Bioresource Technology 119 (2012) 114–122

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Demonstration of laccase-based removal of lignin from wood and non-wood plant feedstocks

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HIGHLIGHTS

- A laccase-mediator treatment removed lignin from whole woody and nonwoody feedstocks.
- A high-redox potential laccase and 1-hydroxybenzotriazole (as mediator) were used.
- This laccase-mediator treatment was combined with an alkaline peroxide extraction.
- 2D NMR analyses revealed oxidative removal of lignin aromatic units and side-chains.
- The enzymatic pretreatment increased the sugar and ethanol yields.

ARTICLE INFO

Article history:
Received 6 February 2012
Received in revised form 3 May 2012
Accepted 22 May 2012
Available online 30 May 2012

Keywords:
Enzymatic deconstruction
Laccase
Bioethanol
Lignocellulose
2D NMR

ABSTRACT

The ability of *Trametes villosa* laccase, in conjuction with 1-hydroxybenzotriazole (HBT) as mediator and alkaline extraction, to remove lignin was demonstrated during treatment of wood (*Eucalyptus globulus*) and non-wood (*Pennisetum purpureum*) feedstocks. At 50 U g^{-1} laccase and 2.5% HBT concentration, 48% and 32% of the *Eucalyptus* and *Pennisetum* lignin were removed, respectively. Two-dimensional nuclear magnetic resonance of the feedstocks, swollen in dimethylsulfoxide-d_6, revealed the removal of *p*-hydroxyphenyl, guaiacyl and syringyl lignin units and aliphatic (mainly β-O-4′-linked) side-chains of lignin, and a moderate removal of *p*-coumaric acid (present in *Pennisetum*) without a substantial change in polysaccharide cross-signals. The enzymatic pretreatment (at 25 U g^{-1}) of *Eucalyptus* and *Pennisetum* feedstocks increased the glucose (by 61% and 12% in 72 h) and ethanol (by 4 and 2 g L^{-1} in 17 h) yields from both lignocellulosic materials, respectively, as compared to those without enzyme treatment.

1. Introduction

Lignin removal is an important technical issue for paper manufacturing and a key challenge for the conversion of lignocellulosic feedstock into liquid transportation fuels such as ethanol. Biofuel production from lignocellulosic material requires deconstruction of the cell-wall matrix into individual polymers, and hydrolysis of the carbohydrate polymers into monomeric sugars. Biomass recalcitrance towards enzymatic hydrolysis is correlated with the content and composition of lignin (Studer et al., 2011). Physical, chemical and biological pretreatments, or combinations of these processes, are being studied for deconstructing lignocellulosic biomass and removing lignin (Alvira et al., 2010; Yu et al., 2011). Most biological pretreatments for delignifying lignocellulosic materials employ lignin-degrading fungi, mainly belonging to the group of white-rot basidiomycetes (Kumar et al., 2009; Salvachúa et al., 2011) but such pretreatments require long application periods and consume a fraction of the plant polysaccharides.

Laccases (phenoloxidases, EC 1.10.3.2) are multicopper oxidases that oxidize substituted phenols using molecular oxygen as the final electron acceptor. The direct action of laccases on lignin is, in principle, restricted to phenolic units that only represent a small percentage of the total polymer, a fact that limits their biotechnological application. However, the discovery that some synthetic compounds can act as electron carriers between the enzyme and

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http://dx.doi.org/10.1016/j.biortech.2012.05.112