Production of ethanol fuel from enzyme-treated sugarcane bagasse hydrolysate using D-xylose-fermenting wild yeast isolated from Brazilian biomes

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Abstract

In this study, we evaluated the potential of yeasts isolated from Amazon to produce second-generation ethanol from sugarcane bagasse delignified with alkaline hydrogen peroxide and hydrolysed with commercial enzyme preparation. The best efficiency savings in glucose and release of xylose were determined by considering the solids and enzyme loads. Furthermore, we selected *Spathaspora passalidarum* UFMG-CM-Y473 strain with the best fermentative parameters. Fermentations used bagasse hydrolysate without any nutritional supplementation, a significant difference from previous studies, which is closer to industrial conditions. Ethanol yield of 0.32 g/g and ethanol productivity of 0.34 g/L h were achieved after the consumption of 78% of the sugar. This hydrolysis/fermentation technology package could represent the input of an additional 3180 L of ethanol per hectare in areas of average sugarcane productivity such as 60 ton/ha. Thus, we concluded that *Sp. passalidarum* UFMG-CM-Y473 has a clear potential for the production of second-generation ethanol from delignified and enzyme-hydrolysed bagasse.

Keywords Bagasse · Enzymatic hydrolysis · Ethanol · Hydrogen peroxide · Xylose fermentation

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Introduction

The search for a renewable and economically viable energy source has encouraged innovative technological research to find alternatives to petroleum such as fuels derived from plant biomass such as sugarcane and crop by-products (Girio et al. 2010). Around 694 million tons of sugarcane were harvested in 2016/2017, an increase of 4.4% compared with the 2015/2016 in Brazil (Conab 2016). Since each ton of cane generates 270–280 kg of bagasse (Canilha et al. 2012), about 187–194 million tons of bagasse were produced and could be used to produce second-generation alcohol, without extending the area used for sugarcane cultivation and hence increasing the productivity of the crop (Stambuk et al. 2008; Soccol et al. 2010; Cadete et al. 2012).

The production of ethanol from this residue requires a pre-delignification treatment for partial or complete removal of lignin, followed by enzymatic hydrolysis to release the fermentable sugars (Correia et al. 2013). *Saccharomyces cerevisiae* yeast is commonly used in the production of first-generation ethanol from hexoses, due to its high

fermentation capacity and adaptation to the industrial process. However, it does not contain the enzymes needed for the conversion of pentoses such as xylose. Xylose is the second most abundant carbohydrate in nature (Kwak and Jin 2017; Girio et al 2010) and an important component in the hemicellulose fraction of bagasse.

An obvious alternative is to modify *S. cerevisiae* by expressing the genes responsible for the conversion of xylose to xylulose (Kwak and Jin 2017). In Brazil, there are pilot initiatives for the use of GMO yeast for the production of second-generation ethanol (<http://www.granbio.com.br/en/>). However, there is clearly room for metabolic improvement for achievement of high yields and productivity. Therefore, it seems reasonable to continue the search for yeasts with a higher capacity for ethanol conversion from xylose, such as those from the genera *Spathaspora* and *Scheffersomyces* (Cadete et al. 2012; Chandel et al. 2014). *Spathaspora passalidarum* has been used in the fermentation of xylose to ethanol, and shows a great potential in the production of lignocellulosic ethanol (Nguyen et al. 2006; Hou and Yau 2011; Cadete et al. 2012; Long et al. 2012).

Innovations in the pre-treatment, enzymatic hydrolysis and fermentation of the hydrolysates stages to produce second-generation ethanol are important for the viability of production. In this context, we examined the potential of several xylose-assimilating yeasts obtained from the Amazon forest to convert xylose to ethanol. Before the screening initial, we examined the efficiency of bagasse pre-treatment with alkaline peroxide followed by enzyme hydrolysis to produce a hydrolysate that can be further fermented by *S. passalidarum* strain UFMG-CM-Y473. We explore the implications of the results for second-generation ethanol production and the improvement of the ethanol fuel sector.

Materials and methods

Bagasse pre-treatment

Sugarcane bagasse produced by the Japungu industrial plant (Santa Rita municipality, Paraíba state, Brazil) was washed three times with water at 50 °C, dried at 45 °C for 48 h in a drying oven (MA 035-1, Marconi Co., São Paulo), to remove the residual impurities and soluble sugars, ground in a Wiley type mill (MA 680, Marconi Co., São Paulo) with a 20 mesh strainer, and stored in a freezer at −20 °C.

Aliquots of 20 g of the ground bagasse were treated with 200 mL of H₂O₂ (7.5%), adjusted to pH 11.5 with 5 M NaOH. Flasks containing the bagasse and hydrogen peroxide solution were incubated in an orbital shaker at 150 rpm, 25 °C for 1 h (Rabelo et al. 2008; Reis et al. 2016). The solid fraction was recovered by filtration and washed with 1.5 L of hot distilled water (70 °C) to remove residual lignin.

After being washed, the pre-treated bagasse was dried at 45 °C for 48 h and the mass loss during pre-treatment was determined as

$$M_L(\%) = [(M_i - M_f)/M_i] \times 100 \quad (1)$$

in which M_i and M_f represent the initial and the recovered masses (g) of bagasse, respectively.

Enzymatic hydrolysis

FibreZyme™ LDI Complex (Dyadic International Inc., Jupiter, USA) was used to hydrolyse the delignified bagasse. This enzyme was kindly provided by the manufacturer. The enzyme activities of avicelase, carboxymethyl cellulase (CMCase), β-glucosidase, xylanase and cellulase (FPU) of this complex were determined in accordance with the method described by Ghose (1987) and Wood and Bhat (1987).

Different amounts of dried delignified bagasse (1, 2, 3, 4, 5 and 7.5 g) were suspended in 50 mL of sodium citrate buffer (50 mM and pH 4.8), resulting to approximate biomass loads of 1.96, 3.84, 5.64, 7.44, 9.0 and 13% (w/v). Enzyme volumes were added so as to have the same enzyme load of 10 or 20 FPU per gram of solid. The flasks were incubated in a rotary shaker at 50 °C and 150 rpm during 72 h, after which the hydrolysed bagasse was filtered (Reis et al. 2016), the liquid fraction was frozen at −20 °C and the solid fraction was dried in an oven at 60 °C for 48 h to determine weight loss (as discussed above) to calculate the efficiency of the enzyme hydrolysis. The hydrolysis assays were performed in triplicate.

Microorganisms

The strains used in this study were isolated from samples of rotting wood from the Amazon forest, collected from two sites in Roraima state, and from the Atlantic Forest, collected in Rio de Janeiro state. They were previously identified as the xylose-fermenting yeasts (Cadete et al. 2012, 2013) *S. passalidarum* UFMG-CM-Y472, UFMG-CM-Y473, UFMG-CM-Y469 and UFMG-CM-Y474, *Scheffersomyces parashehatae* UFMG-CM-Y506 and UFMG-CM-Y507, *Spathaspora xylofermentans* UFMG-CM-Y479, *Spathaspora brasilienses* UFMG-CM-Y353, *Spathaspora suhii* sp. UFMG-CM-Y475 and *Spathaspora roraimanensis* UFMG-CM-Y477. The strains belong to the Culture Collection of Microorganisms, and DNA and cells from the Federal University of Minas Gerais (UFMG). *Scheffersomyces stipitis* NRRL 7124 yeast was used as a positive control for xylose fermentations. The yeasts were grown in YPD medium (yeast extract, 10 g/L; peptone, 20 g/L; dextrose, 20 g/L) and stored at −80 °C in 30% glycerol solution.

Culture and fermentation media

Cell growth of seed cultures were carried out in standard mineral medium (MM) containing a complete Yeast Nitrogen Base (YNB, Difco Co., USA) at 6.7 g/L and glucose at 20 g/L. Yeast screening was performed with the use of mineral medium with xylose (MMX) containing complete YNB (6.7 g/L) and D-xylose (120 g/L) or mineral medium of reference (MMR) containing complete YNB (6.7 g/L) and a mixture of D-glucose (54 g/L), D-xylose (30 g/L), cellobiose (13 g/L) and L-arabinose (3.4 g/L). MMR mimicked the composition of the obtained enzymatic hydrolysate. The yeast cells (seed cells) were cultivated in MM whenever fermentation assays used MMX or MMR, or in YPD whenever fermentations assays used bagasse hydrolysate. The seed cells were prepared by flask cultivations in a rotary shaker at 30 °C and 120 rpm, for 3–5 days. The yeast cells were recovered by centrifugation in graduated conical tubes until an equivalent of 5 mL of wet sediment was accumulated and used for fermentation assays.

Fermentation assays

Fermentations were carried out by re-suspending yeast cells in fermentation media to 50 mL final volume. The wort was transferred to 125-mL flasks and incubated in a rotary shaker at 30 °C and 120 rpm. Samples were taken at regular intervals for 72 h and centrifuged. The supernatant was stored at –20 °C for metabolite analysis. The experiments were performed in independent duplicates for mineral media or in triplicate for bagasse hydrolysate. The following parameters were analysed: (1) xylose consumption (%) defined as the consumption of xylose in relation to the initial concentration; (2) ethanol yield Y_p/s (g/g) as the ethanol produced/xylose consumed; (3) ethanol productivity Q_p (g/L h) as ethanol concentration (g/L)/time (h) fermentation; (4) fermentation efficiency H (%) as the percentage of the maximum theoretical [0.51 (ethanol/L/xylose g/L)] in ethanol.

Analytical methods

Raw pre-treated (de-lignified) bagasse was characterized in accordance with Van Soest (1963) and analysed by Fourier transform infrared spectroscopy (FTIR) in a Vertex 70 spectrometer (Bruker optics, Germany) with a resolution of 4 cm^{−1} and 64 scans per sample, performed in the wave range of 500–4500 cm^{−1} attenuation total reflectance (ATR). The calculations of the conversion efficiency of cellulose and

hemicellulose into glucose and xylose, respectively, were carried out with the equations of Zhao et al. (2014). Samples from enzymatic hydrolysis and fermentations were filtered through 0.22-μm membrane and analysed for sugar, ethanol and glycerol concentrations by HPLC (Agilent HP 1200, Germany) using a Bio-Rad Aminex HPX-87H (300 × 7.8 mm) column at 50 °C, and 5 mM H₂SO₄ at a flow rate of 0.6 mL/min as mobile phase, following by detection using an RI-detector (Agilent). Whenever necessary, samples were diluted with deionised water before analysis. The amount of assimilable nitrogen available in the hydrolysed bagasse was quantified by the ninhydrin method (Abernathy et al. 2009). Cell concentration and weight loss were determined by dry weight. Calculation of the amount of ethanol production per hectare was based on sugarcane productivity and cellulose content, employing the method proposed by Zhao et al. (2009).

Results and discussion

Characterization of sugarcane bagasse

After delignification with alkaline hydrogen peroxide (AHP), the resulting bagasse had 56.1% cellulose, 30.2% hemicellulose and 6.6% lignin. The mass losses of the hemicellulose and lignin fractions were calculated as 20 and 44%, respectively. These biomass constituents were, thus, solubilized in the liquid fraction by the reaction to the hydroxyl and superoxide radicals generated by hydrogen hydroxide in the alkaline medium. The solubilization resulted in a 43% increase in cellulose (Table 1) and preserved most of the hemicellulose, which contains fermentable sugars, unlike other pre-treatments, such as steam explosion or thermal acid procedure. Martín et al. (2012) used the alkaline (NaOH) method to delignify exploded cane bagasse and observed an elimination of around 90% of lignin, with an increase in cellulose of 64% but led to a considerable loss of hemicellulose. The labile characteristics of the hemicellulose binding to cellulose microfibrils renders it susceptible to different pre-treatments but the loss of hemicellulose means a loss of xylose and other sugars that can be used for ethanol production, implying a potential financial loss (Mosier et al. 2004).

In the FTIR spectroscopy measurements of the raw material, the band at 1242.07 cm^{−1} indicated the CO bond elongation that is characteristic of hemicellulose and lignin (Supplementary material Fig. S1). The band was not observed after the alkaline peroxide treatment, confirming the loss of lignin and the existence of only a small amount of hemicellulose. In

Table 1 Characterisation of sugarcane bagasse before and after treatment with alkaline H₂O₂ expressed on dry basis

Bagasse	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Extractives (%)	Ashes (%)
Raw	39.2 ± 1.1	37.9 ± 0.1	11.8 ± 1.5	6.3 ± 0.1	2.2 ± 0.1
Pre-treated	56.1 ± 0.3	30.2 ± 2.4	6.6 ± 0.7	0.0	2.5 ± 0.1

addition, the decrease in the pre-treated material of the band at 1730.16 cm^{-1} , which represents the stretching of unconjugated C=O that is present in hemicellulose and lignin (Chandel et al. 2014), corroborated the loss of these fractions. Furthermore, the presence of the band at 1510.20 cm^{-1} in the raw material is related to vibrations of the lignin aromatic ring (Correia et al. 2013). Thus, the delignified material seemed to be free of lignin bindings. Finally, an increase of the peak in the range of $3000\text{--}3500\text{ cm}^{-1}$ represented an enlargement of the crystalline structure of the cellulose (Chandel et al. 2014).

Enzymatic hydrolysis

The commercial enzyme preparation had five different hydrolase activities: FPase, xylanase, CMCase, avicelase and β -glucosidase (Table 2). To the best of our knowledge, this is the first description of the activities that are present in this enzyme preparation. Enzyme hydrolysis has been performed by other authors with loads of 7–33 FPU per gram of substrate (Sun and Cheng 2002), and the aim is to lower this proportion to reduce the cost of hydrolysis without impairing the efficiency of sugar release. Based on preliminary published results (Reis et al. 2016), we chose concentrations of 10 FPU per gram of substrate.

Additionally, we doubled the enzyme load to evaluate how it affected the sugar release. First, a test was conducted to determine how much of the pre-treated solids were liquefied by the enzymes, and the effect of different concentrations of enzymes on these parameters. The weight loss revealed that from 10 to 18% of the biomass remained as a solid after the enzyme treatment, regardless of its initial load and enzyme concentration (Table 3). The same weight loss was reported when an enzyme load of 0.12 mL of enzyme mixture (Celluclast, Novozyme 188 and 150 Viscostar L) was used per gram of rice hull (Saha and Cotta 2007). A larger amount of sugars (glucose and xylose) was released with 20 FPU than with 10 FPU of the enzymes. However, this efficiency was reduced when the biomass load was increased (Fig. 1). For this reason, a similar efficiency of sugar release (glucose and xylose) was observed for both enzyme loads when 7.5 g of bagasse was used (Fig. 1). Hence, it was decided to reduce the cost of hydrolysis by

Table 3 Effect of biomass and enzyme load on weight loss in sugarcane bagasse pre-treated with alkaline H_2O_2

Biomass load (g/50 mL)	Weight loss (%)	
	10 FPU	20 FPU
1	$86.4 \pm 3.4\%$ (ab)	$90.4 \pm 0.6\%$ (a)
2	$89.7 \pm 0.5\%$ (a)	$88.9 \pm 1.5\%$ (ab)
3	$86.4 \pm 0.1\%$ (ab)	$85.9 \pm 0.8\%$ (bc)
4	$86.2 \pm 3.1\%$ (ab)	$86.9 \pm 1.2\%$ (b)
5	$84.9 \pm 0.9\%$ (ab)	$83.3 \pm 1.6\%$ (cd)
7.5	$83.0 \pm 1.2\%$ (ab)	$82.0 \pm 0.9\%$ (d)

Average values from triplicates ($\pm$ SD)

Values with the same letter in parentheses do not differ by the Tukey test at 5% probability

selecting 10 FPU to prepare the hydrolysates for fermentation. Banerjee et al. (2011) reported a higher efficiency in glucose release than that obtained in this work using the same pre-treatment, probably explained by the type of lignocellulosic biomass (corn stover) and the enzyme cocktail used. In fact, we observed a higher glucose release when hydrolysing sweet sorghum bagasse than sugarcane bagasse (Reis et al. 2016) and higher glucose release from sugarcane bagasse hydrolysed with Celluclast™ (Reis et al. 2016). The xylose release was in the range of previous findings (Weiss et al. 2009 and; Banerjee et al. 2011), which means that the pre-treatment preserved most of the hemicellulose and that FibreZyme™ had high xylanase activity.

Selection of xylose-fermenting yeast

Different yeast strains were tested for assimilation and fermentation of xylose to ethanol in MMX medium (Table 4). The best fermenting yeast was chosen for a fermentation assay with the bagasse hydrolysate among those previously reported by Cadete et al. (2012). In the present work, more than one single fermentative parameter was taken together when considering the potential for industrial application of these yeasts. For example, when the ethanol yield was examined on its own, the *S. xylofermentans* UFMG-CM-Y479 was chosen by its highest $Y_{p/s}$ value (0.34 g/g) among the yeasts tested (Table 4). However, the volumetric productivity was low as was the xylose assimilation, which are undesirable characteristics in industrial processes. On the other hand, *S. passalidarum* UFMG-CM-Y473 seemed to fulfil all the requirements that could make the process feasible among the strains tested: the highest consumption of xylose (42.7%) and the highest rate of ethanol production (16 g/L). Furthermore, the absence of glycerol production indicated the tendency of this strain to produce relatively more ethanol than accumulate its microbial biomass (Table 4). Therefore,

Table 2 Enzymatic activity of the commercial preparation FibreZyme™ LDI

Enzyme	Activity
FPase (filter paper hydrolase)	11.5 FPU/mL
Xylanase	789 U/mL
β -Glucosidase	10 CBU/mL
Avicelase	5.9 U/mL
CMCase (carboxymethyl cellulase)	175 U/mL

Fig. 1 Efficiency of glucose and xylose release from pre-treated sugarcane bagasse with varied amounts of solid and enzyme suspended in 50 mL of buffer. Values from three experiments ($\pm$ SD). Asterisks indicated significant differences by the Tukey test at 5% probability

between 10 FPU and 20 FPU treatments. The coefficient of variance was calculated as 6.26 (glucose) and 5.89 (xylose) for 10 FPU and 8.91 (glucose) and 8.78 (xylose) for 20 FPU

Table 4 Fermentative parameters of xylose-assimilating yeasts isolated from Brazilian Amazon and Atlantic forests in non-supplemented mineral medium containing xylose at 120 g/L after 72 h of cultivation

Yeast species	Yeast strain	Xylose consumed (g/L)	Ethanol (g/L)	$Y_{p/s}$ (g/g)	Q_p (g/L h)	Glycerol (g/L)
<i>Scheffersomyces stipitis</i>	NRRL 7124	26.7	7.8 $\pm$ 0.0	0.29	0.11	0.12 $\pm$ 0.05
<i>Sc. parashehatae</i>	UFMG-CM-Y506	35.8	8.3 $\pm$ 0.33	0.23	0.12	0.48 $\pm$ 0.07
<i>Sc. parashehatae</i>	UFMG-CM-Y507	47.6	12.0 $\pm$ 3.26	0.25	0.16	1.22 $\pm$ 0.38
<i>Spathaspora xylofermentans</i>	UFMG-CM-Y479	15.2	5.2 $\pm$ 3.92	0.34	0.07	1.05 $\pm$ 0.35
<i>Sp. passalidarum</i>	UFMG-CM-Y469	41.4	7.5 $\pm$ 0.43	0.18	0.10	1.0 $\pm$ 0.03
<i>Sp. passalidarum</i>	UFMG-CM-Y474	30.2	7.0 $\pm$ 1.73	0.23	0.10	0.90 $\pm$ 0.14
<i>Sp. passalidarum</i>	UFMG-CM-Y472	47.3	14.3 $\pm$ 0.05	0.30	0.20	0.63 $\pm$ 0.13
<i>Sp. passalidarum</i>	UFMG-CM-Y473	54.7	16.0 $\pm$ 0.85	0.29	0.22	0.0
<i>S. suhii</i>	UFMG-CM-Y475	39.6	4.0 $\pm$ 0.52	0.10	0.05	0.43 $\pm$ 0.03
<i>S. roraimanensis</i>	UFMG-CM-Y477	21.8	2.2 $\pm$ 0.08	0.10	0.03	0.59 $\pm$ 0.01
<i>S. brasiliensis</i>	UFMG-CM-Y353	31.0	2.5 $\pm$ 0.60	0.08	0.04	2.62 $\pm$ 0.25

$Y_{p/s}$ gram of ethanol per gram of xylose consumed, Q_p ethanol-specific productivity

this strain was chosen to ferment the sugarcane hydrolysate, using (testing) the MMX medium.

Fermentation of enzymatically hydrolysed sugarcane bagasse

At the end of 72-h fermentation, 75 g/L of the fermentable sugars (glucose and xylose) was consumed, resulting in 24 g ethanol/L (Fig. 2a). The ethanol yield and volumetric productivity was calculated as 0.32 g of ethanol per gram of sugar consumed and 0.34 g/L h, respectively. As control, fermentations in MMR were performed with approximately the same proportions of the sugars contained in the enzymatic hydrolysate (13 g/L cellobiose, 54 g/L glucose, 30 g/L xylose and 3.36 g/L-arabinose). Under these conditions, the

yeast cells consumed 79% of the fermentable sugars and produced 24.8 g ethanol/L (Fig. 2b), with yield and volumetric productivity calculated as 0.36 g/g and 0.34 g/L h, respectively. In both cases, the glucose was completely assimilated while residual xylose and, in particular, cellobiose were detected in the wort (Fig. 2). Thus *S. passalidarum* UFMG-CM-Y473 apparently co-assimilated glucose, xylose and cellobiose, and this behaviour also characterized *S. passalidarum* NN 245 (Long et al. 2012). However, an accelerated assimilation of xylose was observed after glucose was consumed (Fig. 2), which shows that glucose plays a role in catabolite repression in this yeast. Moreover, the accumulation of xylose and cellobiose at the end of the fermentation could be explained by the dependence on micro-oxygenation for the yeast cells to assimilate these sugars (Long et al. 2012).

Fig. 2 Kinetics of fermentation of sugarcane bagasse hydrolysate (13% biomass load and 10 FPU) (a) or mineral medium of reference (MMR) (b) by *Spathaspora passalidarum* UFMG-CM-Y473 strain

Finally, the yeast biomass remained practically unaltered in the course of the fermentation of both media due to the low amount of nitrogen, which means that the carbon assimilated was converted to ethanol and other minor metabolites. In this case, it is possible to calculate the specific productivity in the first 24 h hours as 1 mM ethanol g DW/h for the hydrolysate, which is half of the specific productivity for the mineral medium. Certainly, new approaches are needed to tackle these problems of co-assimilation and redox imbalance to increase productivity.

The substrates contained sugars at indicated concentrations (Figs. 1, 2) and assimilable nitrogen at a concentration of 12.6 mg/L. The concentration of total nitrogen in sugarcane juice is at least 20 times higher than that observed in our work (De Souza Barros et al. 2015). This very low concentration of nitrogen, leading to a high C:N ratio in

the wort, may reduce cell growth and stimulate fermentative metabolism. In view of this, this yield of 0.32 g/g was reached without any additional nitrogen or vitamin sources. In Table 5, there is a comparison of our results for *S. passalidarum* UFMG-CM-Y473 cultured on sugarcane bagasse hydrolysate with other reports in the literature that consistently supplement the hydrolysates with external source of nitrogen. However, it should be taken into account that the sugar composition varies in these findings and that glucose is the predominant sugar in this study whereas xylose is the major sugar in some others. The experiments with *S. cerevisiae* UFPEDA 1238 using steam-exploded enzyme-treated cane bagasse showed a high yield and volumetric productivity in short fermentation time (Wanderley et al. 2013). However, the authors used yeast extract to supplement the hydrolysate. On the other hand, Da Cunha Pereira et al. (2011) obtained the remarkable yield of 0.45 g/g by fermenting non-supplemented rice hull hydrolysate in co-culture of with *S. arborariae* HM 19.1A and *S. cerevisiae* ICV D254. Thus, *S. passalidarum* UFMG-CM-Y473 presented a good potential for industrial application in terms of second-generation ethanol without nutritional enrichment of the hydrolysates.

In 2016, the use of biomass already accounted for 8.83% of the electric power matrix of Brazil, and a key factor in the use of bagasse was its capacity to produce electricity that is used by the industry itself, with the surplus being sold to the network (<http://www.unica.com.br>). In the harvest season of 2016/2017, it is estimated that 654.54 millions of tons of sugarcane were produced. It is also calculated that there was a production of 280 kg of dry bagasse per ton of harvested sugarcane. Given the fact that half of this biomass could be used for second-generation ethanol by means of the platform proposed in this work (alkaline peroxide pretreatment + enzymatic saccharification + *S. passalidarum* UFMG-CM-Y473 mediated fermentation), this might correspond to 53 L of ethanol per ton of sugarcane. It represents an input of 3180 L/ha of ethanol in areas such as the Northeast Brazil with a sugarcane production of 60 ton/ha. This estimate is quite close to what has been reported for sweet sorghum biomass (Zhao et al. 2009).

Conclusion

It can be concluded that the platform set out in this work showed the feasibility of producing ethanol from sugarcane bagasse. The *S. passalidarum* UFMG-CM-Y473 yeast was able to assimilate the different carbon resources (glucose, xylose and cellobiose) released during the delignification and enzymatic saccharification of pre-treated sugarcane bagasse and convert it to ethanol. The additional advantage of the organism used in this study is that the fermentation

Table 5 Comparative analysis of the efficiency in the conversion of cellulosic biomass to ethanol

Substrate	Treatment	Yeast	Sugar consumed (%)	Ethanol (g/L)	$Y_{s/p}$ (g/g)	Q_p (g/L h)	Medium supplement ^a	Time (h)	References
CB	SE + EH	<i>S. cerevisiae</i> UFPEDA 1238	100	23.38	0.39	0.97	YE + salts	24	Wanderley et al. (2013)
CB	DA	<i>Scheffersomyces stipitis</i> UFMG-IMH-43.2	100	6.4	0.19	0.13	YE	48	Ferreira et al. (2011)
CB	NaOH + AQ + EH	<i>Sc. stipitis</i> NRRL Y-7124	100	17.2	0.29	0.36	YE + urea	15	Nakanishi et al. (2017)
CB	OA + EH	<i>Sc. shehatae</i> UFMG-HM 52.2	100	4.83	0.28	0.20	YE + ME + AS	24	Chandel et al. (2014)
CB	DA	<i>Spathaspora passalidarum</i> UFMG-HMD-1.1	85	8.8	0.20	0.09	YE + PEP	96	Cadete et al. (2012)
CB	DA	<i>Sp. passalidarum</i> UFMG-HMD-14.1	91	9.5	0.18	0.10	YE + PEP	96	Cadete et al. (2012)
CB	NaOH + AQ + EH	<i>Sp. passalidarum</i> NRRL Y-27907	100	23.3	0.46	0.81	YE + urea	15	Nakanishi et al. (2017)
RH	DA	<i>Sp. arborariae</i> HM 19.1			0.45				
CB	AP + EH	<i>Sp. passalidarum</i> UFMG-HMD-14.1	78	24.14	0.32	0.34	None	72	This work
MMR	None	<i>Sp. passalidarum</i> UFMG-HMD-14.1	79	24.8	0.36	0.34	None	72	This work

$Y_{p/s}$ ethanol yield, Q_p ethanol-specific productivity. Substrate: CB cane bagasse, MMR mineral medium of reference. Treatments: DA diluted sulphuric acid, SE steam explosion, OA oxalic acid, AP alkaline peroxide, EH enzyme hydrolysis

^aSupplementation of the hydrolysate with yeast extract (YE), peptone (PEP), salts ((NH₄)₂SO₄ + KH₂PO₄ + MgSO₄), malt extract (ME) and ammonium sulphate (AS)

process does not require nutritional enrichment of the hydrolysates, unlike in most fermentation. This is important because commodities such as ethanol must be produced at a very low cost. The estimates accounted for a considerable increase in ethanol production if only half of the bagasse produced after sugarcane processing is used without the need for nitrogen supplementation, which reduces production costs. In general, the data offer promise for future scale-up of this process.

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Compliance with ethical standards

Ethical approval This article does not contain any studies with human participants or animals performed by the author.

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References

- Abernathy DG, Spedding G, Starcher B (2009) Analysis of protein and total usable nitrogen in beer and wine using a micro-well ninhydrin assay. *J Inst Brew* 115:122–127. <https://doi.org/10.1002/j.2050-0416.2009.tb00356.x>
- Banerjee G, Car S, Scott-Craig JS, Hodge DB, Walton JD (2011) Alkaline peroxide pretreatment of corn stover: effects of biomass, peroxide, and enzyme loading and composition on yields of glucose and xylose. *Biotechnol Biofuels* 4:16. <https://doi.org/10.1186/1754-6834-4-16>
- Cadete RM, Melo KJ, Rodrigues RCLB, Silva SS, Zilli JE, Vital MJS, Gomes FCO, Lachance MA, Rosa CA (2012) Diversity and physiological characterization of D-xylose-fermenting yeasts isolated from the Brazilian Amazonian forest. *PLoS One* 7: 1–11. <https://doi.org/10.1371/journal.pone.0043135>
- Cadete RM, Melo MA, Zilli JE, Vital MJ, Mouro A, Prompt AH, Gomes FC, Stambuk BU, Lachance MA, Rosa CA (2013) *Spathaspora brasiliensis* sp. nov., *Spathaspora suhii* sp. nov., *Spathaspora roraimanensis* sp. nov. and *Spathaspora xylofermentans* sp. nov., four novel (D)-xylose-fermenting yeast species from Brazilian Amazonian forest. *Antonie Van Leeuwenhoek* 103:421–431. <https://doi.org/10.1007/s10482-012-9822-z>
- Canilha L, Chandel AK, Dos Santos-Milessi TS, Antunes FAF, Wagner-Freitas LC, Felipe MGA, Silva SS (2012) Bioconversion of sugarcane biomass into ethanol: an overview about composition, pretreatment methods, detoxification of hydrolysates, enzymatic saccharification, and ethanol fermentation. *J Biomed Biotechnol* 2012:15. <https://doi.org/10.1155/2012/989572>
- Chandel AK, Antunes FA, Anjos V, Bell MJ, Rodrigues LN, Polikarpov I, de Azevedo ER, Bernardinelli OD, Rosa CA, Pagnocca FC, da Silva SS (2014) Multi-scale structural and chemical analysis of sugarcane bagasse in the process of sequential acid–base pretreatment and ethanol production by *Scheffersomyces shehatae* and *Saccharomyces cerevisiae*. *Biotechnol Biofuels* 7:63. <https://doi.org/10.1186/1754-6834-7-63>
- Conab—companhia Nacional de Abastecimento (2016) A companhia de safra brasileira: cana-de-açúcar, terceiro levantamento, dezembro/2016. Companhia Nacional de Abastecimento, Brasília, vol 3, no 3, p 1–74
- Correia JAC, Marques Júnior JE, Gonçalves LRB, Rocha MVP (2013) Alkaline hydrogen peroxide pretreatment of cashew apple bagasse for ethanol production: study of parameters. *Bioresource Technol* 139:249–256. <https://doi.org/10.1016/j.biortech.2013.03.153>
- Da Cunha Pereira F, Hickert LR, Sehnem NT, De Souza Cruz PB, Rosa CA, Ayub MAZ (2011) Conversion of sugars present in rice hull hydrolysates into ethanol by *Spathaspora arborariae*, *Saccharomyces cerevisiae*, and their co-fermentations. *Bioresource Technol* 102:4218–4225. <https://doi.org/10.1016/j.biortech.2010.12.060>
- De Souza RB, de Menezes JAS, de Souza RFR, Dutra ED, de Morais Jr MA (2015) Mineral composition of the sugarcane juice and its influence on the ethanol fermentation. *Appl Biochem Biotechnol* 175:209–222. <https://doi.org/10.1007/s12010-014-1258-7>
- Ferreira AD, Mussato SI, Cadete RM, Rosa CA, Silva SS (2011) Ethanol production by a new pentose-fermenting yeast strain, *Scheffersomyces stipitis* UFMG-IMH 43.2, isolated from the Brazilian forest. *Yeast* 28:547–554. <https://doi.org/10.1002/yea.1858>
- Ghose TK (1987) Measurement of cellulose activities. *Pure Appl Chem* 59:257–268
- Girio FM, Fonseca C, Carvalheiro F, Duarte LC, Marques S, Bogel-Lukasik R (2010) Hemicelluloses for fuel ethanol: a review. *Bioresource Technol* 101:4775–4800. <https://doi.org/10.1016/j.biortech.2010.01.088>
- Hou X, Yau S (2011) Improved inhibitor tolerance in xylose-fermenting yeast *Spathaspora passalidarum* by mutagenesis and protoplast fusion. *Appl Microbiol Biotechnol* 93:2591–2601. <https://doi.org/10.1007/s00253-011-3693-5>
- Kwak S, Jin YS (2017) Production of fuels and chemicals from xylose by engineered *Saccharomyces cerevisiae*: a review and perspective. *Microb Cell Fact* 16:82. <https://doi.org/10.1186/s12934-017-0694-9>
- Long TM, Su Y, Headman J, Higbee A, Willis LB, Jeffries TW (2012) Cofermentation of glucose, xylose, and cellobiose by the Beetle associated Yeast *Spathaspora passalidarum*. *Appl Environ Microbiol* 78:5492–5500. <https://doi.org/10.1128/AEM.00374-12>
- Martín C, Rocha GJM, Santos JRA, Wanderley MCA, Gouveia ER (2012) Enzyme loading dependence of cellulose hydrolysis of sugarcane bagasse. *Quím Nova* 35:1927–1930
- Mosier N, Wyman C, Dale B, Elander R, Lee YY, Holtzaple M, Ladisch M (2004) Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresource Technol* 96:673–686. <https://doi.org/10.1016/j.biortech.2004.06.025>
- Nakanishi SC, Soares LB, Biazzi LE, Nascimento VM, Costa AC, Rocha GJM, Ienczak JL (2017) Fermentation strategy for second generation ethanol production from sugarcane bagasse hydrolyzate by *Spathaspora passalidarum* and *Scheffersomyces stipitis*. *Biotechnol Bioeng* 114:2211–2221. <https://doi.org/10.1002/bit.26357>. (Epub 2017 Jul 6)
- Nguyen NH, Suh SO, Marshall CJ, Blackwell M (2006) Morphological and ecological similarities: wood-boring beetles associated with novel xylose-fermenting yeasts, *Spathaspora passalidarum* gen. sp. nov. and *Candida jeffriesii* sp. nov. *Mycol Res* 110:1232–1241. <https://doi.org/10.1016/j.mycres.2006.07.002>
- Rabelo SC, Maciel-Filho R, Costa AC (2008) A comparison between lime and alkaline hydrogen peroxide pretreatments of sugarcane bagasse for ethanol production. *Appl Biochem Biotechnol* 144:87–100. <https://doi.org/10.1007/s12010-008-8200-9>
- Reis ALS, Damilano ED, Menezes RSC, Morais MA Jr (2016) Second-generation ethanol from sugarcane and sweet sorghum bagasses using the yeast *Dekkera bruxellensis*. *Ind Crop Prod* 92:255–262. <https://doi.org/10.1016/j.indcrop.2016.08.007>
- Saha BC, Cotta MA (2007) Enzymatic saccharification and fermentation of alkaline peroxide pretreated rice hulls to ethanol. *Enzyme Microb Technol* 41:528–532. <https://doi.org/10.1016/j.enzmictec.2007.04.006>
- Soccol CR, Vandemberg LPS, Medeiros ABP, Karp SG, Buckeridge M, Ramos LP, Pitarello AP, Ferreira-Leitão V, Gottschalk LMF, Ferrara MA, Bom EPS, Moraes LMP, Araújo JA, Torres FAG (2010) Bioethanol from lignocelluloses: status and perspectives in Brazil. *Bioresource Technol* 101:4820–4825. <https://doi.org/10.1016/j.biortech.2009.11.067>
- Stambuk BU, Eleutherio EA, Florez-Pardo LM, Souto-Maior A, Bon ES (2008) Brazilian potential for biomass ethanol: challenge of using hexose and pentose cofermenting yeast strains. *J Sci Ind Res* 67:918–926
- Sun Y, Cheng J (2002) Hydrolysis of lignocellulosic bagasses for ethanol production: a review. *Bioresource Technol* 83:1–11. [https://doi.org/10.1016/S0960-8524\(01\)00212-7](https://doi.org/10.1016/S0960-8524(01)00212-7)
- Van Soest PJ (1963) Use of detergents in the analysis of fibrous foods. II. A rapid method for the determination of fibre and lignin. *J Assoc Off Anal Chem* 46:829–835
- Wanderley MCA, Martín C, De Moraes-Rocha GJ, Gouveia ER (2013) Increase in ethanol production from sugarcane bagasse based on combined pretreatments and fed-batch enzymatic hydrolysis. *Bioresource Technol* 128:448–453. <https://doi.org/10.1016/j.biortech.2012.10.131>
- Weiss ND, Nagle NJ, Tucker MP, Elander RT (2009) High xylose yields from dilute acid pretreatment of corn stover under process-relevant conditions. *Appl Biochem Biotechnol* 155:418–428. <https://doi.org/10.1007/s12010-008-8490-y>

- Wood TM, Bhat KM (1987) Methods for measuring cellulase activities. In: Zhao WA, Kellog ST (eds) *Methods in enzymology*, vol 160. Academic Press, San Diego, pp 87–116
- Zhao YL, Dolat A, Steinberger Y, Wang X, Osman A, Xie GH (2009) Biomass yield and changes in chemical composition of sweet sorghum cultivars grown for biofuel. *Fields Crops Res* 111:55–64. <https://doi.org/10.1016/j.fcr.2008.10.006>
- Zhao C, Ding W, Feng-Chen F, Cheng C, Shao Q (2014) Effects of compositional changes of AFEX-treated and H-AFEX-treated corn stover on enzymatic digestibility. *Bioresource Technol* 155:34–40. <https://doi.org/10.1016/j.biortech.2013.12.091>