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Lignocellulose modifications by brown rot fungi and their effects, as pretreatments, on cellulolysis

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ABSTRACT

Brown rot fungi *Gloeophyllum trabeum* and *Postia placenta* were used to degrade aspen, spruce, or corn stover over 16 weeks. Decayed residues were saccharified using commercial cellulases or brown rot fungal extracts, loaded at equal but low endoglucanase titers. Saccharification was then repeated for high-yield samples using full strength commercial cellulases. Overall, brown rot pretreatments enhanced yields up to threefold when using either cellulase preparation. In the best case, aspen degraded 2 weeks by *G. trabeum* yielded 72% glucose-from-cellulose, a 51% yield relative to original glucan. A follow-up trial with more frequent harvests showed similar patterns and demonstrated interplay between tissue modifications and saccharification. Hemicellulose and vanillic acid (G6) or vanillin (G4) lignin residues were good predictors of saccharification potential, the latter notable given lignin's potential active role in brown rot. Results show basic relationships over a brown rot time course and lend targets for controlling an applied bioconversion process.

1. Introduction

Brown rot fungi are a group of wood-degrading fungi with a mechanism relevant for industrial bioconversion. These fungi remove nearly 100% of carbohydrates from wood, but they usually remove little lignin (Eriksson et al., 1990). Brown rot fungi are theorized to initiate decay with a non-enzymatic pretreatment step (Koenigs, 1974; Jensen et al., 2001) that has potential to be chemically reproduced and hastened. Many brown rot species also do not secrete cellobiohydrolase enzymes and lack their genes (Valášková and Baldrian, 2006; Martinez et al., 2009), and many have difficulty efficiently degrading pure cellulose substrates like standard filter paper or delignified wood (Highley, 1980; Blanchette, 1983). This suggests an active role for lignin and a potential for exploiting synergies using simpler, non-traditional hydrolytic suites. Collectively, these biological traits encourage research into the basic brown rot mechanism and into industrial alternatives that harness or emulate these fungi.

Aspects of the brown rot mechanism remain unclear and are often studied individually rather than as part of a system. Brown rot research has focused primarily on early modifications that rapidly reduce wood strength (Winandy and Morrell, 1993; Curling et al., 2002), a key decay issue in load-bearing lumber. Brown rot fungi are theorized to use a non-enzymatic mediated pretreatment combining iron (Fe^{2+}) and hydrogen peroxide to produce hydroxyl radicals (Goodell et al., 1997). Reactive oxygen species (ROS) may play a direct role in demethylating, oxidizing, and cleaving lignin (Kirk, 1975; Filley et al., 2002; Yelle et al., 2008), and brown rot is characterized by early carbohydrate depolymerization starting with side-chain hemicelluloses (Curling et al., 2002; Irbe et al., 2006). Relative to bioconversion interests, however, this is only the first step in metabolizing wood – a pretreatment before saccharification. Although Schilling et al. (2009) showed that early wood modifications made by brown rot fungi broadly facilitate enzymatic saccharification, its underlying mechanism remains unclear and the best application scenario has not been investigated. Therefore, research applying brown rot for bioconversion offers two benefits: (1) improvement in commercial potential, and (2) opportunity to study the mechanism as output from a system rather than reconstruction from individual observations.

Exploiting brown rot fungi for biomass conversion has been attempted in the past, either by inoculating with a fungus as a biological pretreatment or by using a chemical mimick to pretreat wood. Ray et al. (2010) used *Coniophora puteana* as a biological pretreatment to degrade Scots pine blocks for 15 days, then halted

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