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Simultaneous saccharification and co-fermentation of glucose and xylose in steam-pretreated corn stover at high fiber content with *Saccharomyces cerevisiae* TMB3400

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Abstract

The two main sugars in the agricultural by-product corn stover are glucose and xylose. Co-fermentation of glucose and xylose at high content of water-insoluble solids (WIS) without detoxification is a prerequisite to obtain high ethanol concentration and to reduce production costs. A recombinant strain of *Saccharomyces cerevisiae*, TMB3400, was used in simultaneous saccharification and fermentation (SSF) of whole pretreated slurry of corn stover at high WIS. TMB3400 co-fermented glucose and xylose with relatively high ethanol yields giving high final ethanol concentration. The ethanol productivity increased with increasing concentration of pretreatment hydrolysate in the yeast production medium and when SSF was performed in a fed-batch mode.

Keywords: SSF; Ethanol; Steam pretreatment; Glucose and xylose co-fermentation; Recombinant *Saccharomyces cerevisiae*; TMB3400

1. Introduction

Bioethanol contributes to the reduction of greenhouse gases when it is used as a transportation fuel. Bioethanol can be produced from lignocellulosic biomass using pretreatment (Saddler and Brownell, 1983) followed by enzymatic hydrolysis and fermentation (Hahn-Hägerdal and Pamment, 2004; Sondregger et al., 2004). The enzymatic hydrolysis and fermentation can be conducted simultaneously, which is referred to as simultaneous saccharification and fermentation (SSF) (Takagi et al., 1977).

Two factors influence the production cost of ethanol from biomass more than others: the effective conversion of all the sugars to ethanol, and the concentration of ethanol in the fermentation broth prior to distillation. Lignocellulosic materials contain cellulose, hemicellulose and lignin. Cellulose is a highly crystalline

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polymer of glucose. Hemicellulose is a branched polymer, which, in addition to the hexoses glucose, mannos and galactose, also contains the pentoses, xylose and arabinose. The conversion of cellulose and hemicellulose to monomeric sugars is harder to accomplish than the conversion of starch, presently used for ethanol production. However, the price of lignocellulosic material is lower than that of starchy raw material such as corn or wheat (Dumitriu, 1998). Since the raw material cost contributes substantially to the final cost of ethanol (Wingren et al., 2003), the low cost of lignocellulose together with an efficient conversion of all sugars to ethanol could potentially decrease the production cost of ethanol produced from lignocellulose. Therefore, co-fermentation of hexoses and pentoses to ethanol is a prime research target. After fermentation the ethanol is recovered by distillation. The ethanol concentration in the feed has a major effect on the energy demand, especially at concentrations below 4 wt.% (Zacchi and Axelsson, 1989). Increasing the ethanol concentration in the feed to the distillation reduces the production costs considerably (Wingren et al., 2003).

Corn stover is an abundant agricultural by-product with low commercial value (Kadam and Mcmillan, 2003) and it consists of mainly glucose and xylose (Torget et al., 1991). Both sugars can be recovered with high yield using steam pretreatment and subsequent enzymatic hydrolysis (Öhgren et al., 2005), and glucose can be fermented to ethanol with high yield using baker's yeast. This process can also be carried out as an SSF process (Öhgren et al., 2006; Takagi et al., 1977). SSF has been proven superior to separate hydrolysis and fermentation (SHF) at elevated WIS concentrations (Stenberg et al., 2000; Söderström et al., 2005). In addition, combining two process steps reduces capital cost (Wingren et al., 2003), and the increased ethanol concentration achieved in SSF reduces contamination with lactic acid bacteria (Wyman et al., 1992). Ethanol is recovered by distillation, which becomes economically feasible when the concentration exceeds 40 g/L (Zacchi and Axelsson, 1989). This concentration therefore serves as benchmark when different process alternatives are evaluated.

The industrial fermentation of lignocellulose hydrolysate to ethanol requires microorganisms, which have a broad substrate range, and which produce ethanol with high yield and productivity. Such microorganisms must also tolerate ethanol and inhibitors formed in the pretreatment process. When lignocellulose hydrolysates have been fermented with recombinant strains of *Escherichia coli* (Martinez et al., 2001; O'Brien et al., 2004; Saha et al., 2005) and *Zymomonas mobilis* (Mcmillan et al., 1999; Mohagheghi et al., 2004) or with the natural xylose fermenting yeast *Pichia stipitis* (Nigam, 2001) detoxification was required prior to fermentation.

Baker's yeast *Saccharomyces cerevisiae* is the most commonly used microorganism for industrial ethanol production. It has also been shown to efficiently ferment lignocellulosic hydrolysate to ethanol (Öhgren et al., 2005). However, *S. cerevisiae* cannot utilize xylose for growth and ethanol production. When the *P. stipitis* genes *XYL1* and *XYL2* encoding xylose reductase (XR) and xylitol dehydrogenase (XDH), respectively, were introduced in *S. cerevisiae* (Kötter and Ciriacy, 1993) in combination with the endogenous gene *XKS1* encoding xylulokinase (XK) (Ho et al., 1998; Eliasson et al., 2000; Toivari et al., 2001) the resulting strains were able to utilize xylose for growth and ethanol production. *S. cerevisiae* strains with improved capacity to utilize xylose have been acquired through choice of parental strain, random mutagenesis, adaptation and/or breeding (Sonderegger et al., 2004; Wahlbom et al., 2003). Industrial *S. cerevisiae* strains transformed with genes for XR, XDH and XK are capable to ferment non-detoxified lignocellulose hydrolysates (Hahn-Hägerdal and Pamment, 2004). Recently, a haploid laboratory strain of *S. cerevisiae* has been successfully transformed with a xylose isomerase gene (Kuyper et al., 2003). So far the performance of this strain in lignocellulose hydrolysates has not been reported.

In the present investigation, ethanol production by TMB3400 (Wahlbom et al., 2003) and its parental strain USM21 (van der Westhuizen and Pretorius, 1992) were compared with baker's yeast in batch SSF of whole pretreated corn stover slurry at 0.05 g WIS/g SSF slurry. Yeast biomass was produced in the pretreatment hydrolysate. Elevated WIS levels (0.10, 0.11 and 0.12 g/g) were used in batch and fed-batch SSF with TMB3400.

2. Materials and methods

The experimental design is schematically illustrated in Fig. 1.