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Oxidation of lignin model compounds by organic and transition metal-based electron transfer mediators

Dominic Rochefort,[ab] Robert Bourbonnais,[b] Dónal Leech*[c] and Michael G. Paice[b]

[a] *Département de chimie, Université de Montréal, Montréal, Canada. E-mail: dominic.rochefort@umontreal.ca*

[b] *Pulp & Paper Research Institute of Canada, Pointe-Claire, Canada. E-mail: rbourbonnais@paprican.ca; mpaice@paprican.ca*

[c] *Department of Chemistry, National University of Ireland, Galway, Ireland. E-mail: donal.leech@nuigalway.ie*

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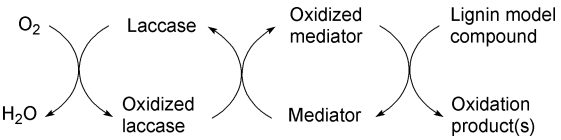
We have studied the oxidation of lignin model compounds by organic and transition metal-based mediators using either an enzyme or an electrolysis cell as the mediator oxidizing agent. Electrolysis of inorganic mediator seems a promising technology for pulp delignification.

The use of a laccase† enzyme with a mediator as a chlorine-free alternative method for pulp delignification (paper bleaching) was proposed by us over a decade ago.[1,2] Although research on such systems has been underway since then, the mechanisms leading to lignin oxidation by the laccase-mediator system are still unclear. A proposed simplified mechanism is presented in Scheme 1.



Scheme 1 Proposed reaction pathways for mediated lignin and model compound oxidation.

Several hypotheses of lignin oxidation mechanisms have been proposed based on results from the oxidation of lignin model compounds (LMC)‡ by radical-forming organic mediators such as 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonate) (ABTS), 1-hydroxybenzotriazole (HBT) and *N*-hydroxyacetanilide (NHA). We recently reported on the use of transition metal-based mediators for pulp delignification.[3] These new mediators are coordination complexes of a transition metal (Fe, Mo or Ru) and ligands such as cyanide or 2,2′-bipyridine. These compounds do not form radicals when oxidized by laccase or by an electrode. The products resulting from the oxidation of lignin and its model compounds with these mediators may then differ from that of the radical-forming organic mediators. We have studied the oxidation of three LMCs, 3,4-dimethoxybenzyl alcohol (veratryl alcohol), 1-(3,4-dimethoxyphenyl)-2-(2-methoxyphenoxy)propane-1,3-diol and 1-(3,4-dimethoxyphenyl)-2-phenylethanediol, the last two being compounds representing β-O-4 and β-1 linkages in the lignin, respectively (see Fig. 1). Bulk electrolysis is used to simulate oxidation of the mediator by laccase.[4] This permits evaluation of the effect of dioxygen on oxidation of LMCs.

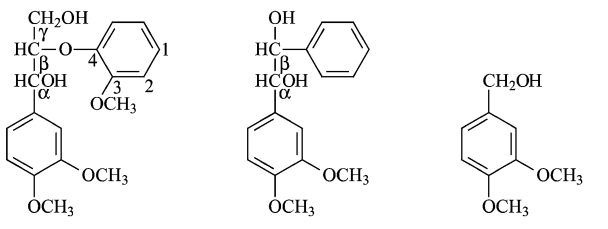


Fig. 1 Structure of the lignin model compounds used in this study.

The reaction mixtures are composed of 2 mM of the LMC and 0.5 mM of mediator dissolved in citrate buffer (100 mM) at pH 4.5. For enzymatic treatments, 0.5 units mL^{-1} of laccase is added to the solution while an electrolysis cell (Bioanalytical Systems Inc.) is used for electrochemical treatments. The laccase activity is measured spectrophotometrically using ABTS as described earlier.[2] All reactions are for 20 hours and the reaction products are analysed by reversed-phase HPLC.[5]

Initially, we compare the ability of four mediators to oxidize the β-1 dimer LMC (Fig. 2). As expected, electrolytic oxidation (E = 615 mV *vs* Ag/AgCl) of mediators possessing oxidation potentials more positive than the applied potential gives similar yields of oxidized LMC to the control, as they are not catalytically oxidized. The control is the % of LMC oxidized directly at the electrode. Potassium octacyanomolybdate(IV) (MoCN) can mediate the LMC oxidation, when electrolytically and enzymatically oxidized. ABTS gives particularly high yields of oxidation, even though the radical cation formed at this applied potential or in the presence of laccase is unreactive towards the LMC.[1] The $ABTS^{\cdot+}$ can undergo a disproportionation reaction (eqn. (1) producing the dication, which is more reactive and oxidizes the LMC efficiently.[6]

$$2\ ABTS^{\cdot+} \leftrightarrows ABTS + ABTS^{2+} \quad (1)$$

HBT gives high LMC oxidation yields with laccase, but not when electrolytically oxidized. HBT oxidation at carbon electrodes is irreversible and occurs at more positive potential than the applied potential. Laccase, however, can rapidly oxidize HBT to the radical cation, which then oxidizes the LMC. Increased LMC oxidation is obtained upon increasing the electrolysis potential. In all cases, the mechanism involved is

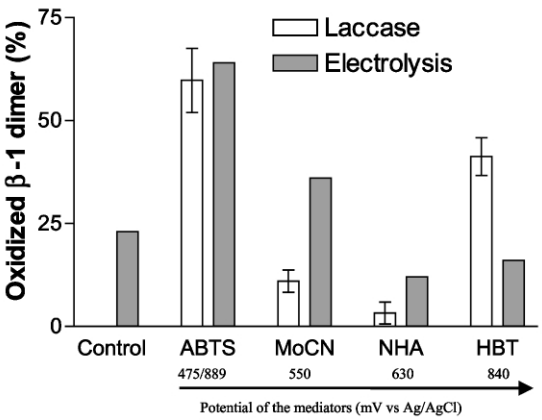


Fig. 2 Percentage of β-1 dimer oxidized after a 20 hour treatment with different mediators, using either laccase (0.5 U mL^{-1}) or electrolysis (E = 615 mV *vs.* Ag/AgCl) as the oxidizing agent. The error bars represent the standard deviation of triplicates.

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Cα–Cβ cleavage (see Fig. 1) and the products identified as veratraldehyde and benzaldehyde in stoichiometric ratio to the amount of LMC oxidized. Removing O_2 during electrolysis at 750 mV has little effect on MoCN-mediated oxidation of the β-1 dimer (86% anaerobic *vs* 77% aerobic), but a decrease in the amount of benzaldehyde formed is observed. This effect has already been reported.[7]

The β-O-4 dimer is often used as a lignin model compound since this linkage is the most abundant (near 50%) in kraft lignin chemical structure. The β-O-4 dimer is not easily oxidized, (oxidation potential 1200 mV *vs.* Ag/AgCl), and only ABTS and HBT mediators are able to do so (Table 1). There is a difference in β-O-4 dimer oxidation mechanism when laccase and electrolytic oxidation of the mediator is used. With laccase only one oxidation product (Cα-ketone) is detected, whilst veratraldehyde, a cleavage product, is detected with electrochemical treatments. It is known that these oxidations occur *via* a two-electron reaction.[6,8] The reaction probably consists of two different electron transfer events: a first structure-specific 1-electron oxidation by a mediator that could be followed by a second oxidation. The oxidation at the electrode is probably non-specific toward an element of structure of the dimer since several products are detected. Two of these products, the most abundant, have been identified (Table 1) but several small unidentified HPLC peaks were also observed. These products could explain the difference existing between the concentration of the dimer oxidized and of the identified products. The mediated laccase oxidation is specific since only one oxidation product is found, and in stoichiometric ratio to the model compound. These issues are presently under further investigation in our laboratories.

Table 1 Comparison of mediated enzymatic (laccase 0.5 U mL^{-1}) and electrolytic (E = 900 mV *vs.* Ag/AgCl) oxidation of the β-O-4 dimer (2 mM) showing amount (percentage) oxidized after 20 h. Amounts of major oxidation products detected are also shown

Oxidizing agent/ mediator (0.5 mM)	Dimer oxidized (mM)	Cα-ketone (mM)	Veratraldehyde (mM)
Laccase/control[a]	0.00 (0 %)	n.d.[b]	n.d.
Laccase/HBT	0.95 (48 %)	0.89	n.d.
Laccase/ABTS	0.39 (19 %)	0.42	n.d.
Electrolysis/control	0.28 (14 %)	0.12	0.07
Electrolysis/HBT	0.85 (42 %)	0.30	0.16
Electrolysis/ABTS	1.68 (84 %)	0.20	0.65

[a] The control represents an assay without mediator. [b] Not detected.

According to the synthesis procedure,[9] the β-O-4 dimer exists in two diastereomeric forms, *erythro* and *threo*, which appear as two resolved peaks by HPLC with the *erythro* being eluted first.[10] Under normal conditions and for all mediators, both isomers are equally oxidized, as both peak areas decrease evenly after the treatment. Under anaerobic conditions, the oxidation of the dimer with HBT becomes stereospecific to the *threo* form. This effect has not been observed with other mediators. When HBT is oxidized in the presence of oxygen, it rapidly forms a dioxide.[11] It is therefore possible that the monoxide, which is the only oxidized form of HBT found under anaerobic conditions, is only reactive toward the *threo* form of the β-O-4 dimer. Since the interphenyl chain in the *threo* form is more exposed than in the *erythro* form,[12] this suggests that the reaction is initiated on this chain and not on the phenyl units, which in both isomers are equally exposed to a reaction with the mediator.

Veratryl alcohol is a non-phenolic LMC that can be irreversibly oxidized at carbon electrodes to veratraldehyde at approximately 1200 mV (*vs* Ag/AgCl). We have used this LMC to study delignification by transition metal-based mediators compared to HBT (Table 2).

Table 2 Amount in millimolar (percentage) of VA oxidized (initial concentration of 2 mM) after a 20 hour treatment using mediators (0.5 mM) with a high $E^{0\prime}$

Mediator ($E^{0\prime}$)[a]	Enzymatic treatment Laccase 0.5 U mL	Electrolysis (E = 900 mV)
No mediator	0.00 (0 %)	0.00 (0%)
HBT (850)[b]	1.10 (55 %)	0.99 (50%)
FeDMBPY (689)	0.01 (~0%)	0.31 (16%)
RuCN (684)	0.01(~0%)	1.98 (99%)

[a] Formal potential measured *vs* Ag/AgCl. [b] HBT oxidation is irreversible (oxidation potential).

Neither of the two mediators, hexacyanoruthenate(II) (RuCN) and tris-(4,4′-dimethyl-2,2′-bipyridine)iron(II) (FeDMBPY) can oxidize the VA when oxidized by laccase, most likely because the overall reaction (Scheme 1) is limited by slow oxidation of mediator by the enzyme.[13] Nevertheless, when electrolysis at 900 mV is used, RuCN oxidizes VA completely. HBT can oxidize only 50% of VA, probably because of the instability of the oxidized HBT, as it is known that the HBT radical decays eventually to benzotriazole, which is unreactive towards the model compounds.[6] Indeed, HPLC analysis shows a benzotriazole concentration corresponding to a 50% decrease in HBT concentration. The results in Table 2 show therefore that electrocatalytic oxidation of VA by the mediators occurs. Oxidation of paper pulps using electrolysis and these inorganic mediators is thus a promising route for chlorine-free delignification.

Notes and references

† Laccase is a blue copper oxidase that can oxidise substrates *via* the four electron-reduction of dioxygen to water. Laccases are commonly found in wood-rotting fungi.[14,15]

‡ Lignin is an irregular, non-soluble biopolymer that surrounds the cellulose fibres in wood. The insolubility of lignin hampers the study of oxidation mechanisms using the intact biopolymer. Model compounds of lignin that are small, soluble and represent some key chemical linkages in pulp lignin, can thus be used to study lignin oxidation mechanisms.

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