Bioresource Technology 146 (2013) 261–266

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Ethanol production from macaúba (*Acrocomia aculeata*) presscake hemicellulosic hydrolysate by *Candida boidinii* UFMG14

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HIGHLIGHTS

- We isolated *Candida boidinii*UFMG14 from macaúba fruits.
- We obtained for the first time a hemicellulosic hydrolysate of macaúba presscake.
- *C. boidinii* UFMG14 was able to growth and ferment on acid hemicellulosic hydrolysate.
- *C. boidinii* UFMG14 reached 0.4 g/g ethanol yield and 0.3 g/Lh volumetric productivity.
- The hydrolysate supplementation affects ethanol yield but not ethanol productivity.

ARTICLE INFO

Article history:
Received 25 April 2013
Received in revised form 15 July 2013
Accepted 17 July 2013
Available online 24 July 2013

Keywords:
Candida boidinii
Ethanol fermentation
Hemicellulosic hydrolysate
Macaúba
Presscake

ABSTRACT

Yeasts capable of growth on xylose were isolated from macaúba (*Acrocomia aculeata*) fruit, a Brazilian palm tree with great potential for use as biodiesel feedstock production. *Candida boidinii* UFMG14 strain achieved the highest ethanol production (5 g/L) and was chosen to ferment macaúba presscake hemicellulosic hydrolysate (MPHH). The MPHH was produced by the first time in this work and the resultant five-fold concentrate showed considerable sugar content (52.3 and 34.2 g/L xylose and glucose, respectively) and low furfural (0.01 g/L) and hydroxymethylfurfural (0.15 g/L) concentrations. *C. boidinii* UFMG14 fermentation was evaluated in supplemented and non-supplemented MPHH containing either 10 or 25 g/L of xylose. The maximum ethanol production (12 g/L) was observed after 48 h of fermentation. The ethanol yield was significantly affected by supplementation and concentration of MPHH while ethanol productivity was affected only by MPHH concentration. This is the first study demonstrating the*C. boidinii* potential for ethanol production from hemicellulose byproducts.

1. Introduction

Biofuels are a sustainable alternative to the global environmental issue. In Brazil, the contribution of renewables in the national energy matrix remained among the highest in the world (44.1%) in 2011. Of these renewable sources, 29.6% of the energy comes from biomass and 13.5% derives from sugar cane. The fraction of the renewable energy used in Brazilian transporting today is 17%, figuring ethanol in approximately 15% of fuel consumption (Ministério das Minas e Energia, 2012). Moreover, between 2005 and 2011, there was a significant increase in the volume of biodiesel produced in the country, from 736m^3 in 2005 to 2.39 million m^3 in 2010, making Brazil the second largest producer of biodiesel in the world (Padula et al., 2012). While these indices put Brazil at the vanguard of alternative fuels use on the planet, they also reinforce the need to find commercial uses for lignocellulosic byproducts of first generation biofuels production.

Brazilian technology of ethanol production from sugarcane is one of the most efficient and well-established of the world and the use of lower cost substrates, such as lignocellulosic byproducts, could result in a more competitive renewable fuel. The second generation ethanol produced from cellulosic and hemicellulosic biomass represents an attractive sustainable energy source that can greatly contribute to the implementation of a clean and environmentally safe energy matrix.

In this context, the palm presscake generated on biodiesel production process may be a new feedstock for second generation ethanol production. The fruits of plants used on biodiesel production contain a mixture of triacylglycerols and comprise an expressive

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http://dx.doi.org/10.1016/j.biortech.2013.07.075

portion of the vast and diverse Brazilian flora. Among the native Brazilian palms, macaúba (*Acrocomia aculeata*) stands out due to its potential for high yields of biodiesel and its occurrence in various regions of Brazil (Tickell, 2000). The technological route for using these raw materials to ethanol production involves steps of pretreatment, saccharification and fermentation, and technical limitations are found in all of them (Hahn-Hägerdal et al., 2007).

One of the challenges for the efficient production of ethanol from lignocellulosic byproducts is the use of microorganisms capable of fermenting pentose to ethanol (Larsson et al., 2000) and capable of growing and fermenting on hemicellulosic hydrolysates containing toxic compounds like furfural and hydroxymethylfurfural (Palmqvist and Hahn-Hägerdal, 2000). *Candida boidinii* is methylotrophic yeast capable of growing on xylose and producing xylitol and ethanol (Vongsuvanlert and Tani, 1989; Vandeska et al., 1995; Kurtzman et al., 2011). However, this yeast has been neglected in the literature regarding their potential use for lignocellulosic residues fermentation to ethanol. Furthermore, *C. boidinii* has been regarded as an expression system suitable for the production of heterologous proteins (Porro et al., 2011).

In this work, we intend to connect and integrate the two most promising Brazilian renewable energy matrices: biodiesel and ethanol. To this aim, yeasts strains were isolated from the macaúba fruit and evaluated for ethanol production in synthetic medium containing xylose. A promising *C. boidinii* strain was selected and used for fermentation of MPHH, a byproduct of biodiesel production plant, obtained by the first time in this work.

2. Methods

2.1. Isolation of xylose assimilating yeasts from samples of the macaúba (A. aculeata) fruit

Fruits were collected in the Serra do Cipó National Park, located 60 miles northeast of Belo Horizonte city, Minas Gerais, in the southern area of Serra do Espinhaço, the parallels between 19–20 S and 43–44 W. Fruits were collected from five palm trees, randomly selected in the region, and stored in plastic bags and processed in a maximum of 24 h after collection. Ten fruits were selected, two from each palm. Each fruit was rinsed with tap water and sterilized by sequential immersion in the following solution: 70% (v/v) ethanol for 1 min, 2% sodium hypochlorite for 5 min; 70% (v/v) ethanol for 30 s. Subsequently, each fruit was rinsed with sterile distilled water and placed in a sterile plastic bag. The epicarp of fruits was broken, providing access to the mesocarp and each broken fruit was placed in a 125 mL Erlenmeyer flask containing 50 mL Yeast Nitrogen Base (YNB, Himedia), 50 g/L xylose and 200 mg/mL chloramphenicol. The flasks were incubated at 25 °C and 150 rpm for a period of seven days. Every 24 h, 1 mL aliquots of the cell suspension from flasks with microbial growth were serially diluted and inoculated by spreading onto plates containing YNB (Himedia) 20 g/L agar supplemented with 50 g/L xylose for obtaining isolated colonies. The plates were incubated at 25 °C for a period of up to five days and the different yeasts morphotypes observed were counted (CFU/g) and purified in new Petri dishes containing the same medium.

Yeasts cells were maintained on Sabouraud agar (10 g/L peptone, 40 g/L dextrose and 20 g/L agar) and maintained in YPD broth (20 g/L glucose, 10 g/L peptone, 10 g/L yeast extract) with 20% (v/v) glycerol at −80 °C.

2.2. Molecular identification of isolated yeasts with xylose assimilation ability

Yeast morphotypes were identified by partial sequence analysis of the 26S rDNA encoding gene. For genomic DNA extraction, yeast colonies from pure culture were grown on YNB agar (YNB, Himedia) containing 50 g/L xylose at 30 °C for 48 h and transferred to 1.5 mL sterile conical tubes (Eppendorf). The DNA extraction was carried out according Brandão et al. (2011) and the resulting DNA was dried overnight at room temperature, suspended in 100 μl TE-buffer (10 mM Tris, 10 mM Na–EDTA, pH 8.0) and stored at −20 °C until processing.

Amplification of D1/D2 domain of the 26S rDNA was performed by PCR using primers NL-1 (5′-GCATATCAATAAGCGGAGGAAAAG-3′) and NL-4 (5′-GGTCCGTGTTTCAAGACGG-3′) according Lachance et al. (1999). The resulting PCR products were purified using 10% (v/v) polyethylene glycol 8000 and 1.25 M NaCl, quantified, and sequenced by Macrogen Inc. (Seoul, Republic of Korea). Yeast strains were identified by databases searching using the BLAST sequence analysis tool (http://.ncbi.nlm.nih.gov/BLAST/).

2.3. Obtainment of macaúba (A. aculeata) presscake hemicellulosic hydrolysate

Oilseed presscake was collected from an experimental biodiesel production laboratory at Universidade Federal de Viçosa, Viçosa, MG. For hydrolysis, the presscake was first crushed and placed in 500 mL Erlenmeyer flasks containing 0.8% sulfuric acid (v/v) as the solid: liquid ratio of 1:10. The hydrolysis was conducted at 121 °C for 60 min. The resulting hydrolysate was cooled, filtered on cotton bags and adjusted to pH 5.5 with sodium hydroxide. Afterwards it was centrifuged at 10,000*g* for 15 min, lyophilized and suspended in distilled water to a fivefold concentration. Then the hydrolysate was adjusted to pH 7.0 with CaO and then adjusted to pH 5.5 with H_3PO_4. The detoxification was done by adding activated charcoal for a final concentration of 2.5% (w/v) and incubated for 3 h at 30 °C and 200 rpm. After further centrifugation at 10,000*g* for 15 min the hydrolysate was sterilized by autoclaving at 121 °C for 30 min and analyzed for concentration of sugars and acids by HPLC. All experiments were performed in triplicate.

2.4. Fermentation conditions

To evaluate the ability to ferment xylose into ethanol, the isolated strains were pre-cultured in YPD broth (glucose 20 g/L, peptone 10 g/L, yeast extract 10 g/L) at 150 rpm and 30 °C. After 24 h, cultures were centrifuged at 10,000*g*, washed with sterile distilled water and part of the obtained cells transferred to fermentation flasks to contain 0.4 g/L biomass. After inoculation, 150 mL Erlenmeyer flasks containing 50 mL supplemented culture medium (xylose 90 g/L, yeast extract 5.0 g/L, MgSO47H2O 1.0 g/L and urea 2.3 g/L) were incubated at 30 °C and stirred using a rotary shaker at 150 rpm until the maximum time of 96 h.

The fermentation assays using MPHH was carried out in 250 mL Erlenmeyer flasks with 100 mL hydrolysate containing 10 or 25 g/L xylose, supplemented or not with 10 g/L yeast extract, 1.5 g/L $MgSO_47H_2O$ and 2.5 g/L urea. Preliminary results (not shown) of our laboratory have indicated that the use of higher hydrolysate concentrations resulted in inhibitory effect on fermentation. Aliquots were taken every 12 h of culture, centrifuged and filtered (0.2 μm) for subsequent ethanol, sugars and acids concentration evaluation. All experiments were performed in triplicate.

2.5. Analytical procedures and determination of fermentation parameters

Ethanol, sugars, acids, furfural and hydroxymethylfurfural (HMF) concentrations were determined using a High Performance Liquid Chromatography (HPLC) Prominence Model LC-20A (Shimadzu). For ethanol sugars and acids was used a SUPELCOGEL C-610H 30 cm × 7.8 mm column (Sigma–Aldrich) and a RID-10A refractive

index detector. The separation occurred at 65 °C and the 5 mM H_2SO_4 mobile phase at a flow rate of 0.6 mL/min, with the detector cell temperature of 45 °C. For furfural and HMF a C18 (4.6 × 200 mm) column (Sigma–Aldrich) and a UV detector (at 276 nm) were used. The separation was performed at 25 °C using acetonitrile:water (1:8 with acetic acid) as mobile fase at 0.9 mL/min flow rate.

The biomass concentration was determined by obtaining a linear regression of the absorbance plot (600 nm) versus dry mass (g/L). Culture samples from the exponential growth phase (10 mL) were centrifuged at 3000g for 5 min and the cell pellet was resuspended in 6 mL of deionizer sterile water, transferred to a weighed conical tube and dried at 105 °C until constant weight. One absorbance at 600 nm unit was found to be equivalent to 1.44 g/L of the dry mass of *C. boidinii* UFMG14.

The fermentation parameters ethanol yield, $Y_{P/S}$ (g/g); volumetric productivity, Q_P (g/L.h) and total sugars consumption, Y (%) were calculated according to Eqs. (1)–(3), respectively:

$$Y_{P/S} = \frac{\Delta P}{\Delta S} \quad (1)$$

where $\Delta P = (P_{ethanol} - P_i)$, $P_{ethanol}$ is the maximum ethanol production (g/L) at the corresponding cultivation time, t_f; P_i is the ethanol production (g/L) at the initial of fermentation time, t_i; $\Delta S = (S_i - S_f)$, S_f is the total sugar content (g/L) at cultivation time t_f; S_i is the initial concentration of total sugars (g/L) at fermentation time t_i.

$$Q_P = \frac{\Delta P}{\Delta t} \quad (2)$$

where $\Delta t = (t_f - t_i)$, t_f is the time (h) corresponding to the fermentation time at which the parameter has been calculated, and t_i is the time (h) at the initial fermentation time.

$$Y\ (\%) = \frac{\Delta S}{S_i}\ x\ 100. \quad (3)$$

2.6. Statistical analysis

Tukey's test was performed to compare ethanol production (g/L) means from three repetitions of isolated yeast strains cultivation.

To study the *C. boidinii* UFMG14 fermentation on MPHH, each treatment combination of supplementation and non-supplementation with 10 or 25 g/L xylose concentration on MPHH was performed three times and fermentation parameters were calculated as described. The effects of supplementation and concentration of MPHH at fermentation parameters was accessed by ANOVA (Analysis of Variance) followed by a *t*-test for the adjusted effects of each factor. The analysis and graphing were performed with MINITAB15 using a cutoff of 95% confidence ($\alpha = 0.05$).

3. Results and discussion

3.1. Isolation and identification of xylose assimilating yeasts from samples of the macaúba (A. aculeata) fruit

The yeast isolation from macaúba fruits and the following selection on medium containing xylose as sole carbon source strategy allows the identification of twelve different morphotypes which were submitted to genetic identification. Sequences corresponding to PCR products of 26S rDNA D1/D2 region of the yeast morphotypes were evaluated using the BLAST tool: Basic Local Alignment Search Tool, available at gateway NCBI (http://www.ncbi.nlm.nih.gov/blast/), for comparison with the sequences deposited in GenBank. NCBI accession numbers were considered, as well as the authors of the deposit sequence of type strains, listed in Kurtzman et al. (2011). All obtained sequences showed similarity greater than 99% with the yeast sequences previously deposited in GenBank. Table 1 shows the relationship between different morphotypes identified from the species and the corresponding type strain NCBI accession number. Five yeast species were identified, all of them belonging to the phylum Ascomycota, and were previous described as capable of growth on xylose, ferment glucose but not xylose (Kurtzman et al., 2011). To confirm this information, the twelve isolates were evaluated for ethanol production in synthetic medium containing xylose.

3.2. Fermentation of xylose to ethanol in synthetic medium by isolated yeasts

The fermenting xylose to ethanol ability of the isolated yeast strains in this study was assessed by a period of 72 h and the fermentation parameters obtained are shown in Table 2. Among the isolated yeasts, *C. boidinii* UFMG14 exhibited the highest xylose consumption rate (Y %) and ethanol production ($P_{ethanol}$) (p-value <0.05) and was selected for further experiments.

Yeast strains capable of xylose fermentation typically use a redox reaction in two steps, involving reductases and dehydrogenases that catalyze the metabolism of pentoses to xylulose-5P. Reductases use preferentially NADPH as cofactor, whereas dehydrogenases prefer NADH. As these enzymes have different cofactors, redox imbalance occurs and can prevents the anaerobic use of pentoses, which can results in the production of xylitol instead of ethanol (Tai and Stephanopoulos, 2012).

Vongsuvanlert and Tami (1989) first described xylitol production by *C. boidinii*, and Vandeska et al. (1995), in turn, first reported the production of ethanol from xylose by the same yeast. Since then, the facultative methylotrophic yeast *C. boidinii* has been studied as an alternative system for heterologous gene expression, using strong and inducible promoters derived from methanol metabolism pathway genes (Gellissen, 2000; Yurimoto and Sakai, 2009), however has been neglected for the production of ethanol from xylose rich feedstock.

Such information associated with the results of the present work suggest a positive outlook towards fermentation of hemicellulosic hydrolysates by *C. boidinii* UFMG14 and open a promising investigation perspective on ethanol production optimization from xylose by this strain.

3.3. Composition of macaúba (A. aculeata) presscake hemicellulosic hydrolysate

In this work a hemicellulosic hydrolysate of macaúba presscake was obtained by acid hydrolysis and the hydrolysate was detoxified for comparison with the non-detoxified hydrolysate. The chemical composition of fivefold concentrated detoxified and non-detoxified MPHH is shown in Table 3. The predominant sugars were xylose (>50%) and glucose, not being detected the presence of arabinose. Results indicate that the detoxification process using activated charcoal was not effective in reducing the concentrations of furfural and acetic acid in the MPHH, while the same process was able to reduce the 5-hydroxymethyl furfural (HMF) concentration by half. Both treatments resulted in a hydrolysate containing acetic acid, furfural and HMF, which have been demonstrated to show a negative effect on growth of yeast and ethanol fermentation (Palmqvist and Hahn-Hägerdal, 2000). At high temperature and pressure, the molecule of xylose is converted into furfural and, similarly, hexoses are converted to HMF. During fermentation, the reduction of furfural to furfuryl alcohol is achieved at high rates (Diaz de Villegas et al., 1992), resulting in inhibition of aerobic yeasts growth (Weigert et al., 1988). These compounds reduce the biological enzymatic activities, cause DNA and cell wall

Table 1
Identification of yeasts isolated from macaúba fruits. Relationship of the different morphotypes isolates identified in relation to the species and the corresponding type strain NCBI accession number.

Species	Isolated strain	Type strain NCBI access number
Meyerozyma guilliermondii	UFMG1, UFMG2, UFMG4, UFMG10, UFMG12, UFMG15	JQ689047
Wickerhamomyces anomalus	UFMG6	EF550341
Meyerozyma caribbica	UFMG7, UFMG8	EU348786
Zygoascus hellenicus	UFMG11, UFMG13	AJ508566
Candida boidinii	UFMG14	U70242

Table 2
Fermentation parameters for xylose to ethanol fermentation in synthetic medium after 72 h by isolated yeasts.

Strain	$P_{ethanol}$ (g/L)	$Y_{P/S}$ (g/g)	Q_P (g/L.h)	Y (%)
Meyerozyma guilliermondii UFMG1	–	–	–	47.45 ± 2.38
Meyerozyma guilliermondii UFMG2	–	–	–	51.20 ± 2.64
Meyerozyma guilliermondii UFMG4	–	–	–	47.63 ± 0.77
Wickerhamomyces anomalus UFMG6	–	–	–	13.39 ± 0.53
Meyerozyma caribbica UFMG7	–	–	–	53.34 ± 4.57
Meyerozyma caribbica UFMG8	–	–	–	54.54 ± 1.42
Meyerozyma guilliermondii UFMG10	–	–	–	46.30 ± 2.01
Zygoascus hellenicus UFMG11	0.42 ± 0.12	0.01 ± 0.00	0.01 ± 0.00	37.17 ± 1.39
Meyerozyma guilliermondii UFMG12	–	–	–	49.15 ± 0.70
Zygoascus hellenicus UFMG13	0.40 ± 0.05	0.01 ± 0.00	0.01 ± 0.00	39.89 ± 0.59
Candida boidinii UFMG14	5.36 ± 1.03	0.11 ± 0.02	0.07 ± 0.01	54.67 ± 0.99

Table 3
Composition of fivefold concentrated detoxified and non-detoxified macaúba presscake hemicellulose hydrolysates.

Composition (g/L)	Detoxified hydrolysate (g/L)	Non-detoxified hydrolysate (g/L)
Xylose	50.5 ± 1.6	46.6 ± 4.3
Glucose	32.4 ± 1.0	30.1 ± 2.9
Acetic acid	3.1 ± 0.2	2.3 ± 0.3
Glucuronic acid	15.9 ± 0.5	14.6 ± 1.5
Glycerol	10.9 ± 0.3	9.7 ± 0.9
Furfural	0.01 ± 0.00	0.01 ± 0.00
HMF	0.15 ± 0.01	0.31 ± 0.02

damage and inhibit RNA and protein synthesis (Khan and Hadi, 1994; Modig et al., 2002; Liu et al., 2008). Lower concentrations of furfural to 1 g/L have been described as virtually harmless to microorganisms in fermentation processes. Additionally, acetic configured as toxic to the microorganism at concentrations above 3 g/L, whereas degradation products of lignin (phenolic compounds) are toxic to microorganisms even when their concentrations are low (Felipe et al., 1995; Parajó et al., 1998).

Although the results show the presence of fermentation inhibitory compounds, acetic acid, furfural and HMF, their concentrations were relatively small compared to those commonly observed for other hemicellulosic hydrolysates. Lin et al. (2012) using rice straw hydrolysates found furfural and acetic acid concentrations of 0.5 and 3.6 g/L, respectively and Hande et al. (2013) found a furfural and HMF concentrations of 0.03 and 0.43 g/L, respectively. The high concentrations of sugars and low concentrations of inhibitory compounds obtained in this work indicate a MPHH as a hydrolysate with great potential for use as a substrate for successful ethanol production.

3.4. Fermentation of macaúba presscake hemicellulosic hydrolysate to ethanol by C. boidinii UFMG14

C. boidinii UFMG14 was investigated for its ability to ferment the detoxified MPHH. The fermentation was evaluated in supplemented and non-supplemented medium containing either 10 or 25 g/L of xylose. Preliminary results (not shown) of our group have indicated that the use of higher hydrolysate concentrations resulted in inhibitory effect on fermentation. Sugar fermentation profile (Fig. 1) of *C. boidinii* UFMG14 on the four treatment combinations revealed that this strain is able to use both glucose and xylose to ethanol production on MPHH. All evaluated conditions show that ethanol production starts together to the simultaneous consumption of hexose and pentose. Interestingly, except on non-supplemented hydrolysate containing 10 g/L xylose (Fig. 1A), in all other analyzed conditions (Fig. 1B, C and D) ethanol production continues to increase even after the total consumption of glucose. This suggests that the ethanol produced is also due to xylose fermentation. The presence of glucose in small concentrations has been reported as a major factor for increased fermentation of xylose and consequent increase in ethanol production. This effect has been related to increased carbon flux through glycolysis and pentose phosphate pathway in these conditions (Hector et al., 2011; Kim et al., 2012).

Cell growth during fermentation of MPHH to ethanol by *C. boidinii* UFMG14 is show in Fig. 2. The growth (Fig. 2) and fermentation profile (Fig. 1) of *C. boidinii* UFMG14 on MPHH reveals that this yeast was able to growth and produce ethanol even in the presence of inhibitory compounds as furfural, HMF and acetic acid. These results reaffirm the potential of *C. boidinii* UFMG14 for ethanol production on hemicellulosic hydrolysates obtained by acid treatment and open perspectives to test this strain in hydrolysates derived of others biomass sources.

A comparison of the cellular dry weight in supplemented and non-supplemented detoxified MPHH suggests that cell growth is influenced by the hydrolysate supplementation. In fact, when using detoxified hydrolysate supplemented with yeast extract, $MgSO_4 7H_2O$ and urea and containing 25 g/L xylose was observed the largest ethanol production (12 g/L) after 48 h of cultivation. Previous studies have indicated that the simultaneous addition of these three nutritional sources to the fermentation medium showed to be important to improve the ethanol production in detriment of the substrate conversion to cell. These nutritional sources are commonly used to compose fermentation media for different microorganisms, and have been demonstrated to be of great importance for different yeast strains development (Ferreira et al., 2011; Silva et al., 2012).

Fermentation parameters for *C. boidinii* UFMG14 on supplemented and non-supplemented detoxified MPHH are present in Fig. 3. The significance of hydrolysate supplementation and concentration effects on fermentation parameters was accessed by analysis of variance, which showed that yield of sugar to ethanol conversion, $Y_{P/S}$, was significantly affected by supplementation and hydrolysate concentration (p-value = 0.012 and 0.019,

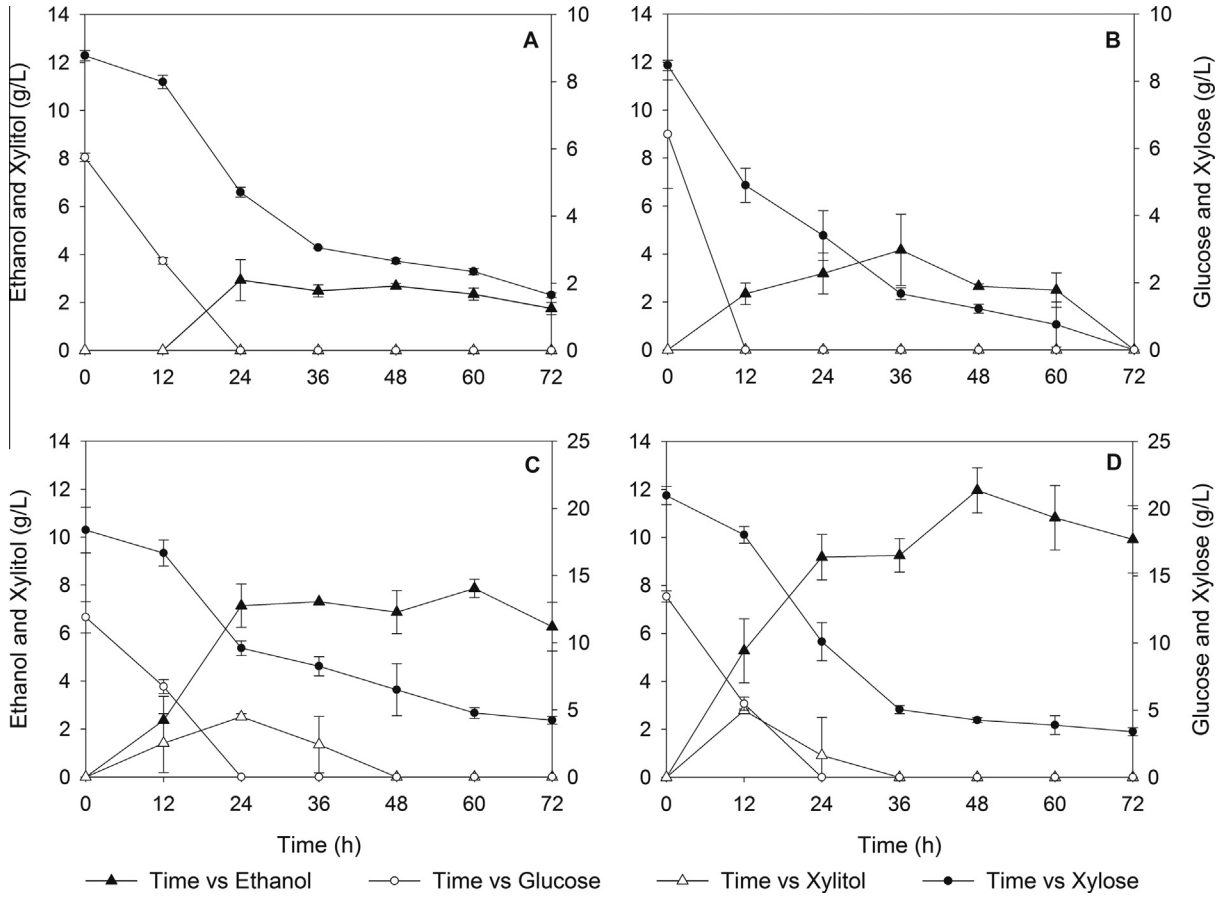

Fig. 1. Fermentation profile of macaúba presscake hemicellulosic hydrolysate to ethanol by *C. boidinii* UFMG14. (A) Detoxified non-supplemented hydrolysate containing 10 g/L xylose. (B) Detoxified supplemented hydrolysate containing 10 g/L xylose. (C) Detoxified non-supplemented hydrolysate containing 25 g/L xylose. (D) Detoxified supplemented hydrolysate containing 25 g/L xylose.

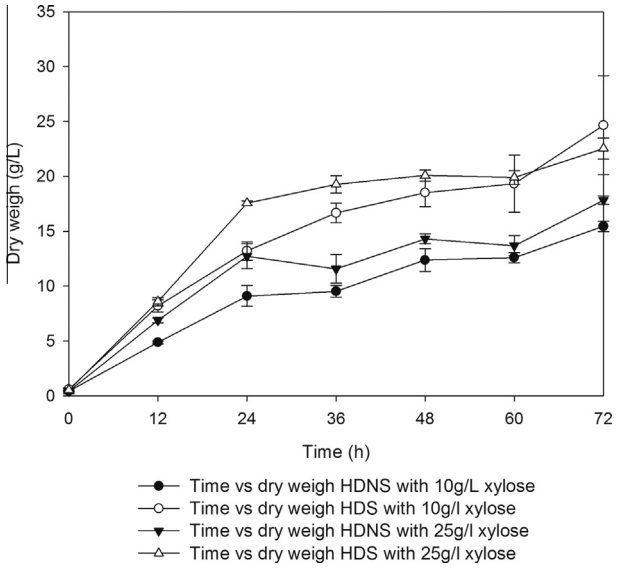

Fig. 2. Cell growth during fermentation of macaúba presscake hemicellulosic hydrolysate to ethanol by *C. boidinii* UFMG14. HDNS: Detoxified non-supplemented hydrolysate. HDS: Detoxified supplemented hydrolysate.

respectively). Both factors present positive effects on $Y_{P/S}$. The best $Y_{P/S}$ was 0.40 ± 0.02 g/g after 48 h of fermentation on supplemented detoxified hydrolysate with 25 g/L of xylose. On the other hand, only the hydrolysate concentration had influence on the volumetric productivity, Q_P, (p-value = 0.001), affecting it positively. Despite there were no significant differences between Q_P in hydrolysates supplemented and non-supplemented, the best volumetric productivity was 0.33 ± 0.04 g/L.h, after 24 h of fermentation on non-supplemented detoxified hydrolysate with 25 g/L of xylose. Once hydrolysate supplementation appears to stimulate cell growth (Fig. 2), it is possible that this is the main reason it also increases $Y_{P/S}$ providing a better substrate use. This does not occur with Q_P, which is not directly affected by cell growth.

These results indicate that MPHH supplementation could be suppressed for ethanol production by *Candida boidinii* UFMG14 and ethanol productivity would not be affected. Thus, the feedstock cost could be further reduced. Indeed, the most important economic factors for maintaining low distillation costs in the ethanol industry are ethanol volumetric productivity, ethanol yield and feedstock cost (von Sivers and Zacchi, 1996; Chovau et al., 2013).

Surprisingly, *C. boidinii* that is referred as a xylitol-producing yeast and neglected with respect to fermentation of xylose to ethanol reached values of $Y_{P/S}$ and Q_P similar or even superior to those observed in fermentations by *Sheffersomyces stipitis*, the best xylose to ethanol fermenting yeast described. *S. stipitis* NRRL Y-7124 growth on hemicellulosic hydrolysate sunflower seed hull presented $Y_{P/S}$ 0.32 g/g and Q_P 0.07 g/L.h (Telli-Okur and Eken-Saraçoglu, 2008), while another strain *S. stipitis* UFMG-IMH 43.2 was able to reach $Y_{P/S}$ 0.17 g/g and Q_P 0.13 g/L.h after culturing in sugarcane bagasse hemicellulosic hydrolysate (Ferreira et al., 2011). There are

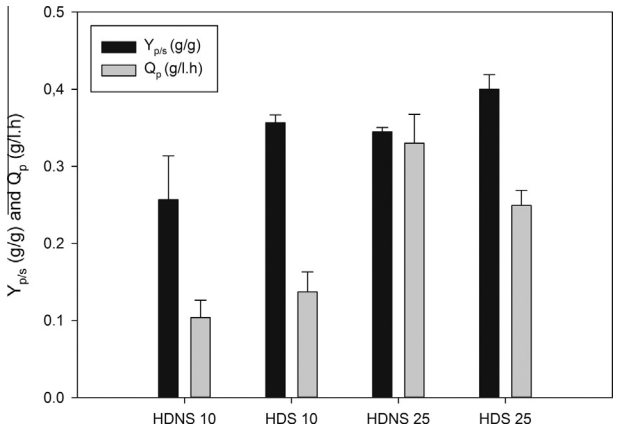

Fig. 3. Fermentation parameters of macaúba presscake hemicellulosic hydrolysate to ethanol by*C. boidinii* UFMG14. HDNS: HNSH 10: Detoxified non-supplemented hydrolysate containing 10 g/L xylose. HSH 10: Detoxified supplemented hydrolysate containing 10 g/L xylose. HNSH 25: Detoxified non-supplemented hydrolysate containing 25 g/L xylose. HSH 25: Detoxified supplemented hydrolysate containing 25 g/L xylose.

also reports of higher fermentation parameters, as $Y_{P/S}$ 0.44 g/g and Q_P 0.22 g/L.h after cultivation of *S. stipitis* in rice straw hemicellulosic hydrolysate (Lin et al. 2012) or $Y_{P/S}$ 0.45 g/g and Q_P 0.33 g/L.h obtained by *Pichia strain* BY2 cultured in sugarcane bagasse hemicellulosic hydrolysate (Hande et al., 2013).

The present study demonstrates the potential of the *C. boidinii* UFMG14 for ethanol production from xylose rich feedstock and suggests that further optimizing studies can improve the fermentation parameters on hemicellulosic hydrolysates by this yeast. Additionally, the available knowledge about *C. boidinii* genetic manipulation opens perspectives to genetic engineering of *C. boidinii* UFMG14 aiming their utilization for hemicellulosic hydrolysates fermentation.

4. Conclusion

Macaúba presscake is a byproduct of biodiesel production. Macaúba presscake hemicellulosic hydrolysate (MPHH) was obtained for the first time in this work and *C. boidinii* UFMG14, isolated from macaúba fruit, was able to produce ethanol on MPHH. This may represent the first step in integrating production technologies of ethanol and biodiesel, two important Brazil's renewable energy matrices. This is the first study demonstrating the potential of *C. boidinii* for ethanol production on a xylose rich feedstock. Optimization of fermentation by *C. boidinii* UFMG14 and genetic engineering of this yeast are promising strategies for improvement of ethanol production from hemicellulosic hydrolysates.

Acknowledgements

The authors would like to thank Dr. José Antonio Saraiva Grossi and MSc. Anderson Barbosa Evaristo for providing macaúba presscake. This research was supported by Brazilian agencies Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and by Petróleo Brasileiro S.A., PETROBRAS.

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