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Saccharification of woody biomass using glycoside hydrolases from *Stereum hirsutum*

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ABSTRACT

Enzymatic saccharification of woody biomasses was performed using glycoside hydrolases from *Stereum hirsutum*, a newly isolated fungal strain found to secrete efficient glycoside hydrolases. The strain showed the highest β-glucosidase, cellobiohydrolase, endoglucanase, endoxylanase, laccase, and filter paper activity of 10.3, 1.7, 10.3, 29.9, 0.12, and 0.58 U/ml, respectively. Among the various biomasses tested for saccharification, pine biomass produced maximum reducing sugar. Response surface methodology was used to optimize the hydrolysis of pine biomass to achieve the highest level of sugars. The parameters including enzyme, substrate concentration, temperature and pH were found to be critical for the conversion of pine biomass into sugars. Maximum saccharification of 49.7% (435 mg/g-substrate) was obtained after 96 h of hydrolysis. A close agreement between the experimental results and the model predictions was achieved. *S. hirsutum* could be a good choice for the production of reducing sugars from cellulosic biomasses.

1. Introduction

Lignocellulosic biomass, in the form of agricultural residues, forestry remnants, and herbaceous energy crops, can serve as low cost feed-stocks for production of fuel ethanol and other value-added commodity chemicals. Cellulose, the major fraction of lignocellulosic biomass, can be hydrolyzed to glucose by cellulase enzymes (Cassman and Liska, 2007). The enzyme system for the conversion of cellulose to glucose involves at least three types of cellulases including endo-glucanase (EG, E.C.3.2.1.4), cellobiohydrolase (CBH, E.C.3.2.1.91) and β-glucosidase (BGL, 3.2.1.21). EGs act randomly along the cellulose chains to produce cellulose fragments. CBH acts as exoglucanase to release the disaccharide cellobiose. The BGLs then hydrolyze cellobiose to yield glucose. Since cellobiose is an inhibitor of EG or CBH activity, the BGL reaction allows the cellulolytic enzymes to function more efficiently (Béguin and Aubert, 1994; de Palma-Fernandez et al., 2002; Workman and Day, 1982). The most widely investigated source of cellulase is *Trichoderma reesei* (Persson et al., 1991). However, the *T. reesei* cellulase system is deficient in BGL activity, making it an inefficient system for the complete hydrolysis of cellobiose. This in turn causes serious inhibition of the cellulolytic enzymes (Holtzapple et al., 1990). Although this problem can be overcome by the addition of an extra source of BGL, e.g. from *Aspergillus niger* (Wright et al., 1986), the hydrolysis of lignocelluloses to fermentable monosaccharides is still technically problematic.

In lignocellulose, the linear cellulose polymers are highly crystalline and are usually surrounded by lignin and xylan, which reduce their accessibility to hydrolytic enzymes. Several pretreatment techniques to remove lignin and xylan have been used to increase the hydrolysis of lignocellulosic biomass: enzymes, dilute acid, ammonia recycle percolation, lime, steam explosion (Hendriks and Zeeman, 2009), alkaline and acidic wet oxidation (Varga et al., 2004). Biodegradation of lignin and xylan requires laccase (EC.1.10.3.2), lignin peroxidase (EC. 1.11.1.14), endo-1,4-β-D-xylan xylanohydrolase (EC.3.2.1.8), β-xylosidase (EC.3.2.1.37) and several other accessory enzymes (Baldrian, 2006; Beg et al., 2000; Polizeli et al., 2005). Renewable energy demand in the future will only be met if we can utilize lignocellulosic biomasses. Critical to successful use of these biomasses is development of enzyme cocktails that will break the plant cell wall down into usable fractions. Nonetheless, several other factors still remain that can affect the enzymatic hydrolysis of lignocellulose, including the substrate type, enzyme activity, and hydrolysis conditions such as pH and temperature. Most research has therefore focused on optimization of the hydrolysis process and on enhancement of the enzyme activity in order to

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