Co-production of ethanol and xylitol from sugarcane hemicellulose hydrolysate by yeasts isolated from the Atlantic Forest and the Brazilian Amazon Forest

Souza, J. P [1], Fogarin, H.M.[1], Almeida, S.G.C.[1], Santos, B.C.S[1], Silva, D.D.V.[2], Dussán, K.J.[1,*]

[1]Department of Engineering, Physics and Mathematics, Institute of Chemistry, São Paulo State University-UNESP, Araraquara, Av. Prof. Francisco Degni, 55 – Jardim Quitandinha, 14800-900, Brazil.
[2]Department of Biochemistry and Organic Chemistry, Institute of Chemistry, São Paulo State University-UNESP, Araraquara, Av. Prof. Francisco Degni, 55 – Jardim Quitandinha, 14800-900, Brazil.
Keywords: Xylitol, Bioethanol, *Spathaspora sp.*, Sugarcane hemicellulose hydrolysate.
*Presenting author email: kelly.medina@unesp.br

1. Introduction

In the recent years the term 'zero-waste' has been brought up by researchers and companies as part of their efforts to promote changes in the energy matrix approaching renewable energy sources such as the lignocellulosic biomass, and one strategy that has been intensively applied is the use of sugarcane bagasse as raw material in biorefineries, because of its availability and its potential to meet current energy consumption (Dahmen et al. 2019; Farias et al. 2022). The sugarcane bagasse is mainly composed of cellulose (33-36%), hemicellulose (28-30%) and lignin (18-20%) (Liao et al. 2016), which the first two components are important sources of fermentable sugars for the biorefineries, moreover one of the most efficient pre-treatment techniques to release those fermentable sugars is the diluted acid hydrolysis (Laopaiboon et al. 2010), however it also releases compounds such as aliphatic acids, phenolic compounds, and furan derivates, which acts as inhibitors to the microorganisms while in the fermentation process. In reference to the inhibitors and the variables that play an important role in the fermentation, such as: the biomass, nitrogen concentration, temperature, pH, aeration, reactor's operation, and downstream processes; there is the need to identify microorganism that can overcome those hindrance (Sànchez Nogué and Karhumaa 2015). Three yeast species have been isolated from the Brazilian ecosystem, *Spathaspora girioi*, *Spathaspora boniae* and *Spathaspora brasiliensis*, and they have shown promising result for xylose fermentation to obtain value-added products as xylitol. Thus, the aim of this study is to investigate the fermentative capacity of the yeasts *Spathaspora girioi*, *Spathaspora boniae*, and *Spathaspora brasiliensis* to produce xylitol and/or bioethanol from sugarcane biomass hemicellulose hydrolysate by the screening of different organic and inorganic nitrogen sources.

2. Material and methods

2.1 Sugarcane bagasse hemicellulose hydrolysate preparation

A hemicellulose hydrolysate was obtained by pre-treating a straw/sugarcane bagasse mixture (50:50 by weight) with diluted sulfuric acid. A solid:liquid ratio of 1:10, 140 °C, an acid concentration of 0.5% (w/v), for 15 min in a stainless-steel reactor (250L) were used in the pretreatment step. The hydrolysate was detoxified by adjusting the pH with CaO to pH 7.0 and then with H_3PO_4 to pH 2.5, then adding 1.0% (w/v) activated carbon and incubating for 30 minutes at 60°C and 100 rpm. The detoxified hydrolysate (24 g/L of xylose, 3.5 g/L of glucose, 3.0 g/L of arabinose, 4.08 g/L of acetic acid, and 0.008 g/L of furfural) was autoclaved at 110 °C and 0.5 atm.

2.1 Organisms and Fermentation conditions

The yeasts *Spathaspora girioi* (UFMG-CM-Y302), *Spathaspora boniae* (UFMG-CM-Y306), and *Spathaspora brasiliensis* (UFMG-HMD19.3) were kindly donated and obtained from the Federal University of Minas Gerais Microorganism Collection in Belo Horizonte, Minas Gerais, Brazil. Determining the best nitrogen source for xylitol and ethanol production, central composite designs (2^{4-1}) were used: Urea (0.05 – 1.3 g/L), Yeast extract (0.23 – 6.1 g/L), Peptone (0.19 – 4.8 g/L), and Ammonium sulfate (0.11 – 2.9 g/L) in non-detoxified and detoxified hydrolysate. The experiments were carried out in 250 mL Erlenmeyer flasks containing 100 mL of sugarcane bagasse hydrolysate at pH 5.5 and an initial cell concentration of 1 g/L for 72 hours at 30°C.

2.3 Analytical procedures

The concentrations of sugars, acetic acid, and formic acid were determined using high-performance liquid chromatography (HPLC). A BIO-RAD AMINEX HPX-87H analytical column (300 X 7.8 mm) was used for chromatography, with the mobile phase H_2SO_4 0.01 N flowing at a rate of 0.6 mL/min.

3. Results and discussion

In non-detoxified hemicellulose hydrolysate, yeasts *S. girioi* and *S. brasiliensis* consumed glucose but were unable to consume xylose and produce ethanol or xylitol, while *S. boniae* not only assimilated glucose and xylose as produce ethanol and xylitol. This different behaviour of yeast to toxic compounds in the hydrolysate led to the to use 2 different experiments to determine the better nitrogen source. The ability of yeasts to convert sugars into ethanol and xylitol is shown in Table 1. *S. boniae* consumed a high percentage of sugars (97.05%) when non-detoxified hydrolysate was supplemented with urea (0.7 g/L), yeast extract (6.1 g/L), peptone (2.5 g/L), and ammonium sulfate (1.5 g/L), accompanied by the production of xylitol (10.74 g/L) with $Y_{P/S}$ and Q_P of 0.437 g/g and 0.149 g/L, respectively. In contrast to *S. boniae*, the yeast *S. girioi* produced more ethanol (6.49 g/L) than

xylitol in hydrolysate detoxified supplemented with urea (0.05 g/L), yeast extract (6.1 g/L), peptone (4.8 g/L), and ammonium sulfate (0.11 g/L), with a high sugar consumption (96.33%). *S. brasiliensis*, like the yeast *S. boniae*, produced more xylitol (11.22 g/L) than ethanol, but in detoxified hydrolysate supplemented with urea (1.3 g/L), yeast extract (6, 1 g/L), peptone (0.19 g/L), and ammonium sulfate (0.11 g/L) with the highest sugar consumption (99.28%). Even preliminary, these results agree with reported by authors (Silva et al., 2020) working with new yeast *Scheffersomyces amazonensis* and can be improved to be competitive to that presented by model yeasts.

Table 1. Evaluation of fermentation parameters for ethanol and xylitol production using *S. boniae*, *S. girioi* and *S. brasiliensis* in sugarcane bagasse hemicellulosic hydrolysate.

		Ethanol			Xylitol		
		Ethanol (g/L)	Yp/s (g/g)	Qp (g/Lh)	Xylitol (g/L)	Yp/s (g/g)	Qp (g/Lh)
Non-detoxified hydrolysate	*S. boniae*	3.86	0.157	0.054	10.74	0.437	0.149
	S. girioi	*Nd*	*Nd*	*Nd*	*Nd*	*Nd*	*Nd*
	S. brasiliensis	*Nd*	*Nd*	*Nd*	*Nd*	*Nd*	*Nd*
Detoxified hydrolysate	*S. girioi*	6.49	0.220	0.090	2.76	0.093	0.038
	S. brasiliensis	1.40	0.054	0.019	11.22	0.437	0.156

Nd = Not detected.

Conclusions

Spathaspora boniae demonstrated a superior capacity to convert sugars into xylitol in hemicellulose hydrolysate without detoxification, indicating that it can tolerate the toxic compounds. The other 2 new xylose-fermenting yeasts, *S. girioi* and *S. brasiliensis* however, need toxic levels to be reduced in the detoxification step to be able in bioconvert sugars into xylitol and ethanol. Despite their preliminary nature, the results demonstrated that these yeasts from the Brazilian ecosystem have a high potential for industrial bioproducts production from sugarcane bagasse hydrolysate.

Acknowledgments

We gratefully acknowledge Dr. Carlos A. Rosa from Universidade Federal de Minas Gerais (UFMG), Brazil, for providing us the yeast strain and CAPES for financially supporting the current study.

References

Dahmen N, Lewandowski I, Zibek S, Weidtmann A (2019) Integrated lignocellulosic value chains in a growing bioeconomy: Status quo and perspectives. GCB Bioenergy 11(1):107–117. https://doi.org/10.1111/gcbb.12586

Farias D, de Mélo AHF, da Silva MF, Bevilaqua GC, Ribeiro DG, Goldbeck R, Forte MBS, Maugeri-Filho F (2022) New biotechnological opportunities for C5 sugars from lignocellulosic materials. Bioresour Technol Rep 17:100956. https://doi.org/10.1016/j.biteb.2022.100956

Laopaiboon P, Thani A, Leelavatcharamas V, Laopaiboon L (2010) Acid hydrolysis of sugarcane bagasse for lactic acid production. Bioresour Technol 101(3):1036–1043. https://doi.org/10.1016/j.biortech.2009.08.091

Liao JC, Mi L, Pontrelli S, Luo S (2016) Fuelling the future: microbial engineering for the production of sustainable biofuels. Nat Rev Microbiol 14(5):288–304. https://doi.org/10.1038/nrmicro.2016.32

Sànchez Nogué V, Karhumaa K (2015) Xylose fermentation as a challenge for commercialization of lignocellulosic fuels and chemicals. Biotechnol Lett 37(4):761–772. https://doi.org/10.1007/s10529-014-1756-2

Silva, D.D.V., Dussán, K.J., Idarraga, A., Grangeiro, L., Silva, S.S., Cardona, C.A., Quintero, J. and Felipe, M.G.A. (2020), Production and purification of xylitol by *Scheffersomyces amazonenses* via sugarcane hemicellulosic hydrolysate. Biofuels, Bioprod. Bioref: (14): 344-356. https://doi.org/10.1002/bbb.2085