Fermentation Kinetics for Xylitol Production by a *Pichia stipitis* d-Xylulokinase Mutant Previously Grown in Spent Sulfite Liquor

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**Abstract** Spent sulfite pulping liquor (SSL) contains lignin, which is present as lignosulfonate, and hemicelluloses that are present as hydrolyzed carbohydrates. To reduce the biological oxygen demand of SSL associated with dissolved sugars, we studied the capacity of *Pichia stipitis* FPL-YS30 (xyI3Δ) to convert these sugars into useful products. FPL-YS30 produces a negligible amount of ethanol while converting xylose into xylitol. This work describes the xylose fermentation kinetics of yeast strain *P. stipitis* FPL-YS30. Yeast was grown in rich medium supplemented with different carbon sources: glucose, xylose, or ammonia-base SSL. The SSL and glucose-acclimatized cells showed similar maximum specific growth rates (0.146 h\(^{-1}\)). The highest xylose consumption at the beginning of the fermentation process occurred using cells precultivated in xylose, which showed relatively high specific activity of glucose-6-phosphate dehydrogenase (EC 1.1.1.49). However, the maximum specific rates of xylose consumption (0.19 g\(_{\text{xylose}}\)/g\(_{\text{cell}}\) h) and xylitol production (0.059 g\(_{\text{xylitol}}\)/g\(_{\text{cell}}\) h) were obtained with cells acclimatized in

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glucose, in which the ratio between xylose reductase (EC 1.1.1.21) and xylitol dehydrogenase (EC 1.1.1.9) was kept at higher level (0.82). In this case, xylitol production (31.6 g/l) was 19 and 8% higher than in SSL and xylose-acclimatized cells, respectively. Maximum glycerol (6.26 g/l) and arabitol (0.206 g/l) production were obtained using SSL and xylose-acclimatized cells, respectively. The medium composition used for the yeast precultivation directly reflected their xylose fermentation performance. The SSL could be used as a carbon source for cell production. However, the inoculum condition to obtain a high cell concentration in SSL needs to be optimized.

Keywords Xylitol · Yeast · Xylose · Ammonia spent sulfite liquor-SSL · Inoculum adaptation · Enzymes

Abbreviations
XR xylose reductase
XDH xylitol dehydrogenase
G6PDH glucose-6-phosphate dehydrogenase
 XK xylulokinase
 $\mu_x$ maximum specific cell growth rate (h$^{-1}$)
 $\mu_s$ maximum specific xylose consumption rate (g$_{xylose}$/g$_{cell}$ h)
 $\mu_p$ maximum specific xylitol production rate (g$_{xylitol}$/g$_{cell}$ h)
 $Y_{p/s}$ xylitol yield coefficient (g$_{xylitol}$/g$_{xylose}$)
 $Y_{x/s}$ cell yield coefficient (g$_{cell}$/g$_{xylose}$)
 $Q_p$ xylitol volumetric productivity (g$_{xylitol}$/l h)

Introduction

In the sulfite-pulping process, about 50% of the wood (hemicellulose and lignin) is dissolved to produce cellulose for paper along with the effluent ("spent sulfite liquor," SSL). SSL is the only lignocellulosic hydrolysate available today in large quantities (about 90 billion liters annually worldwide) [1], and it is produced at a rate of 1 ton (dry basis) per ton of pulp [2]. Many investigators have studied the possibility of using microbes to convert SSL into usable products. Its sugar content ranges from 3 to 4%, depending on the source of wood being pulped [3]. Softwoods have been the traditional feedstock, and their hexose sugars constitute 74% of these hydrolysates. However, hardwood sulfite pulping is becoming more popular, and the pentose sugars, principally xylose, in hardwood SSL can be up to 50% [2].

Recently, the conversion of xylose into value-added chemicals, such as xylitol, ethanol, and lactic acid have made this process attractive to the fermentation industry [4]. In particular, bioconversion for xylitol production has been intensively studied during the last decade because xylitol can be used as a functional sweetener [5].

The xylose reductase (XR) catalyzes the first step of a fungal pathway that allows certain organisms to metabolize xylose, such as Candida boidinii [6], Candida guilliermondii [7], Candida tropicalis [8], Candida parapsilosis [9], and Debaryomyces hansenii [10]. After the reduction of xylose to xylitol by XR in a manner that can utilize nicotinamide adenine dinucleotide (reduced form; NADH) or nicotinamide adenine dinucleotide phosphate (reduced form; NADPH), xylitol is re-oxidized to xylulose by xylitol dehydrogenase, which is often specific for nicotinamide adenine dinucleotide (NAD)$^+$ [11]. Xylulose is then phosphorylated. An efficient, pathway should recycle the cosubstrate such that there is no