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ScienceDirect
Bioresource Technology 98 (2007) 1767–1773
BIORESOURCE TECHNOLOGY

Adaptation of a recombinant xylose-utilizing *Saccharomyces cerevisiae* strain to a sugarcane bagasse hydrolysate with high content of fermentation inhibitors

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Received 22 March 2006; received in revised form 3 June 2006; accepted 3 July 2006
Available online 24 August 2006

Abstract

Adaptation of a xylose-utilizing genetically engineered strain of *Saccharomyces cerevisiae* to sugarcane bagasse hydrolysates by cultivation during 353 h using medium with increasing concentrations of inhibitors, including phenolic compounds, furaldehydes and aliphatic acids, led to improved performance with respect to ethanol production. The remaining xylose concentration in the medium at the end of the cultivation was 5.2 g l^{-1}, while it was 11 g l^{-1} in the feed, indicating that approximately half of the xylose was consumed. The performance of the adapted strain was compared with the parental strain with respect to its ability to ferment three bagasse hydrolysates with different inhibitor concentration. The ethanol yield after 24 h of fermentation of the bagasse hydrolysate with lowest inhibitor concentration increased from 0.18 g g^{-1} of total sugar with the non-adapted strain to 0.38 g g^{-1} with the adapted strain. The specific ethanol productivity increased from 1.15 g ethanol per g initial biomass per h with the non-adapted strain to 2.55 g g^{-1} h^{-1} with the adapted strain. The adapted strain performed better than the non-adapted also in the two bagasse hydrolysates containing higher concentrations of inhibitors. The adapted strain converted the inhibitory furaldehydes 2-furaldehyde (furfural) and 5-hydroxymethyl-2-furaldehyde (HMF) at a faster rate than the non-adapted strain. The xylose-utilizing ability of the yeast strain did not seem to be affected by the adaptation and the results suggest that ethanol rather than xylitol was formed from the consumed xylose.

Keywords: Ethanol; Sugarcane bagasse; *Saccharomyces cerevisiae*; Adaptation; Fermentation inhibitors; Xylose utilization

1. Introduction

Ethanol is a possible alternative liquid fuel for vehicles. Traditional raw materials for ethanol production, such as sugarcane and maize, have the drawback that they serve as food and would represent a major part of the total production cost (Claassen et al., 1999). Lignocellulosic waste materials are abundant and represent a promising feedstock for industrial production of low-cost fuel ethanol (reviewed by Wheals et al. (1999)). Sugarcane bagasse represents one of the major lignocellulosic materials to be considered in most tropical countries since it has high carbohydrate and low lignin content, is readily available at the sugar mill site, and the cost for harvest, transport and storage has been borne by the sugar production (Triana et al., 1990; Pandey and Soccol, 2000). The use of bagasse for ethanol production would contribute to the diversification of the sugar industry, which is an urgent requirement for the sugarcane-based economies (Olguin et al., 1995; Gálvez, 2000).

Hydrolysis is required for the conversion of the polysaccharides in lignocellulose to fermentable sugars. However,

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0960-8524/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2006.07.021

during acid hydrolysis, compounds that inhibit fermenting microorganisms are formed as well (reviewed by Parajó et al. (1998); Palmqvist and Hahn-Hägerdal (2000); Hahn-Hägerdal et al. (2001)). Inhibitory compounds in lignocellulose hydrolysates include aromatic, primarily phenolic, compounds, furaldehydes, such as 2-furaldehyde (furfural) and 5-hydroxymethyl-2-furaldehyde (HMF), as well as aliphatic acids, such as formic, acetic and levulinic acid (Larsson et al., 1999). Removal or dilution of these compounds improves the fermentability of lignocellulose hydrolysates. Several different detoxification methods have been described and the effects on the chemical composition of lignocellulose hydrolysates have been investigated (Larsson et al., 1999). Although detoxification improves the fermentability of hydrolysates, it is for economical reasons desirable to limit the requirements for detoxification to a minimum (von Sivers et al., 1994). Adaptation of fermenting microorganisms to inhibitory hydrolysates is one of several possible strategies for dealing with inhibitor problems.

Saccharomyces cerevisiae (baker's yeast), the microorganism that has traditionally been used for producing alcoholic beverages, has several positive features for ethanol production from lignocellulose. However, a disadvantage associated with *S. cerevisiae* is that it cannot metabolize xylose, an abundant sugar in hydrolysates of hardwoods and agricultural residues (Jeffries and Shi, 1999; Hahn-Hägerdal et al., 2001). Metabolic engineering can be used for the development of recombinant *S. cerevisiae* strains able to ferment xylose (Stephanopoulos et al., 1998; Hahn-Hägerdal et al., 2001). Previous investigations on fermentation of bagasse hydrolysates with a genetically engineered xylose-utilizing *S. cerevisiae* strain showed that removal of inhibitors was necessary for achieving good fermentation performance (Martín et al., 2002a,b).

The aims of the present work were to adapt a genetically engineered xylose-utilizing strain of *S. cerevisiae* to enzymatic hydrolysates of sugarcane bagasse and to compare the fermentative performance of the adapted strain with that of the non-adapted parental strain using bagasse hydrolysates with different inhibitor content.

2. Methods

2.1. Yeast strain

The strain used in this study, TMB 3001, is a CEN.PK derivative that expresses xylose reductase and xylitol dehydrogenase from the chromosomally integrated *Pichia stipitis* genes *XYL1* and *XYL2*, respectively, and over-expresses the homologous *XKS1* gene encoding xylulokinase (Eliasson et al., 2000). The strain was transferred from −80 °C and maintained on YPD-agar plates containing 10 g/l yeast extract (Merck, Darmstadt, Germany), 20 g/l peptone (Difco, Detroit, MI, USA), 20 g/l glucose (BDH, Poole, UK), and 20 g/l agar–agar (Merck).

2.2. Media

For the adaptation procedure, five different media with increasing inhibitor concentration (Table 1) were prepared by mixing three different bagasse hydrolysates. The hydrolysates were prepared by H_2SO_4-catalysed steam pretreatment of sugarcane bagasse followed by enzymatic hydrolysis as previously described (Martín et al., 2002a). The pH of all media was adjusted to 5.5 with 2 M NaOH and the media were filter sterilized and enriched with $(NH_4)_2SO_4$, $MgSO_4 \cdot 7\,H_2O$, KH_2PO_4, vitamins and minerals (Verduyn et al., 1992). All chemicals used were purchased from Merck (Darmstadt, Germany) if not otherwise stated. A sterile solution of glucose (BDH, Poole, England) and xylose (Acros Organics, New Jersey, USA) was added to the adaptation media to final concentrations of 30 and 11 g l^{-1}, respectively.

For evaluation of the adapted strain, three different media were used (Table 1). One of the media consisted of hydrolysate that was not diluted and is hereafter referred to as Hydrolysate SA100 (sulfuric acid, 100%). The two other media were prepared by dilution of Hydrolysate SA100 to a concentration of 50% and 75% (v/v) and are hereafter referred to as Hydrolysate SA50 and SA75, respectively. The concentrations of glucose and xylose in

Table 1
Sugar and inhibitor concentrations in the media used for adaptation of the yeast strain to bagasse hydrolysates (Inoculum, initial batch, feed 1–3) and for evaluation of the adapted strain (SA50, SA75, SA100)

Medium	Glucose (g l^{-1})	Xylose (g l^{-1})	Furaldehydes[a] (g l^{-1})	Aliphatic acids[b] (g l^{-1})	Phenols[c] (g l^{-1})
Inoculum	30	11	0.7	2.5	1.5
Initial batch	30	11	1.0	3.9	2.2
Feed 1	30	11	1.5	5.4	2.2
Feed 2	30	11	2.1	6.2	2.0
Feed 3	30	11	3.4	8.7	2.3
SA50	25.8	5.9	2.2	5.0	1.4
SA75	25.5	5.3	3.8	7.6	2.1
SA100	25.4	4.9	4.5	10.1	2.8

[a] Furfural and HMF.
[b] Formic, acetic and levulinic acid.
[c] Total phenolics determined using the Folin-Ciocalteu method.