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Pilot-scale ethanol production from rice straw hydrolysates using xylose-fermenting *Pichia stipitis*

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ABSTRACT

Ethanol was produced at pilot scale from rice straw hydrolysates using a *Pichia stipitis* strain previously adapted to NaOH-neutralized hydrolysates. The highest ethanol yield was 0.44 ± 0.02 g_p/g_s at an aeration rate of 0.05 vvm using overliming-detoxified hydrolysates. The yield with hydrolysates conditioned by ammonia and NaOH was 0.39 ± 0.01 and 0.34 ± 0.01 g_p/g_s, respectively, were achieved at the same aeration rate. The actual ethanol yield from hydrolysate fermentation with ammonia neutralization was similar to that with overliming hydrolysate after taking into account the xylose loss resulting from these conditioning processes. Moreover, the ethanol yield from ammonia-neutralized hydrolysates could be further enhanced by increasing the initial cell density by two-fold or reducing the combined concentration of furfural and 5-hydroxymethyl furfural to 0.6 g/L by reducing the severity of operational conditions in pretreatment. This study demonstrated the potential for commercial ethanol production from rice straw via xylose fermentation.

1. Introduction

Rice straw is the most abundant agricultural residue in Taiwan and the amount available has been estimated at approximately 2.49 million tons each year (Chen et al., 2011b). Currently, most rice straw is discarded or burned on farmland, but utilization as a feedstock for ethanol production has been suggested as a more economic and eco-friendly alternative (Guo et al., 2008).

The biochemical processes for lignocellulosic ethanol production usually include pretreatment, enzyme hydrolysis, and fermentation of pentose and hexose sugars (Fig. 1). Hydrolysis using dilute sulfuric acid is effective as the hemicelluloses are broken down and the products are released into solution (Srilekha Yadav et al., 2011). Pretreatment with diluted acid can convert hemicelluloses to xylose with a yield of 75–90% (Eggeman and Elander, 2005; Mosier et al., 2005; Sun and Cheng, 2002).

Pichia stipitis (Jeffries and Jin, 2000), *Candida shehatae* (Eken-Saraçoğlu and Arslan, 2000), and *Pachysolen tannophilus* (Jeffries and Jin, 2000; Sánchez and Cardona, 2008) are capable of converting xylose to ethanol. But *P. stipitis* is considered the most promising naturally occurring microorganism for the fermentation of hemicellulosic hydrolysates since it generally produces the highest ethanol yield from xylose (Agbogbo and Coward-Kelly, 2008). Although several genetically engineered organisms, such as *Saccharomyces cerevisiae*, *Escherichia coli*, and *Zymomonas mobilis*, capable of fermenting pentose sugars have been developed (Ahmed et al., 2009; Katahira et al., 2008; Matsushika et al., 2009), their effectiveness in large-scale fermentations has not been established.

There are several obstacles in the fermentation of hemicellulosic hydrolysates for ethanol production. Inhibitors, such as weak acids and furans, are often generated during pretreatment with diluted acid. Although concentrations of these inhibitors are usually limited when pretreatment is conducted at a relatively low temperature, such as 130 °C (Huang et al., 2009). The dilute acid-pretreatment is normally performed at 180–200 °C to increase the conversion efficiency during subsequent enzymatic hydrolysis (Neureiter et al., 2002; Saha et al., 2005; Sun and Cheng, 2002, 2005). Hydrolysates produced under those conditions usually require a detoxification step before fermentation of hemicellulosic hydrolysates (Mohagheghi et al., 2006; Ranatunga et al., 2000). Currently, overliming is considered the most promising method for eliminating inhibitors, such as furfural, in hydrolysates; however, overliming is time-consuming and costly. Alternative methods such as ammonia/sodium hydroxide (NaOH)-neutralization to improve the efficacy of hydrolysate conditioning for ethanol production have been proposed due to no gypsum generated and reduced xylose loss (Pienkos and Zhang, 2009). The aim of the present study was to identify suitable fermentation conditions for rice straw hydrolysate fermentation based on the conversion of xylose to ethanol using *P. stipitis*, with an objective of integrating these findings into lignocellulosic ethanol production processes.

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