Bioresource Technology 117 (2012) 92–98

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

A viable method and configuration for fermenting biomass sugars to ethanol using native *Saccharomyces cerevisiae*

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ARTICLE INFO

Article history:
Received 8 November 2011
Received in revised form 2 April 2012
Accepted 3 April 2012
Available online 10 April 2012

Keywords:
Xylose
Xylulose
Simultaneous isomerization and fermentation
Hollow fiber
Saccharomyces cerevisiae

ABSTRACT

A system that incorporates a packed bed reactor for isomerization of xylose and a hollow fiber membrane fermentor (HFMF) for sugar fermentation by yeast was developed for facile recovery of the xylose isomerase enzyme pellets and reuse of the cartridge loaded with yeast. Fermentation of pre-isomerized poplar hydrolysate produced using ionic liquid pretreatment in HFMF resulted in ethanol yields equivalent to that of model sugar mixtures of xylose and glucose. By recirculating model sugar mixtures containing partially isomerized xylose through the packed bed and the HFMF connected in series, 39 g/l ethanol was produced within 10 h with 86.4% xylose utilization. The modular nature of this configuration has the potential for easy scale-up of the simultaneous isomerization and fermentation process without significant capital costs.

1. Introduction

Complete and efficient sugar utilization is one of the prerequisites to cost effectively produce ethanol from biomass (Hahn-Hägerdal et al., 2007a). *Saccharomyces cerevisiae* has traditionally been used in large-scale ethanol fermentation of sucrose and starch-based glucose; however, this species is unable to ferment xylose, the major pentose sugar released from the hydrolysis of hemicellulose, to ethanol, since the metabolic pathways in native *S. cerevisiae* convert xylose solely to xylitol (Traff et al., 2001).

Genetic engineering approaches have been developed to introduce key enzymes into the yeast in order to achieve fermentation of both glucose and xylose (Kuyper et al., 2005; Hahn-Hägerdal et al., 2007b; Brat et al., 2009; Bera et al., 2010); however, the success of these approaches has been limited by co-factor imbalances, unfavorable intracellular xylose-to-xylulose isomerization equilibrium, and decreased robustness of the yeast. To circumvent the complexity and uncertain outcome associated with genetic modification, xylose can be exogenously isomerized to xylulose. However, the isomerization reaction (whether it occurs intracellularly or extracellularly) does not have a favorable equilibrium (xylose:xylulose ~6:1) (Hsiao et al., 1982; Rao et al., 2008). While simultaneous isomerization and fermentation (SIF) can increase the total amount of xylose isomerized to xylulose, the optimal pH for the fermentation is in the range of 4–5 while commercially-available xylose isomerase (XI) works best at near-neutral pH. The large pH disparity between the two steps requires SIF operation under compromised conditions which slows both reactions (Gong et al., 1981). In addition, large quantities of by-products, particularly xylitol (Gong et al., 1981; Hahn-Hägerdal et al., 1986), are produced and bacterial contamination of the fermentation broth is more likely. Hahn-Hägerdal et al. (1986) showed that in an SIF mode (at a *compromised* pH of 6), native *S. cerevisiae* was able to convert xylose to ethanol in the presence of *high* concentrations of *soluble* XI and 4.6 mM sodium azide (Hahn-Hägerdal et al., 1986). Soluble XI efficiently converts xylose to xylulose while azide inhibits respiration and suppresses by-product formation and bacterial contamination. However, the authors concluded that in the absence of a viable recovery scheme for the high amounts of XI used in the process, the economics are non-viable. Moreover, azide affects xylulose utilization kinetics in ethanol fermentation (Yuan et al., 2011).

In a novel bi-layered enzyme approach recently proposed by this group, high conversion of xylose to xylulose under conditions optimal for fermentation was achieved (Rao et al., 2008). In this approach, urease is immobilized on commercially available XI pellets and dispersed in a fermentation broth containing urea and a small quantity of sodium tetraborate. Urea serves as a nitrogen source for the yeast and hydrolysis of urea to ammonia by urease results in a localized pH gradient within the pellet; thus sugar isomerization occurs at close to neutral pH while sugar is fermented at pH 4.5. Borate plays an important role in xylulose complexation at neutral

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http://dx.doi.org/10.1016/j.biortech.2012.04.005