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Effects of laccase on lignin depolymerization and enzymatic hydrolysis of ensiled corn stover

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HIGHLIGHTS

- TMAH–GC–MS can effectively elucidate molecular changes in plant cell wall lignin.
- Laccase directly contributed to lignin decomposition at a molecular level.
- Laccase increased cellulose digestibility in ensiled stover.

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ABSTRACT

The aim of this study was to explore the synergies of laccase, a ligninolytic enzyme, with cellulose and hemicellulase amendments on ensiled corn stover. Molecular signals of lignin decomposition were observed by tetramethylammonium hydroxide thermochemolysis and gas chromatography–mass spectroscopy (TMAH–GC–MS) analysis. The significant findings suggest that ensilage might provide a platform for biological pretreatment. By partially hydrolyzing cellulose and hemicellulose into soluble sugars, ensilage facilitates laccase penetration into the lignocellulose complex to enhance lignin degradation. Downstream cellulose hydrolysis was improved 7% with increasing laccase loading rate. These results demonstrate the potential of enzymes, either directly amended or expressed by microbes during ensilage, to maximize utilization of corn stover for cellulosic biofuels and other downstream fermentations.

1. Introduction

Ensilage amended with cellulases and/or hemicellulases has been demonstrated as an effective and stable long-term storage strategy for lignocellulosic biomass, as well as a beneficial platform for downstream pretreatment (Ren et al., 2007; Richard et al., 2002; Shinners et al., 2007; Vervaeren et al., 2010). However, saccharification of cellulose during and after ensilage is limited due to the presence of lignin in biomass. Lignin is a semi-random three-dimensional aromatic polymer composed of phenylpropanoid subunits linked together by a variety of ether and carbon–carbon bonds. Lignin is always intimately interlaced with hemicellulose in the plant cell wall, forming a matrix to envelop the crystalline cellulose microfibrils (Kirk and Farrell, 1987). Its complex structure and high molecular weight make lignin degradation very difficult (Call and Mücke, 1997; Ke et al., 2011; Shi et al., 2009). To overcome the recalcitrance of lignocelluloses associated with lignin, delignification strategies such as supercritical fluid extraction and hydrogenolysis have attracted increasing attention (Gosselink et al., 2012; Moilanen et al., 2011; Torr et al., 2011). Chemical and biological delignification are two major methods of lignin depolymerization. The latter is considered superior to the former due to its friendly environmental characteristics and lower energy demand. However, within the category of biological delignification strategies, microbial treatment is usually slower than enzymatic treatment, and is often achieved at the expense of considerable dry matter loss (Lechner and Papinutti, 2006; Pinto et al., 2012; Shi et al., 2009). Although enzymatic treatment is challenging due to the high molecular mass of lignin degrading enzymes, their high cost, and the requirement of enzyme co-factors, it has advantages over microbial treatment because it targets specifically selected reactions and minimizes the interference of side reactions (Roberts et al., 1995).

Among the limited number of enzymes known to participate in delignification, the laccase system has been studied most extensively. Laccase (EC 1.10.3.2) is a class of copper containing enzymes produced by fungi, bacteria and plants (Call and Mücke, 1997;

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