. Hardwood lignosulfonate and softwood lignosulfonate are obtained from waste pulping liquor concentrate by the Howard process [56] after stripping and recovery of the sulphur. They exhibit monomer molecular weights of 188 Da, and 215–154 Da, respectively. The number-average molecular

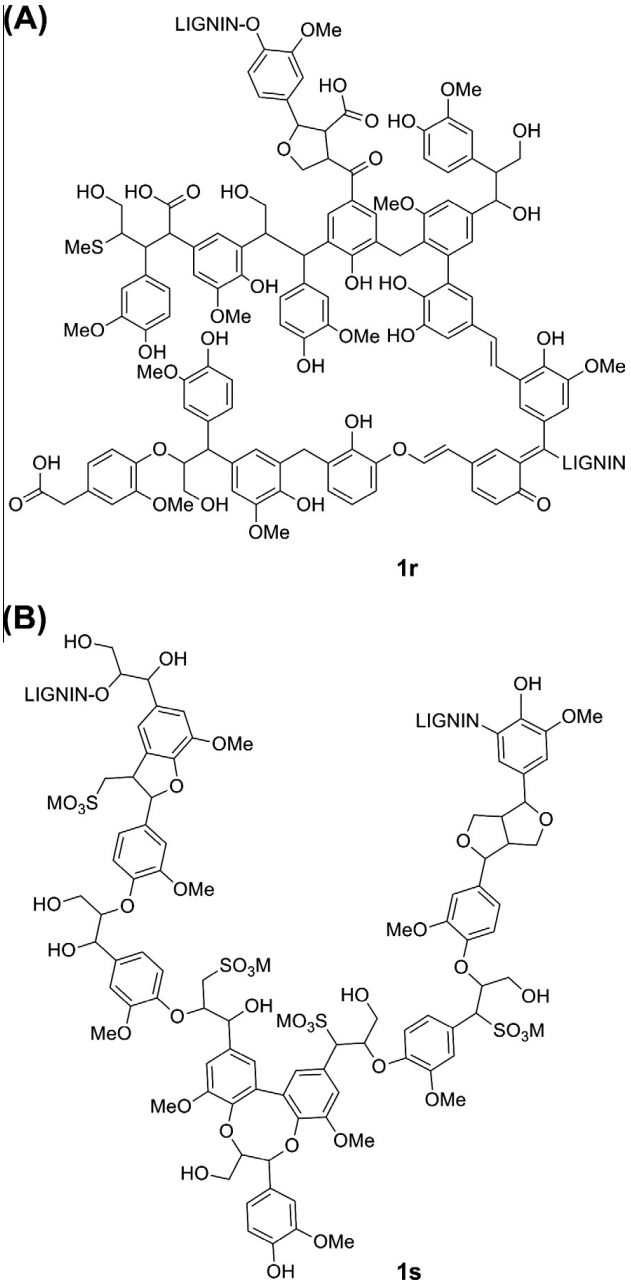


Fig. 4. Structure of kraft lignin (A), and lignosulfonate (B).

weight (M_n) can vary from 1000 Da to 140,000 Da, with the majority lying between 5000 Da and 20,000 Da. Lignosulfonate is soluble in acidic and basic aqueous solutions, and in highly polar organic solvents, but hydrolysis reactions, and eventually excessive sulfonations can occur. Neither kraft lignin nor lignosulfonate are suitable for studying the behaviours and the characteristics of natural lignin. They are, however, important as industrial by-products, and their valorisation is subject to different studies.

3.7. Organosolv lignin

Organosolv lignin is obtained as a separate process stream after the separation of wood components through

treatment with organic solvents in the organosolv pulping process [57]. The best known variant is the Allcel process which uses ethanol or an ethanol–water mixture [58]. A wide variety of solvents and combinations have been proposed for organosolv pulping; many combinations include acidic or alkaline aqueous components to enhance pulping rates. Organosolv lignin can be easily separated from the pulping solvents either by solvent removal and recovery, or by precipitation with water accompanied by distillation to recover solvent. Most organosolv lignin is insoluble in acidic aqueous solutions, but will dissolve in basic solutions, and in many polar organic solvents. Number-average molecular weights are typically less than 1000 Da, and polydispersity may range from about 2.4 to 6.4, with a calculated monomer molecular weight of 188 Da.

3.8. Pyrolisis lignin

Pyrolytic processes (thermal decompositions occurring in the absence of oxygen) can be used to produce a lignin stream that can be used in biorefinery processes [46,47]. Pyrolytic processes require relatively high temperatures (723 K); short vapour residence times of up to only two seconds are characteristic. Char and gases are typical by-products; these are used within the process to meet energy requirements within the overall process. There are no waste streams other than flue gas and ash. The main disadvantage lies in the high carbohydrate consumption required to fuel the process. The largest difference between pyrolytic lignins and the lignin in biomass is the very low average molecular weights found for pyrolytic lignin indicating the high degree of depolymerisation caused by the drastic conditions. On the other hand, this finding suggests that pyrolysis may be useful as a technology for the controlled molecular weight reduction of lignin. Molecular weights of 600–1300 Da, and number-average molecular weights of 300–600 Da were reported for pyrolytic lignins. Pyrolisis lignin offers unique opportunities to produce specific aromatic hydrocarbons not available from other processes.

3.9. Steam explosion lignin

Steam explosion consists in biomass impregnation with steam (180–230 °C) under high pressures (14–35 bar) at short contact times (1–20 min) followed by rapid pressure release [48,59]. The steam explosion process allows release of individual biomass components, and the process has generally been used as a method for preparing cellulose pulp. Alkali washing or extraction with organic solvents allow recovery of hardwood lignins in yields of up to 90%. Steam explosion lignin shows a lower molecular weight and higher solubility in organic solvents than, for example, kraft lignin.

4. Methods for characterising lignin, lignin extracts, and lignin model compounds

Due to the structural variety that is inherent to biomass lignin as such, as well as the structural changes that can be caused by the various methods used to obtain lignin extracts (*vide supra*), several methods for characterising lignin samples were developed. Comprehensive overviews have been published before [60,61]. Generally, characterisation can be performed either directly using unmodified lignin sample, or lignin extracts, or indirectly *via* chemical modification of the samples and extracts. Instrument-based analyses often benefits from chemical modifications of the lignin samples of interest prior to analysis.

A purely qualitative analysis that aims at highlighting the presence of the characteristic functional groups in the lignin sample, is often possible based on direct colorimetric reactions of the lignin sample. Using suitable reagents, additional, or concomitant quantitative analyses are possible (e.g. DPPH assay [62]), both *via* direct and indirect methods. One of the most prominent direct methods for analysing lignin is the Klason method [63]: Following the original protocol, after treatment of lignocellulosic material with 72% sulfuric acid, the amount of acid-insoluble lignin content of lignocellulose material is determined. Indirect methods are, for example [50]: (i) the determination of the amount of consumed oxidant (depending on the oxidant used, a qualitative result can be obtained simultaneously); (ii) the determination of the kappa number; (iii) the nitrosation method.

Quantification of specific functional groups in a lignin extract can be achieved using instrument-based methods, after derivatising the functional groups using appropriate reagents; examples are the esterification of hydroxyl groups into phosphites to allow for ^{31}P NMR-based analyses (*vide infra*), or the esterification of the alcohol functionalities into carboxylates to ease gel permeation chromatographic analyses [64]. Gas chromatography, alone or coupled with mass spectrometry, as well as size extrusion chromatography are other routinely used analytical tools for qualitatively and quantitatively analysing lignin, modified lignins, or lignin degradation products.

As mentioned before, structural information regarding the lignin sample, the lignin extract, or their degradation and valorisation products, can be obtained *via* nuclear magnetic resonance spectroscopy. Apart from two-dimensional NMR experiments (e.g., HSQC experiments) [65], ^{31}P NMR spectroscopy proofed to be a versatile tool in lignin research [66–69]. The different hydroxyl groups are simply converted to the corresponding phosphites using 2-chloro-4,4′,5,5′-tetramethyl-1,3,2-dioxaphospholane. This derivatisation allows to simultaneously discriminate between aliphatic and phenolic hydroxyl groups, as well as the 4-O-5′ and the 5–5′ condensed forms (*vide supra*, Fig. 2C), diphenylmethoxy, and carboxylic acid groups. It also allows quantification against an internal standard, such as cholesterol, by running a specifically developed pulse-sequence. Another advantage is that the phosphite derivatives are stable enough to guarantee a great reproducibility of the results even hours after the derivatisation was performed.

Noteworthy, powerful methods for characterizing lignin are also useful with respect to the exploitation of woody samples for archaeological studies, besides the well-known radiocarbon dating technique for age determinations [70–72]. Based on structural findings concerning the lignin component, the origin of a woody sample can be narrowed down, for example.

5. Oxidative valorisation of lignin and lignin model compounds

5.1. Organometal catalysed oxidation methods

5.1.1. Methyltrioxo rhenium (MTO)

MTO (**17**) is one of the simplest structures that can serve to catalytically activate the truly oxidising species, molecular oxygen or hydrogen peroxide [73,74]. Activation of hydrogen peroxide happens *via* the formation of two peroxorhenium intermediates, a mono-peroxo η^2-complex ([MeRe(O_2)O_2] **(18)**), and a bis-peroxo η^2-complex ([MeRe(O_2)$_2$O] (**19**)); both stability and reactivity of these species highly depend on the reaction conditions [75]. The transfer of oxygen from these peroxo-complexes to the substrate occurs *via* a concerted mechanism that includes a butterfly-like transition state, **20**, which prevents the formation of intermediate radical species (Scheme 2A). MTO-activated hydrogen peroxide is able to oxidise even challenging substrates such as activated phenols like **21** or methoxybenzene species like **26** (Scheme 2C, D) *via* a rather complex cascade of bond-breaking and bond formation steps [76–81]. Depending on the substitution pattern of alkyl-substituted phenols, different regioisomeric benzoquinones like **22** and **23** are obtained, apart from conjugated diacids like **24**, and bicyclic ethers like **25**, both resulting from ring cleavage reactions. The MTO-hydrogen peroxide system proved suitable for oxidatively transforming lignin model compounds like **30** [82], **8** [83], **13** [84], and **41** [85] (Scheme 2E and H), and lignin samples [82]. Phenolic model compound **30**, resembling one of the most common bonding patterns in lignin (4-O-5′, *vide supra*), was converted to the corresponding monoaromatic benzoic acid derivative **31**, phenyl methyl ketone derivative **32**, syringol (**33**), and unsaturated lactone **33**, which indicate ring-cleavage reactions [82]. Ring cleavage reactions were also found in the MTO-catalysed oxidations of phenolic diphenyl model **8**.

Also neolignans [85] like **38** could be oxidatively transformed, undergoing demethylation (**39**, **40**, **41**), oxidation of the benzylic position (**40**), and ring opening reactions (**41**) (Scheme 2G) [86,87].

In order to further model the complexity of the structure of lignin, a selected array of monomeric phenols and dimeric neolignans, resembling the main bonding patterns in native and technical lignins, were successfully studied (not shown graphically) [82]. Phenolic and non-phenolic monomeric model compounds, vanillyl alcohol, as well as veratryl alcohol, were treated with an MTO / hydrogen peroxide mixture in acetic acid. Complex mixtures of products were obtained, including both aldehyde and carboxylic acid derivatives that must originate form oxidation of side-chains. Benzoquinones and muconolactones, derived from oxidative ring cleavage of the aryl groups were detected in significative yields.

Various lignin samples (sugar cane lignin, red spruce kraft lignin, hardwood lignin extract), that contained a representative mixture of *para*-hydroxyphenyl–guaiacyl, guaiacyl–syringyl, as well as simple guaiacyl motifs (*vide supra*) (Fig. 2), showed extensive modification of the polymeric structure when treated with MTO-activated hydrogen peroxide, including a high degree of oxidation of the aliphatic side chain, aromatic ring-cleavages, and opening of hexahydrofuro[3,2-*b*]furan motifs. These degradation reactions are accompanied by an increase in functionalisation of the residual lignin polymers [67,68].

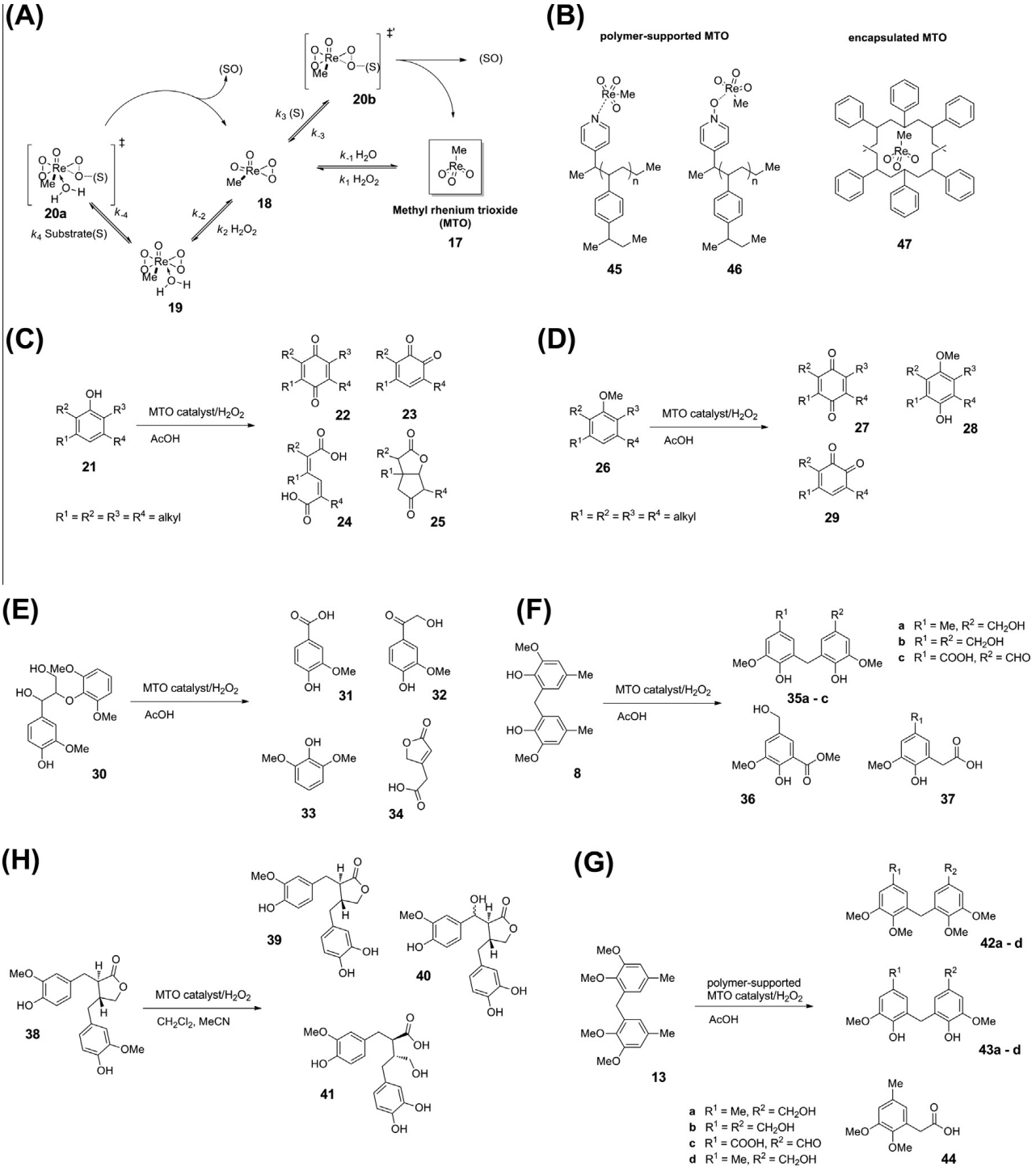
Although the oxidation of phenolic substrates can be achieved under mild reaction conditions, the occurrence of the benzoic acid derivatives, and the newly formed phenols, indicate side reactions such as over-oxidation, and hydroxylation of the aromatic ring.

Slightly superior results were obtained when MTO was immobilised using polystyrene or poly(4-vinylpyridine) beads: The polymer-supported heterogeneous catalysts, namely (i) 2% and 25% cross-linked poly(4-vinylpyridine) / MTO (PVP2/MTO and PVP25/MTO, respectively) (**45**); (ii) 2% cross-linked poly(4-vinylpyridine-*N*-oxide)/MTO (PVPN2/MTO) (**46**);iii) MTO microencapsulated in 2% cross-linked polystyrene (PS2/MTO) (**47**) (Scheme 2B). These species oxidised both phenolic and non-phenolic lignin model compounds, while exhibiting a superior lifetime, and a handle – in form of the polymeric support – to tune reactivity [88–90]. This tuning handle allowed to lower the Lewis acidity of the MTO catalyst, which served to direct reactivity toward the oxidation of aliphatic C–H-groups, with concomitant Dakin reactions, rather than oxidation of the aromatic rings in lignin and its models. Noteworthy, the polymer-support also served to supress the formation of over-oxidized products Scheme 2G shows the results obtained in the polymer-supported MTO-hydrogen peroxide effected oxidation of diphenyl lignin model **13** [90].

5.1.2. Salen complexes

Salen complexes of various transition metals ([M(salen)]) are widely used in organic chemistry to oxidise a great variety of substrates by activated molecular oxygen or hydrogen peroxide [91]. Especially cobalt salen ([Co(salen)]) complexes, which had been shown to be compatible with aqueous reaction media [92], were successfully used in various studies on oxidative lignin transformations. The oxidation proceeds mechanistically *via* the initial formation of a phenoxy-radical, which reacts with molecular oxygen to ultimately form oxidised lignin model compounds (EPR studies, Scheme 3) [93–96]. Both conversions and yields obtained in the [Co(salen)]-based oxidation reactions are generally very high for a broad variety of substrates. Various lignin model compounds, ranging from basic cynnamic ester (**48**, Scheme 3A), to more complex phenolic and non-phenolic phenylcoumaranes like **54** were oxidised smoothly, producing benzoquinone derivatives, alkyl–phenyl ketones, benzoic acid derivatives, as well as densely functionalized phenoxyacrylaldehydes **53** and benzofuran **56** (Scheme 3B and C).

Recent studies have shown that varying the substitution pattern of the aromatic ring in the salen ligand can serve as a handle to modify the reactivity of the [Co(salen)] complex [97]: Studies on models of G- and S-type lignin suggest that the yields of the corresponding benzoquinones depend on, and vary with, the electronic and steric



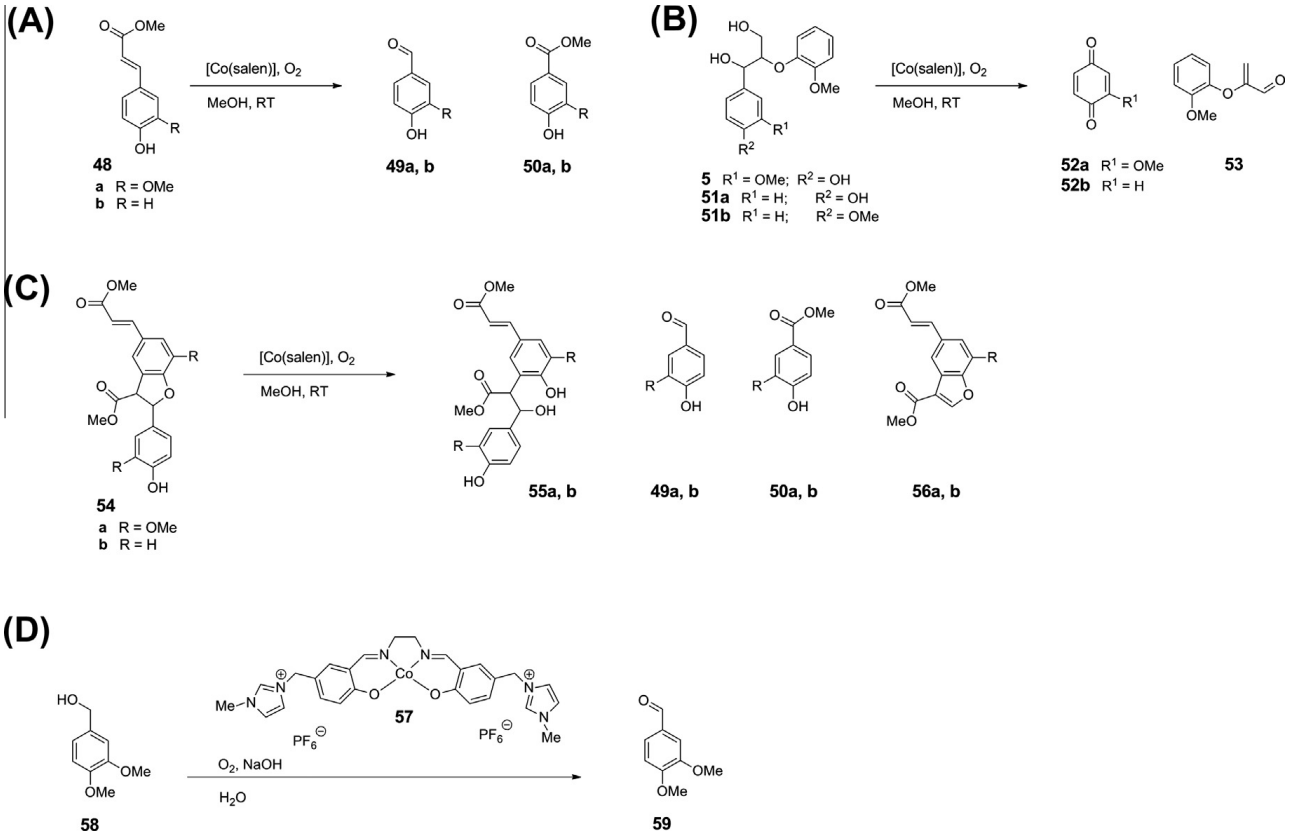
Scheme 2. Oxidation of lignin model compounds using MTO-activated hydrogen peroxide. (A) Activation of hydrogen peroxide by MTO. (B) Structures of polymer-supported and encapsulated MTO. (C)–(G) Exemplary reactions with various phenolic and non-phenolic lignin models, including the oxidation of a neolignan compound (H).

demand of the salen ligand. The sterical demand of the ligand thus bears the potential to control catalyst performance in order to preferentially oxidise one type of lignin in the presence of another.

The use of immidazolium-tagged salen ligands (IL-salen) in cobalt salen-mediated oxidative transformations of lignin model compounds has been reported as well (Scheme 3D) [98]. The products obtained with the tagged catalyst were shown to be the same as those obtained in case of the non-tagged parent ligand system, with the noteworthy exception of the benzoic acid derivatives, which were no longer found. The latter finding underlines the tuning possibility offered by the additional substituents on the salen ligands (*vide supra*).

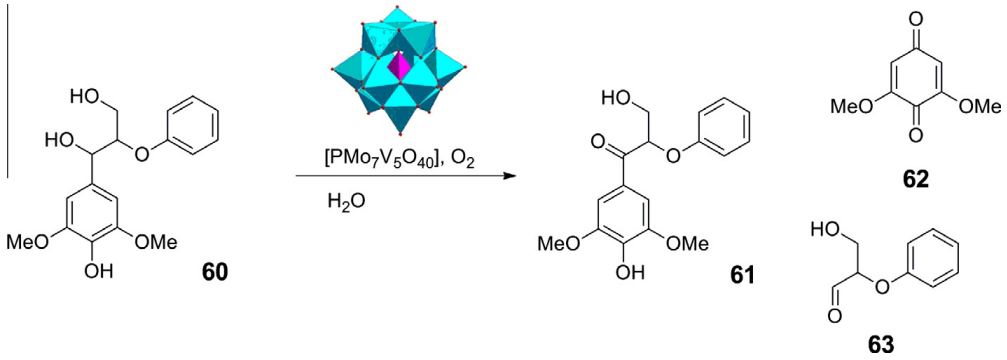
5.1.3. Polyoxometalates (POMs)

Polyoxometalates can activate hydrogen peroxide and molecular oxygen for the oxidative valorisation of lignin



Scheme 3. Oxidation of lignin model compounds using [Co(salen)] complexes. (A) Oxidation of cynnamic ester. (B) Oxidation of phenolic and non-phenolic lignin model compounds for β-O-4′ linkages. (C) Oxidation of phenolic and non-phenolic lignin models for β-5′ linkages. (D) Oxidation of veratryl alcohol using a IL-salen cobalt complex.

and its various model compounds, and the procedures developed are reported as "green" alternatives for the oxidative valorisation of lignin. [99–102] Keggin-type POMs with the general formula $[PM^1_xM^2_{12-x}O_{40}]$ – M^1 or M^2 stands for the catalytically active element (Scheme 4) – were found to be best suited [103]. Accomplishments in this field have just recently been reviewed in dedicated articles, so that we will only briefly introduce the field here [100]. While the use of POMs in lignin degradation initially suffered from the fact that the regenerable POMs had to be used in stoichiometric amounts, it soon became apparent that it was possible to depolymerise lignin catalytically in the presence of molecular oxygen, using Keggin's HPA-5 POMs, such as $[PMo_7V_5O_{40}]$ (Scheme 4). The POM-based oxidation proceeds *via* different pathways for phenolic and non-phenolic substrates. Phenolic substrates like **60** react supposedly under formation of a phenoxy radical, that is immediately further oxidised by a second POM equivalent to form a cyclohexadienyl cation, leading to the depolymerisation products shown in Scheme 4, mainly quinones (**62**) and benzoic acid derivatives [104]. Both phenolic and non-phenolic substrates were also shown to react under successive oxidation of the benzylic position. As observed for the immobilised MTO system, the heterogeneity of the POM-based system influences the mechanism of the depolymerisation and the products that are obtained when compared to, for example, non-POM vanadium catalysts [105].



Scheme 4. Example for a Keggin HPA-5 POM-mediated oxidative valorisation of a phenolic lignin model compound.

The effects of substitution pattern of the lignin model substrates, the effects of solvents, and the effect of additives on the reaction have been investigated: Noteworthy, the addition of aliphatic alcohols and radical scavengers can effectively reduce the amount of undesired re-polymerisation of just-generated, lignin-based radical monomers [106].

5.2. Biomometic oxidation methods

5.2.1. Metalloporphyrins

In nature, modification of lignin is mainly accomplished by enzymes such as laccase, lignin peroxidase (LiP), and manganese dependent peroxidases (MnP), which use molecular oxygen [107]. Before reviewing studies on the oxidative upgrade of lignin using these enzymes (*vide infra*), we wish to summarise results obtained by using biomimetic catalysts that can activate hydrogen peroxide for the oxidative degradation of the complex lignin structure.

Synthetic metalloporphyrins represent biomimetic systems for both LiP and MnP, since they can yield highly oxidised metallo-oxo species similar to those found in LiP I, and LiP II [108]. Highly functionalized porphyrins, bearing aryl substituents in the *meso*-positions of the heme motif, represent tunable catalyst systems: both the redox potential and the solubility of the complexes can be tuned by carefully varying the nature of the *meso*-substituents. Several tunable metal porphyrin catalysts were found to be capable of oxidising lignin and lignin model compounds, and the efforts in this field have been summarised before [109,110]. Nevertheless, we wish to review some of the most important aspects here as well, since these catalyst systems exhibit an advantage over the enzyme-based variants employing the same reactive metal core: the metalloporphyrins are not as sensitive towards an excess of hydrogen peroxide, and are thus potentially more interesting with respect to industrial applications.

Anionic manganese and iron *meso*-tetra(2,6-dichloro-3-sulphonatophenyl porphyrin chlorides (TDCSPPMnCl and TDCSPPFeCl, respectively), anionic *meso*-tetra-4-sulphonatophenyl porphyrin chloride (TSPPMnCl), as well as cationic manganese *meso*-tetra(*N*-methylpyridinio)porphorin pentaacetate **68** (TPyMePMn(MeCOO)$_5$) (Scheme 5A) were shown to effectively oxidise residual kraft lignin and lignin model compounds yielding the usual degradation products – benzoquinones, benzyl alcohols, and phenyl alkyl ketones in acidic environments (pH 3–6) (Scheme 5B) [111].

All porphyrin catalysts produced comparable mixtures of products, hinting at comparable reaction mechanisms despite the different metal centres and porphyrin moieties. The cationic manganese complex TPyMPMn(MeCOO)$_5$ (**68**), however, performed best, and in general, manganese complexes performed better than the corresponding iron complexes. The water soluble manganese porphyrin derivative **68** distinguished itself through high yields of products originating from oxidations of both the aromatic ring and the side chains, as well as products indicative of demethylation reactions; only low yields of undesired products resulting from radical recombination reactions.

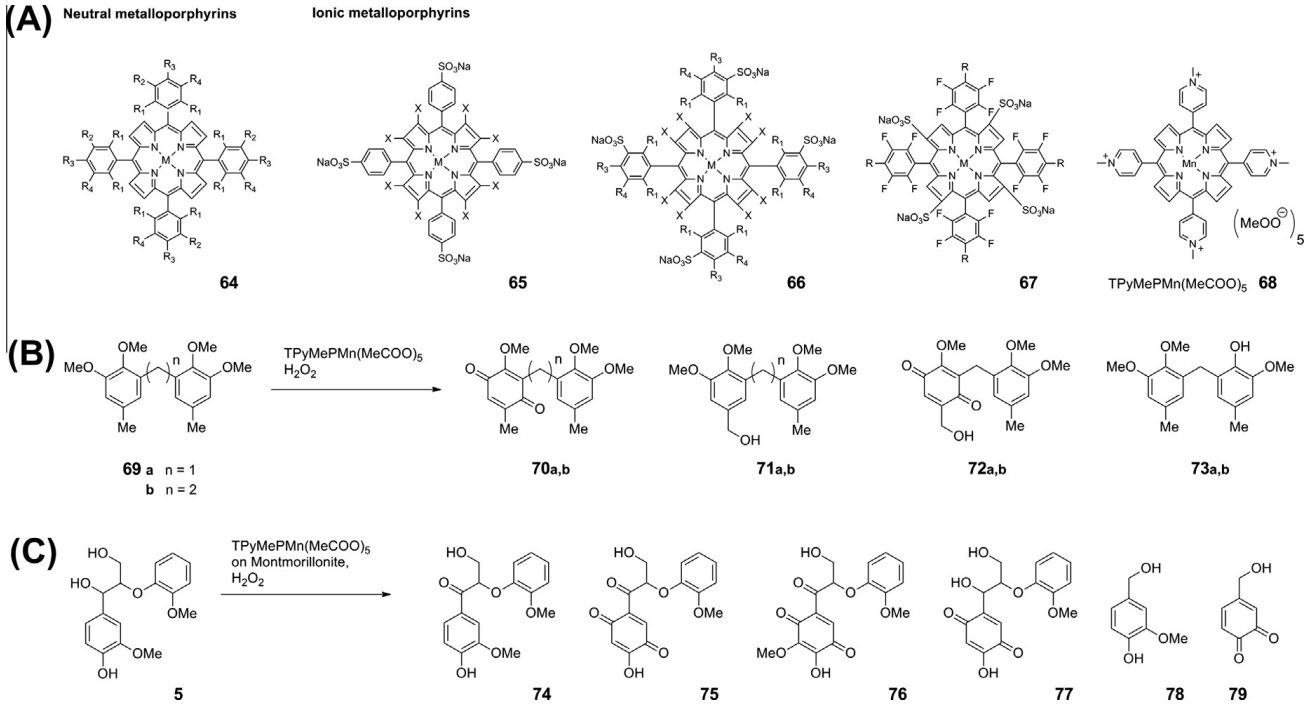
5.2.2. Immobilised metalloporphyrins

The major disadvantage of the (not easy to synthesise) porphyrin complexes – with respect to large scale/industrial applications – is their liability under the reaction conditions typically used for lignin degradation processes. This problem can be solved by developing a mimic of the polypeptide envelope that protects the active centre in natural enzymes. One simple way to achieve such an envelope mimic is to immobilise the porphyrin-based catalyst systems on solid supports such as silica gel [112], naturally occurring clays [111], or artificial polymers [113]. These immobilisation methods, which also significantly ease recovery of the catalysts, do not cause any loss in reactivity [111] Clays from the smectite family proved to be very versatile due to an inherent structural feature: two-dimensional oxyanions are separated by layers of hydrated metal cations, which can be exchanged using simple ion-exchange protocols [109,114]. For example, Montmorillonite-supported TPyMPMn(MeCOO)$_5$ activated hydrogen peroxide for the oxidative decomposition of lignin model compound **5** into the same small molecules that were obtained using non-immobilised complex **68**, namely products originating from oxidation of the side chains, of the phenolic hydroxyl groups, of the aromatic moieties and from cleavages of the side chain (Scheme 5C). The identical compositions of the product mixtures obtained for both the immobilised catalyst and its free analogue suggest identical reaction mechanisms underlying the oxidations.

More recently, it has been reported that the porphyrin-skeleton can be attached to typical ionic liquid cations such as immidazolium derivatives, which allow solubilising the porphyrin-based catalysts in ionic liquids [115], which have been used before in studies on lignin degradation. Unfortunately, more detailed results have not been published yet.

5.2.3. Mediator-containing systems

The immobilisation of metalloporphyrins on the surface of porous clays, despite all the advantages mentioned above, reduces the accessibility of the active metal core for bulkier substrates. In order to maintain high turnover numbers, mediators can be used. These are ideally small enough to easily diffuse through the pores of the clay to access the activated metal core, to become activated, and to subsequently diffuse back into the medium where they can readily react with the bulkier substrates. The use of meta-stable mediator systems is generally beneficial with respect to the oxidative functionalisation and depolymerisation of lignin, since they can readily diffuse into / in between the polymeric structures of lignin. Immobilised metalloporphyrin-mediator systems were shown to oxidise phenols and lignins by side-chain oxidation/fragmentation and depolymerisation, as spectroscopically (^{31}P NMR spectroscopy) validated by decreasing intensities of the signals corresponding to aliphatic hydroxyl groups, and increasing intensities of signals corresponding to carboxyl groups [114]. Noteworthy, no products originating from oxidative coupling reactions were observed. More detailed mechanistic studies on the effects of the mediators on the oxidative functionalisation of lignin are covered in the following paragraphs.



Scheme 5. Metalloporphyrin-mediated oxidative valorisation of lignin model compounds. (A) Various neutral and ionic metalloporphyrins. (B) Oxidation of various lignin model compounds using $TPyMePMn(MeCOO)_5$. (C) Oxidation of lignin model **5** using clay-supported $TPyMePMn(MeCOO)_5$.

5.3. Enzyme-based oxidation methods

As indicated, in nature, lignin is selectively oxidised by white-rot fungi that utilise a series of enzymes to selectively oxidise lignin in the present of cellulose and hemicellulose, which are enzymatically decomposed by soft-rot fungi and/or red-rot fungi species [107]. The most active lignin degrading enzymes are laccases, manganese peroxidases, and lignin peroxidases. The use of lignolytic enzymes in industrial processes has recently been discussed [116]. In this review, we will briefly summariseresults that have been accumulated in literature on the oxidative degradation of lignin by laccases and peroxidases. We wish to highlight important mechanistic aspects, and to show possibilities of how to further enhance the industrial exploitation of these enzymes.

5.3.1. Laccase

Laccase (EC 1.10.3.2) is a multicopper oxidase that oxidises suitable substrates under concomitant reduction of oxygen to water [117,118]. The thermally very stable enzyme – no denaturation is observed at temperatures up to 60 °C – contains four copper atoms, organised in three different copper centres, one type 1 copper centre (T1 site), one type 2 copper centre (T2 site), and a coupled binuclear type 3 copper centre (T3 site). The T2 and T3 sites form a trinuclear copper cluster onto which molecular oxygen is reduced [119]. The T1 copper atom oxidises the reducing substrate and transfers electrons to the T2 and T3 copper atoms. Within an outer-sphere electron-transfer mechanism [120], a radical cationic species is initially generated from a phenolic substrate. This radical cation undergoes subsequent deprotonation to yield a phenoxy radical, which initiates the depolymerisation of lignin following an exo-depolymerisation mechanism [107,120–123]. The variety of substrates that can be oxidised by laccases mainly depends on the oxidation potential of the substrates, and to a lesser extent on their steric demands. Although different fungi produce laccases with different redox potentials, ranging from $E^\circ = 0.43$ V (tree laccase from *Rhus vernicifera*) to 0.78 V (fungal laccase from *Polyporus versicolor*) [124], it was not yet possible, for example, to find a laccase that is able to directly oxidise blocked phenolic substrates.

Blocked phenolic substrates can, however, be *indirectly* oxidised by laccases, when these are used in combination with a radical mediator species, such as 1-hydroxybenotriazole (HBT, **80**) [125], *N*-hydroxyacetanilide (NHA) [126], violuric acid [126], or 2,2′-azinobis-3-ethyl-benzthiazoline-6-sulfonate (ABTS) [127,128]. The use of these mediators helps to improve the efficacy of laccase-based depolymerisations of lignin for two reasons: Firstly, as mentioned before, these mediators can readily diffuse into the lignin fibres after being activated by the enzyme. Secondly, they can serve to alter the mechanism underlying the enzymatically-induced depolymerisation cascades by altering their starting point.

Mechanistic studies involving the mediator HBT (**80**) revealed that laccase initially converts HBT to an oxybenzotriazolyl radical, which substracts a hydrogen atom from either the phenolic or the benzylic position – semiemperical calculations (PM3) suggest a comparable spin density for the two radical species – of phenolic and non-phenolic lignin model compounds, respectively (Scheme 6) [129]. Oxidation thus does no longer occur *via* an electron transfer process in these cases, but *via* a hydrogen atom

abstraction process, which is potentially easier to control (*Vide infra* for a more detailed discussion of the influence of the radicals.) [130]. Vanillyl alcohol (**78**), and derivatives thereof, were used as model compounds in comparative studies aiming at elucidating the effects of radical mediators in the laccase-based modification of phenolic and non-phenolic lignin subunits [131]: It was found that the mediator effectively changes the composition of the product mixture obtained upon oxidative treatments using the enzyme mediator system. In laccase-only systems, products originating from oxidation of the alkyl side-chain oxidation (**49**), as well as of oxidative coupling reactions (**81** and **82**) were obtained (Scheme 6). These products suggest that laccases equally well oxidise side-chains (as indicated by the decrease of aliphatic hydroxyl groups present in lignin side chains) and aromatic rings of the lignin structure, as indicated by the increase of condensed phenolic species. When HBT (**80**) is added, products originating from oxidation of the aromatic rings, like *ortho*- and *para*-benzoquinones **83** and **84**, catechol **86**, and muconic acid derivative **85** were found in appreciable yields (Scheme 6), beside products like **49**, which are indicative of oxidations of the side-chains. The formation of the products found upon addition of HBT cannot be explained on the basis of the reaction of oxygen centred radicals such as phenoxy radicals with molecular oxygen, since the addition of molecular oxygen to phenoxy radicals is slow (*vide infra*) [132]; the addition of a superoxide anion to a phenoxy species is faster, and thus more likely to be the reason for the formation of the observed product mixtures. The additionally postulated benzyl radical would kinetically be suitable to bind molecular oxygen, resulting in a peroxo radical species that could decompose under formation of a superoxide anion, which would then act as reaction partner for the phenoxy radical present in the mixture.

5.3.2. Peroxidases

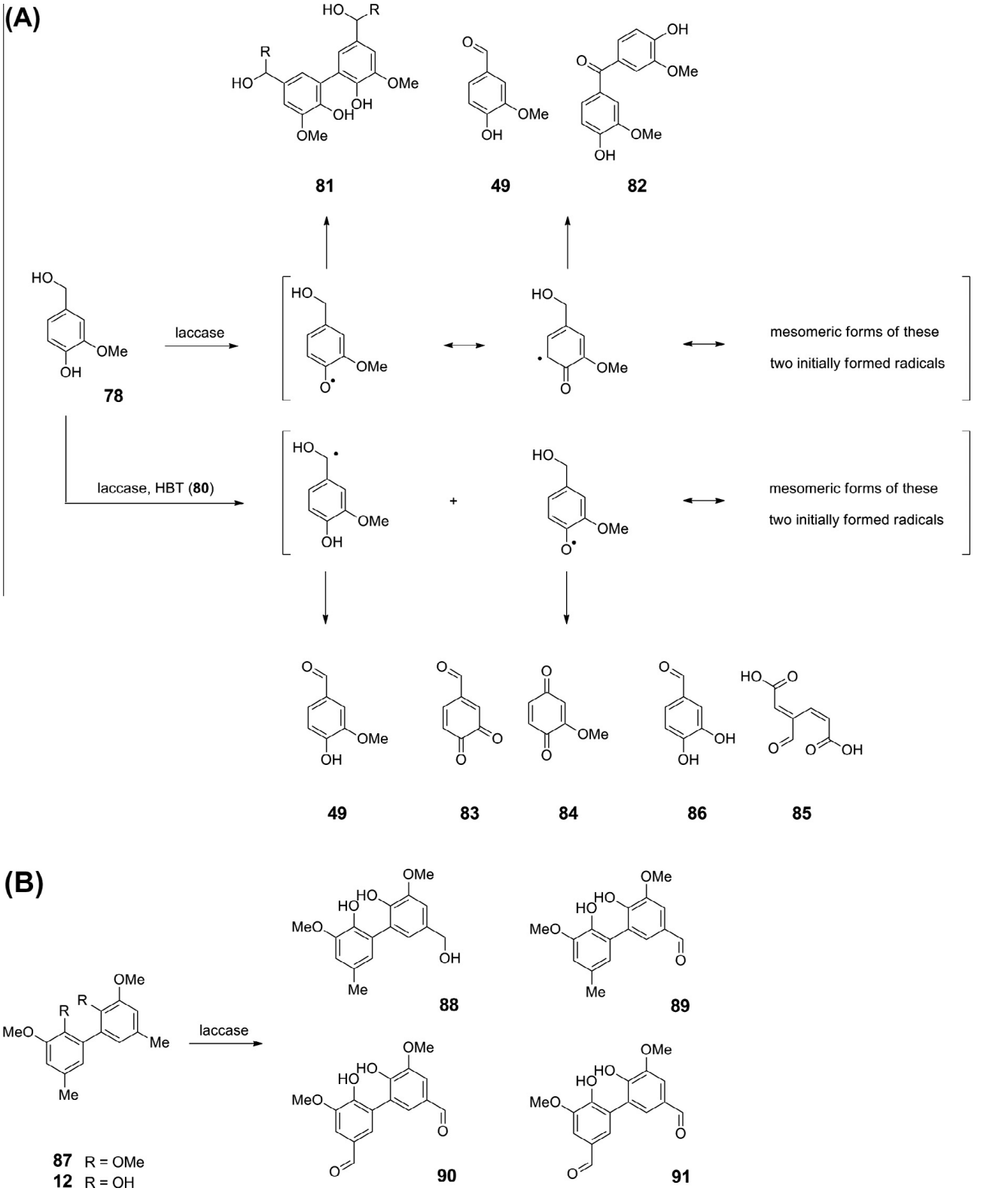
Manganese peroxidases represent the second class of enzymes, that were shown to oxidatively depolymerise lignin under concomitant reduction of molecular oxygen or hydrogen peroxide to water [133]. Activation of molecular oxygen or hydrogen peroxide is achieved *via* a two-step process, in which an iron protoporphyrin IX activates the oxidant first for the oxidation of the manganese co-factor from Mn(II) to Mn(III) [134]. The Mn(III)-centre is subsequently chelated by carboxylic acid anions, and forms thus a small, freely diffusible species – comparable to the activated mediator in the laccase-mediator system – that ultimately oxidatively depolymerises lignin and lignin model compounds [135,136]. The depolymerisation is initiated by the formation of a phenoxy radical species *via* hydrogen abstraction [133]. Different lignin model compounds, mimicking the recalcitrant phenolic arylglycerol β-aryl ether and diarylpropane motifs, could be cleaved successfully using MnP [135,136]. Condensed phenolic lignin model compounds displaying 5–5′, α-5′, and diphenylmethane subunits were also efficiently oxidised by MnP [137]. When 5–5′ models were treated with MnP isolated from white-rot fungus *Lentinula edodes*, products of oxidations of the alkyl side-chain were detected; an effective overall substrate conversion was observed [138]. The best substrate conversion was found for the α-5 model compound 2,4′-dimethoxy-3,3′-dimethoxy-5-methyl-diphenylmethane (**9**), when it was treated with MnP (Scheme 7). The isolated products confirm the expected oxidation of the alkyl side-chain, and they showed that oxidative cleavage preferentially occurs at the carbon-bridging position. The combined MnP results suggest that the reactive manganese species is selectively attacking methyl and methylene groups in *para*-position to the phenolic OH-groups in lignin. As in case of laccase, an exo-depolymerisation mechanism is assumed based on the analyses of the residual lignin.

Peroxidases could also be used to catalyse the formation of lignans. "Unnatural" dihydrobenzofuran lignans could be synthesised *via* an oxidative cross-coupling protocol using HRP. The construction of "unnatural" lignans, using the very same enzymes that can also degrade natural lignin, holds – in principal – the potential to develop all-encompassing processes for the production of tailor-made synthetic polymers from lignin monomers as renewable resource [139].

5.3.3. Immobilised enzymes

The enzyme-based valorisation of lignin suffers from the same drawbacks as the organometal-catalysed and the biomimetic methods discussed earlier. Large scale applications seem to be prohibitive since the need to recycle the costly, active ingredient is compromised by a rapid loss of activity of the enzymatic catalyst [140]. As for the other methods (*vide supra*), a protecting immobilisation of the enzymes, should be suitable to solve both issues simultaneously. Extensive reports exist in literature detailing the immobilisation of laccase and other enzymes [141–145], but although immobilisation indeed eases any recycling process, a wide-spread application of these protocols is compromised by the fact that the supported enzymes still lose their activity too rapidly. A recently developed process for the immobilisation and subsequent protection of laccase, termed layer-by-layer (LbL) technique [146–150], relies on successive deposition of ultra-thin layers of alternatingly charged polyelectrolytes on various surfaces that were functionalized before with the enzyme of choice, by conventional means (Scheme 8). For example, laccase was immobilised on alumina particles (*via* consecutive silanisation and crosslinking using glutyraldehyde) [151], before it was protected by alternate layers of poly(allylamine) hydrochloride and polystyrene sulfonate [152]. The protected immobilised enzyme could be used in numerous (>10) oxidation cycles in which it retained 70–80% of its original activity.

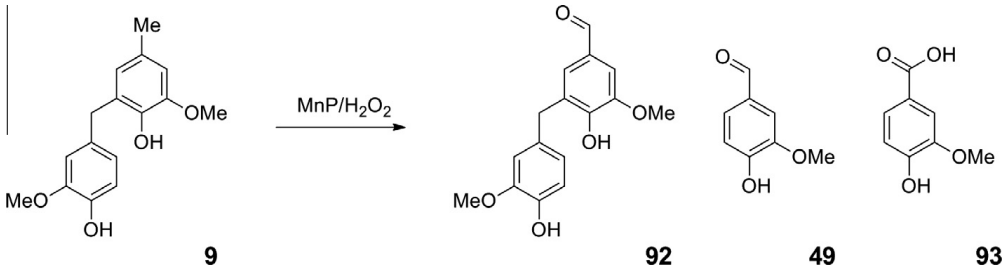
The LbL-technique can also be used in order to create hollow spheres, which can serve as containers for enzymes (Scheme 9) [152]. Once the sphere, – comprised of three alternatively charged layers of polyelectrolytes – has been obtained after dissolving the core particle around which it was initially built up, variation of the pH of the solution allows opening and closing of pores within the capsule walls, thus ultimately allowing loading of the enzyme at one pH, and diffusion of substrates into the sphere at a different pH; the latter conditions do not cause the pores to become wide enough to accidentally free the enzyme. The laccase



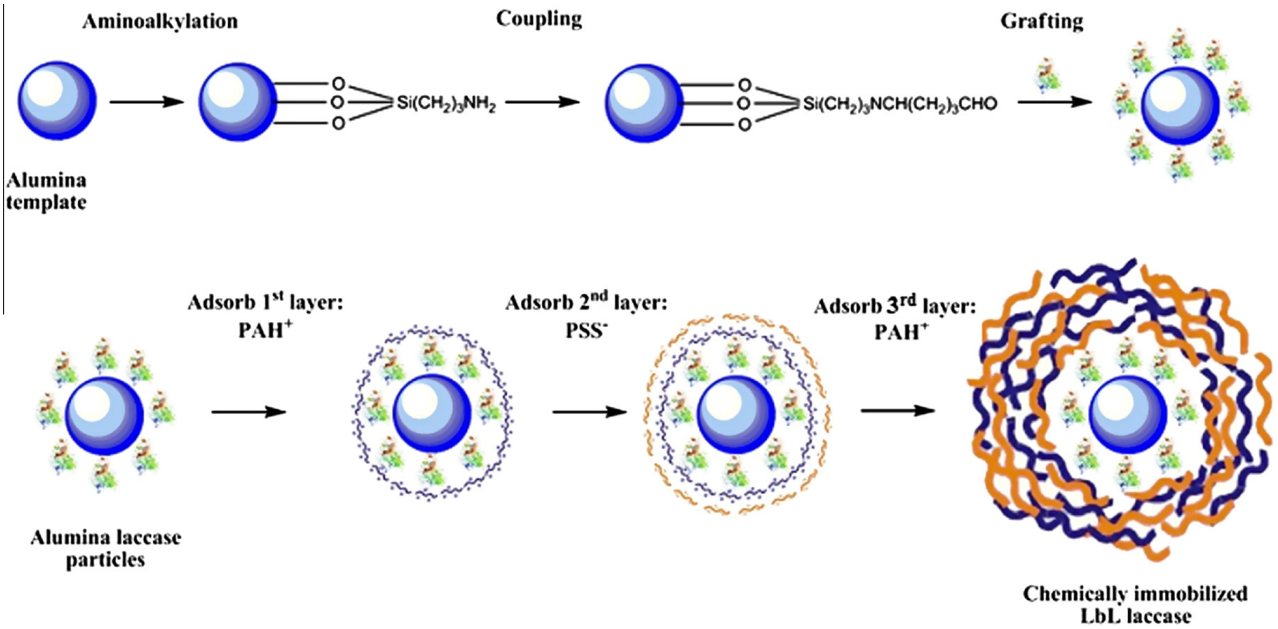
Scheme 6. Comparison of product distributions obtained from the oxidative depolymerisation of lignin and phenolic lignin model compounds using laccase and a laccase-HBT mediator system.

loaded into the capsules displayed an activity, and a stability that was comparable to those found for the laccase that was immobilised on the solid spheres using the LbL-technique.

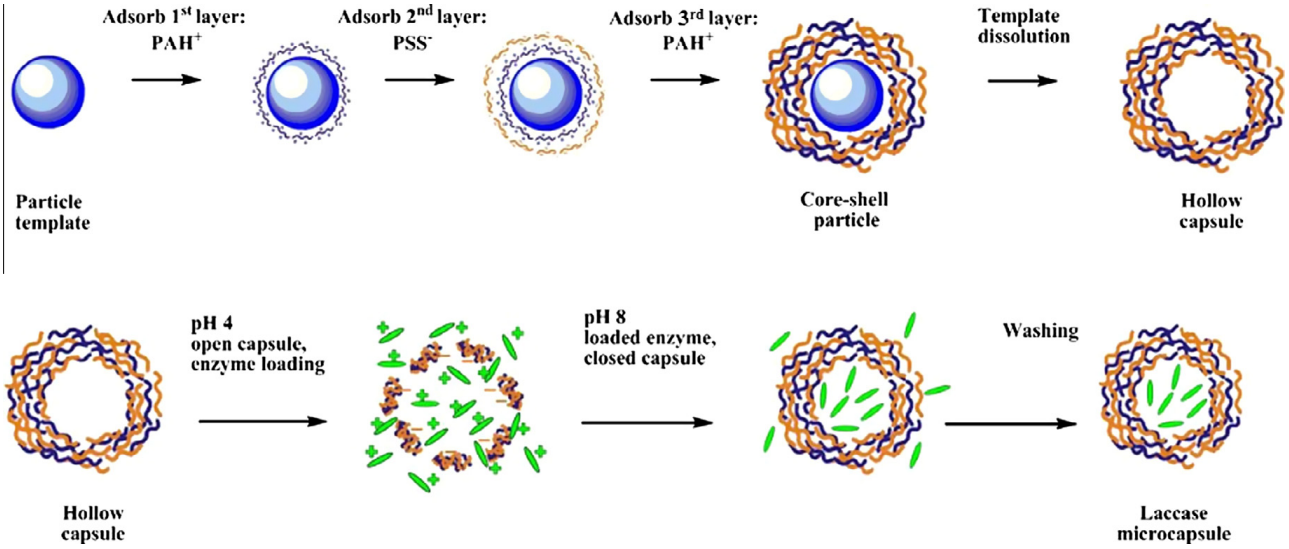
Both the LbL-coated, and the encapsulated laccase were studied in the direct oxidation of lignin and lignin model compounds [152]. The samples were found to be much more efficiently oxidised by the supported and



Scheme 7. Reaction of a α-5′ motif model compound **9** with MnP.

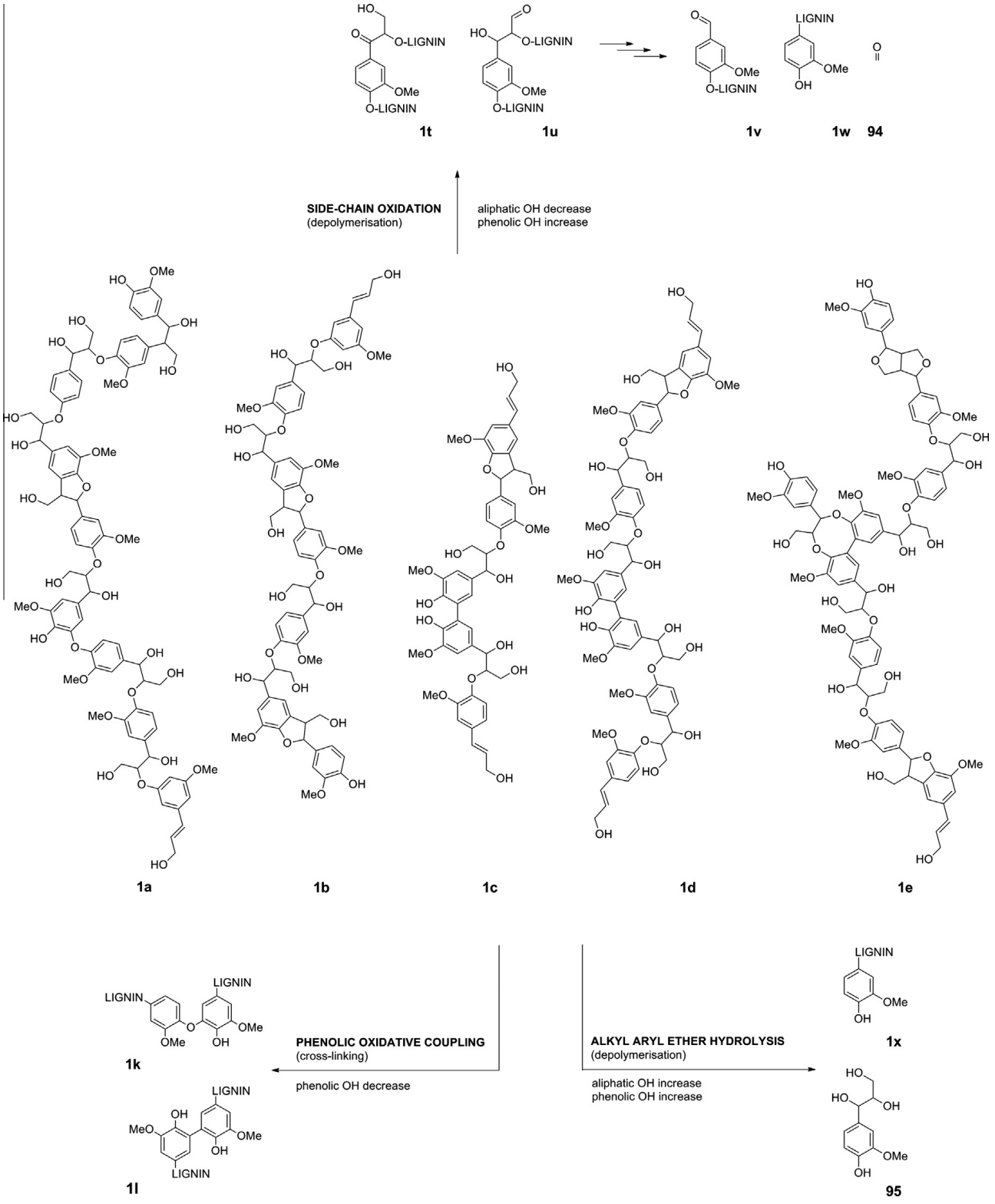


Scheme 8. LbL-coating procedure for immobilising enzymes, e.g. laccase, on the surface of an alumina particle.



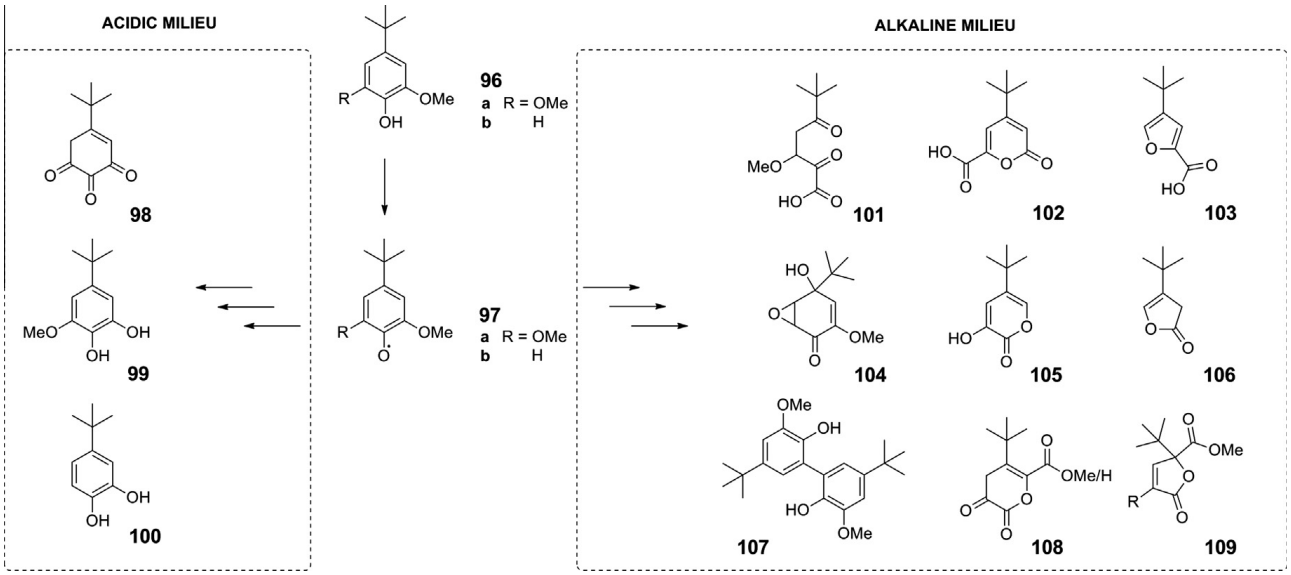
Scheme 9. Encapsulation procedure using the LbL-coating technique.

encapsulated species than by the free enzymes: conversions were found to double. A closer look at the product mixture and the residual lignin revealed interesting differences in comparison to the product mixture obtained with the free enzyme: (i) The number average molecular weight of the residual lignin was decreased, probably due to

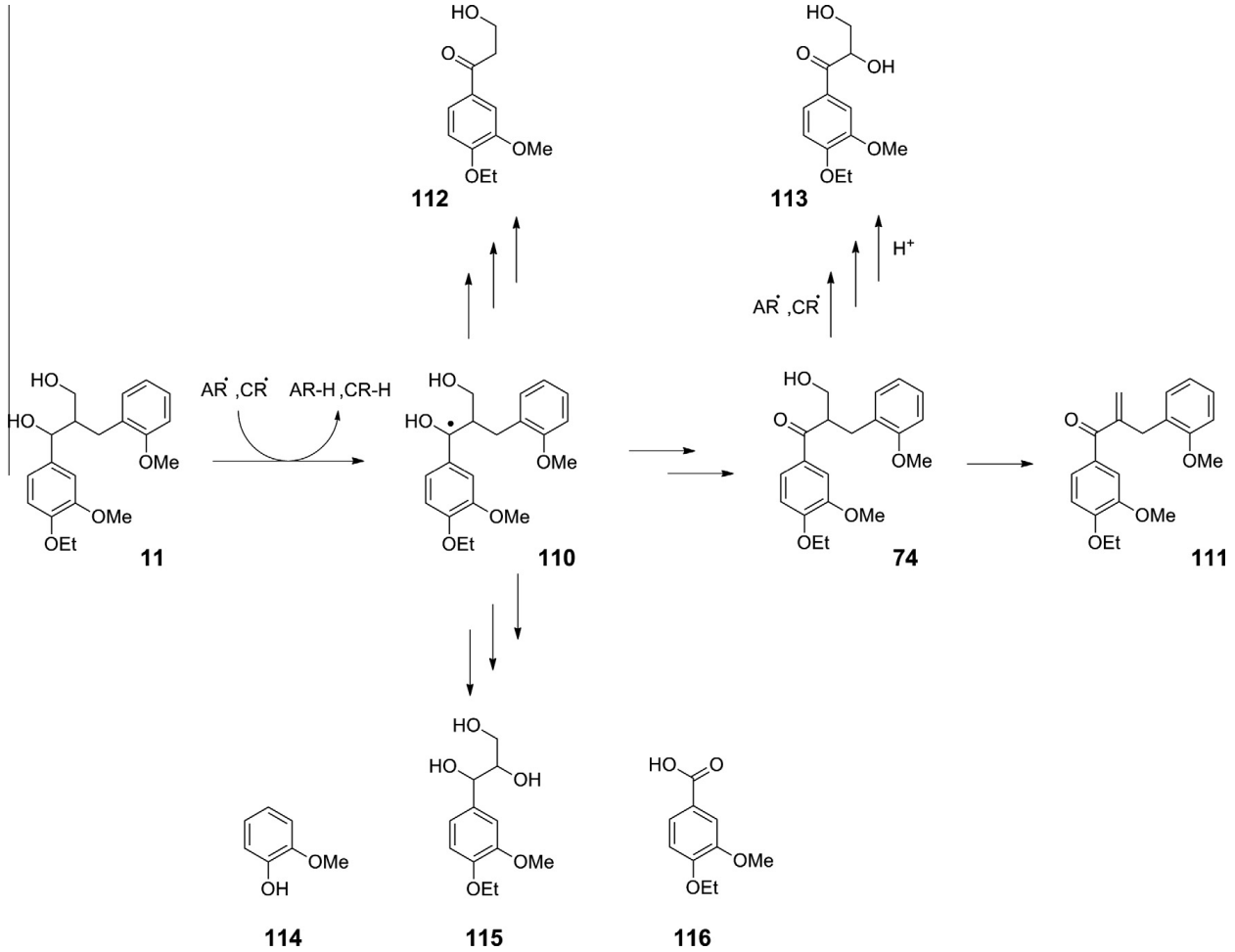


Scheme 10. Different pathways of the oxidative depolymerisation of lignin by co-immobilised laccase and HRP.

hydrolysis reactions; (ii) NMR analyses suggested an increase in both aliphatic and phenolic hydroxyl groups; (iii) the soluble fractions were characterised by the presence of a significant amount of lower molecular weight polymeric material and not only by oligomers. All these findings suggest that the immobilised enzymes cause *endo*-depolymerising hydrolytic processes, e.g., cleavage of alkyl aryl ethers. As mentioned before, ree laccase, as well as free peroxidase, were found to be *exo*-depolymerising enzymes. The oxidative decomposition of lignin results here – *via* aromatic ring-cleavage, side-chain oxidations, and demethylations – in low molecular weight fractions,



Scheme 11. Proposed paths leading to the oxidative decomposition of lignin model compound **94** *via* peroxo radicals.



Scheme 12. Proposed paths leading to the oxidative decomposition of lignin model compound **11** without peroxo radicals.

under concomitant further polymerisation of the remaining lignin polymer, as it has to be concluded from increased average molecular weight numbers found for residual lignin after exposure to laccase.

The LbL-technique is not only compatible with a variety of enzymes [153,154], it also allows for the simultaneous immobilisation of multiple enzymes [155]. Interestingly, this does not only serve to extend the range of

the theoretically available electrochemical potential that can be covered, thus potentially extending the substrate range, but also seems to affect the mixture of depolymerisation products, the nature of the oligomers of residual lignin, and, most noteworthy, the apparent overall mechanism of the enzymatic depolymerisation [155]: When, for example, laccase and HRP are immobilised simultaneously on the same particles, both *endo*- and *exo*-polymerisation products are obtained (Scheme 10).

6. Digression on the importance of the nature of intermediate radical species and the reaction mechanisms for the oxidative depolymerisation of lignin

The desire to oxidatively functionalize lignin comes with the burden that one has to deal with potentially highly reactive species, which usually make it difficult to control product selectivity. With exception of MTO, that undergoes a concerted oxygen transfer mechanism (*vide supra*), all of the above presented methods for an oxidative valorisation of lignin run *via* intermediate oxygen-based radicals of different types and reactivities, causing the formation of a wide variety of products. This variety of products, which potentially represents a significant problem with respect to the straightforward industrial applications of suitable oxidative valorisation processes, is only partly caused by the fact that the biopolymer lignin does not follow a regular polymer pattern. This variety also originates from a rather un-selective reactions of the initial oxygen-based radical species. It is thus important to identify ways to control the reactivity, or the type of the main oxygen-based radical species, in order to ultimately control the product formation upon oxidative lignin depolymerisation. A series of mechanistic studies suggest that the product mixture can be influenced (i) by carefully controlling the pH of the reaction medium [156]; (ii) by controlling – potentially *via* the use of mediators (*vide supra*) – whether initially an oxygen-centred radical, for xample a phenoxy radical, or a carbon-centred radical, for example a benzylic radical is formed; and (iii) by controlling the atmosphere under which the reactions are carried out (Scheme 11) [157].

In the presence of oxygen, both the oxygen-based and the carbon-centred radical will react with hydroperoxyl or superoxide anion radicals to hydroperoxides, which in turn can originate from reactions between phenolate structures with oxygen or hydrogenperoxides in the presence of transition metal catalysts, from UV-radiation, or from reaction with other radicals [156]. The intermediate hydroperoxides undergo further reactions that are depending on the pH of the reaction mixture as well as the position of the peroxo motif in the structure (Scheme 11). In acidic environments, mainly dealkoxylations are observed. In basic environments, the phenolic peroxide species undergo mainly ring opening reactions [156]. Unfortunately, the questions whether a benzylic radical would have formed under the various conditions, and how it it could have reacted, has not been addressed in this study. This would have been interesting with respect to newer studies on the treatment of lignin model compounds with radical species:

These studies suggest, that both the oxygen-centred and the carbon-centred radicals can directly contribute to the depolymerisation of lignin, and that the intermediate peroxo-radical species is not as essential as originally anticipated [157]. The alternative mechanistic pathway leading to oxidised lignin "monomers" is shown in Scheme 12. These findings hint at the fact, that it should be possible to achieve a controlled oxidative lignin depolymerisation process that does not require the use of peroxo-species.

Also the rough comparison of the number and nature of different products generated upon depolymerising lignin, or lignin model compounds, using organometalli catalysts, biomimetic catalysts, or oxidative enzymes reveals the necessity to focus on the underlying mechanism for the development of lignin based products. If product selectivity is less important, and the depolymerisation as such is the main objective, an effective method for the generation of free hydroxyl radicals should be chosen. Selective oxidative oxidative valorisation of lignin, however, can only be achieved when no hydroxyl radicals are present. In this case the formation of phenoxy radicals and / or benzylic radicals will occur in the presence of inorganic or biomimetic catalysts and oxidative enzymes. Further improvements of the selectivity within oxidative transformations of lignin are only possible by using transformations, that work *via* concerted mechanisms in the oxidation reactions, thus preventing the extensive formation of free radicals. The MTO-catalyzed oxidative valorisation of lignin is one example for such a process (*vide supra*).

7. Conclusions

To date, lignin represents the main waste stream from modern saccharification processes. As such its valorisation is necessary in order to develop sustainable biorefinery processes. Lignin constitutes a rich renewable source of aromatic compounds. Its complex structure offers unique routes to produce fine and bulk chemicals either by adjustment of already developed petroleum processes or by new technologies.

Catalysis plays a major role in the conversion of lignin, and a considerable number of efforts have been devoted to the development of catalytic routes for its specific oxidation/functionalisation. Despite of this, a general description of the chemical reactivity of lignin is still lacking, mainly due to three fundamental issues: Lignin sources are heterogeneous by nature. Different lignin pre-treatments, often originally developed for pulping and paper production purposes, provide fundamentally different lignin streams with variable chemical characteristics. Another factor responsible for the comparably slow understanding of lignin reactivity relies on its structural variability according to the plant source. The third factor affecting a possible general understanding of lignin chemistry is the analytical challenge associated with its structural characterisation.

The variable and unpredictable structure of lignin requires the development of strategies aimed at controlling and tailoring its multi-functionality, namely processes of selective oxidation, reactive groups protection and functionalisation.

Inorganic catalysts, such as MTO, salen-complexes, or POMs achieve an efficient degradation of lignin and lignin model compounds; however, the aforementioned concerted radical mechanism is only given in case of MTO. Biomimetic catalysts also offer suitable reactivities, but reactions do not proceed in concerted mechanisms avoiding free radical species. Neither do the enzymatic processes that have been studies so far; these did prove to be very effective for lignin degradation, though. More research efforts are needed in order to exploit the initial findings presented in this and other reviews, and to develop robust protocols that avoid free radical species.

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