Influence of mediators on laccase catalyzed radical formation in lignin - DTU Orbit (17/06/2018)

**Influence of mediators on laccase catalyzed radical formation in lignin**

Laccases (EC 1.10.3.2) catalyze oxidation of phenolic groups in lignin to phenoxyl radicals during reduction of $\text{O}_2$ to $\text{H}_2\text{O}$. Here, we examine the influence on this radical formation of mediators which are presumed to act by shuttling electrons between the laccase and the subunits in lignin that the enzyme cannot approach directly. Treatments of three different lignins with laccase-mediator-systems (LMS) including laccases derived from *Trametes versicolor* and *Myceliophthora thermophila*, respectively, and four individual mediators, 1-hydroxybenzotriazole (HBT), N-hydroxyphthalimide (HPI), 2,2,6,6-tetramethylpiperidin-1-yloxy (TEMPO), and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) were assessed by real time electron paramagnetic resonance measurements. Radical steady state concentrations and radical formation rates were quantified. LMS treatments with 500 μM N-OH type mediators (HPI or HBT) did not affect the lignin radical formation, but increased doses of those mediators (5 mM) surprisingly led to significantly decreased radical formation rates and lowered steady state radical concentrations. Laccase-TEMPO treatment at a 5 mM mediator dose was the only system that significantly increased steady state radical concentration and rate of radical formation in beech organosolv lignin. The data suggest that electron shuttling by mediators is not a significant general mechanism for enhancing laccase catalyzed oxidation of biorefinery lignin substrates, and the results thus provide a new view on laccase catalyzed lignin modification.

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