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Co-fermentation of hexose and pentose sugars in a spent sulfite liquor matrix with genetically modified *Saccharomyces cerevisiae*

Vera Novy, Stefan Krahulec, Karin Longus, Mario Klimacek, Bernd Nidetzky *

Institute of Biotechnology and Biochemical Engineering, Graz University of Technology, Petersgasse 12/I, A-8010 Graz, Austria

HIGHLIGHTS

- ▶ Xylose fermenting *S. cerevisiae* strain IBB10B05 for SSL fermentation.
- ▶ Fermentation of 70% (v/v) SSL without detoxification.
- ▶ Ethanol yields between 0.31 and 0.44 g/g total sugar.
- ▶ Correlation between xylose uptake rates and molar xylitol and glycerol yields.

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ABSTRACT

Spent sulfite liquor (SSL) is a by-product of pulp and paper manufacturing and is a promising substrate for second-generation bioethanol production. The *Saccharomyces cerevisiae* strain IBB10B05 presented herein for SSL fermentation was enabled to xylose utilization by metabolic pathway engineering and laboratory evolution. Two SSLs from different process stages and with variable dry matter content were analyzed; SSL-Thin (14%) and SSL-S2 (30%). Hexose and pentose fermentation by strain IBB10B05 was efficient in 70% SSL matrix without any pretreatment. Ethanol yields varied between 0.31 and 0.44 g/g total sugar, depending on substrate and process conditions used. Control of pH at 7.0 effectively reduced the inhibition by the acetic acid contained in the SSLs (up to 9 g/L), thus enhancing specific xylose uptake rates (q_{Xylose}) as well as ethanol yields. The total molar yield of fermentation by-products (glycerol, xylitol) was constant (0.36 ± 0.03 mol/mol xylose) at different q_{Xylose}. Compound distribution changed with glycerol and xylitol being chiefly formed at low and high q_{Xylose}, respectively.

1. Introduction

Environmental concerns and the issue of limited fossil fuel resources have called attention from public, politics and research all over the world. Amongst others, major objectives are containment of climate change and quest a way to fuel the world in future. Therefore a sustainable, environmentally compatible and economically feasible fossil fuel substitute has to be found and research has to aim towards large scale production. One promising renewable energy source is second generation bioethanol, produced from lignocellulosic biomass such as municipal, agricultural or forestry waste (Chundawat et al., 2011; Hahn-Hägerdal et al., 2006; Sims et al., 2010).

Lignocellulosic materials consist of the two major fractions cellulose and hemicellulose. They are composed of glucose and a variety of hexose and pentose sugars, respectively, which can be liberated by hydrolysis and subsequently fermented to ethanol (Alvira et al., 2010; Hahn-Hägerdal et al., 2006; Sims et al., 2010). Spent sulfite liquor (SSL) is a by-product of pulp and paper industry. As a waste stream, SSL has the advantages of being abundantly available and low-priced. Consequently SSL has been employed for ethanol generation for a long time, and research has been accomplished since the 1960s (Björling and Lindman, 1989; Helle et al., 2004, 2007, 2008; Holderby and Moggio, 1960; Yu et al., 1987). The pulping process includes the treatment of wood with sulfurous acid and magnesium bisulfite which solubilizes lignin and part of the hemicellulose fraction, and out leaches the lignin. The process provides low yields but high purity of cellulose, which then is processed further to pulp or paper. After recovery of the chemicals the liquor contains a high fraction of organic compounds including sugars, which are released during acid treatment. The main drawbacks in SSL fermentation are low sugar content and variations in hexose and pentose composition (Helle et al., 2004, 2007, 2008).

In order to facilitate efficient conversion of SSL to ethanol, robust and reliable co-fermentation of xylose and hexoses must be

* Corresponding author. Tel.: +43 316 873 8400; fax: +43 316 873 108400.
E-mail address: bernd.nidetzky@tugraz.at (B. Nidetzky).

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