Orbital: The Electronic Journal of Chemistry
journal homepage: www.orbital.ufms.br
ISSN 1984-6428

| Vol 10 | | No. 1 | | Special Issue January 2018 |

Full Paper

Potential of Saccharine Substrates for Ethanol Production

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Article history: Received: 02 June 2017; revised: 19 September 2017; accepted: 26 September 2017. Available online: 21 January 2018. DOI: http://dx.doi.org/10.17807/orbital.v10i1.1013

Abstract: Brazil, which has a great territorial extension, with fertile soils and availability of water and, in this way, can produce a diversity of vegetal products with bioenergetics potential. Thus, this study aims to evaluate the technological characteristics of saccharine substrates of direct fermentation for ethanol production as well as to analyze the fermentative profile of FT-858 yeast in different culture conditions. For this, a bibliographic survey was carried out comparing the technological qualities of the analyzed biomasses. To evaluate the fermentative capacity, the saccharine substrates were used at concentrations of 18 and 32°Brix at 30 °C for 24 hours in test tubes containing Durhan tubes. For the analysis of the fermentation profile the yeasts were grown in 2% YPD medium and the biomass obtained was used in the saccharine substrates of direct fermentation for 50 hours. The saccharine substrates presented potential for ethanol production: sugar cane and saccharine sorghum. The yeast FT-858 presented fermentation capacity in the different substrates in the concentrations of Brix analyzed, presenting a better fermentative performance in sugarcane broth and the highest concentrations of ethanol occurring in the times of 10 and 15 hours of fermentation for both substrates.

Keywords: fermentation; *Saccharomyces cerevisiae*; fermentation parameters; biofuel

1. INTRODUCTION

The renewable biomass emerges in the global scenery as an alternative to non-renewable natural resources. Thus, the countries that have the capacity to produce large quantities of natural resources with energy potential are in more promising conditions [1], such as Brazil, which has large territorial extension with fertile soils and water availability, thus, producing a variety of plant products with bio-energetic potential [2].

One of these biomass is sugarcane (*Saccharum officinarum*) which has a major role in Brazilian energy matrix, since its production is intended both for production of sugar and for biofuels and electricity generation [3]. Another biomass that emerges as a bioenergetics crop is sorghum (*Sorghum bicolor* (L.) Moench) because it is a plant with characteristics similar to sugarcane and possesses potential for ethanol and also generate bioenergy [4, 5]

This biomass consists of a number of compounds with low and high molecular masses, with the main one being sucrose, on average 23%, a carbohydrate formed in the photosynthesis process consisting of two monosaccharides, D-glucose and D-fructose, which are joined by glycosidic bonds (Figure 1) [6]. This carbohydrate is also found in other matrices of vegetable origin as beet (*Beta vulgaris*), sorghum (*Sorghum bicolor* (L.) Moench) according to [7]. Sorghum, as well as sugar cane, has stems composed on average of 75 to 86% water, 14 to 25% fiber and sugar content ranging from 10 to 18%, corresponding to sucrose, glucose and fructose [8, 9].

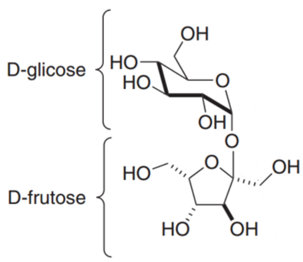

Figure 1. Structural formula of sucrose. Source: Adapted from [10].

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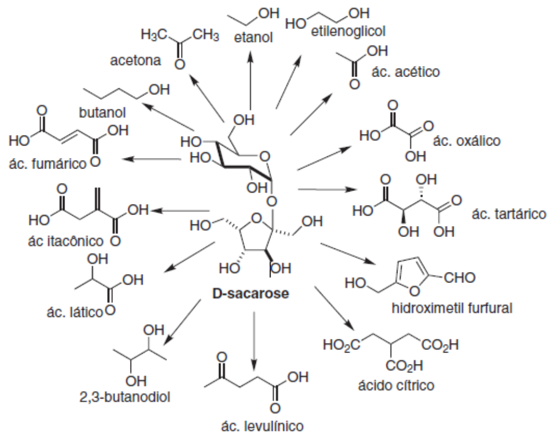

Figure 2. Sucrose and the formation of chemical molecules of low molecular weight. Source [10]

This economically attractive carbohydrate undergoes numerous structural changes within the industrial process, which enable the formation of new compounds that add value from the industrial point of view and great economic interest, and ethanol is the most representative in terms of quantity produced by fermentative process (Figure 2). This conversion process can be biological, chemical or physical [10].

The production of fuel ethanol from renewable sources is based on the biotechnological process that is performed by microorganisms, yeasts, which have the ability to metabolize sucrose in a process called fermentation [11]. This process is used both in Brazil and in United States for the production of this important biofuel, as long as the raw-material used in fermentation contains a fermentable sugar content, which is generally found in starch compounds (corn, rice, cassava and others), cellulosic (wood, agricultural residue) and sugar (sugarcane, beet, molasses and sorghum) [12,13].

In alcoholic fermentation occurs the process of converting sugar into ethanol and CO_2 by yeasts under conditions of anaerobiosis [13]. These microorganisms are unicellular, heterotrophic, facultative (aerobic and anaerobic metabolism) and reproduce by budding. The species used, due to the economic importance of biotechnological processes of industrial scale in Brazil, is the yeast *Saccharomyces cerevisiae*, in this sense, the most studied [15].

This biochemical process consists of the incomplete oxidation of sugar, sucrose, which undergoes hydrolysis promoted by the action of invertase enzyme resulting in glucose and fructose. These carbohydrates enter the glycolytic pathway and suffer numerous reactions being converted to pyruvate, which is decarboxylated by the action of the enzyme pyruvate decarboxylase, producing acetaldehyde and releasing CO_2. The acetaldehyde is reduced to ethanol by enzymatic action of alcohol dehydrogenase [15].

In this sense, understanding the fermentation process and the intrinsic and extrinsic factors that guide it is necessary, mainly because in this process, carbon sources obtained from renewable natural resources are being used, which has aroused the interest of researchers worldwide in the development of new technologies both for the interior of the process and for the acquisition of new raw materials. Therefore, this study aimed to evaluate the technological characteristics of saccharine substrates of direct fermentation for ethanol production, as well as analyze the fermentative capacity and fermentation profile of the FT-858 yeast under different culture conditions.

2. MATERIAL AND METHODS

Evaluation of the technological parameters of the saccharine substrates

The evaluation of the saccharine substrates was carried out in a qualitative exploratory way through a comparative bibliographical study of the technological characteristics of the saccharine substrates.

Collection and preparation of the substrate

The wort was obtained in a plant, packed in sterilized bottles and transported at a low temperature (4 ºC). This material was filtered on filter paper in order to remove the impurities to the max. Brix was concentrated by evaporation and was accompanied by a portable refractometer. The sorghum broth was donated and processed by Embrapa being transported to the laboratory and concentrated under the same conditions.

Strain used

Saccharomyces cerevisiae strain FT-858 used for the fermentation process for the production of ethanol in Brazilian industries.

Test fermentative capacity

For the preparation of the saccharine substrates, these were filtered in cotton, to remove larger impurities, and subsequently filtered on filter paper, the substrates were concentrated by evaporation and were monitored with a portable refractometer at concentrations (18 and 32°Brix). In the evaluation of the fermentative capacity, 10.0 mL of the saccharine substrates were added in test tubes containing inverted Durhan tube, and the whole was autoclaved at 120ºC for 20 minutes. With a platinum loop a yeast colony was inoculated into each tube and incubated in an oven at temperatures of 30ºC for 24 hours. The criterion for the selection of yeast as fermentative capacity was medium turbidity of the medium and production and gas retention in the Durham tube.

Cell growth

To obtain cell mass, it was used classical cultivation medium YPSAC 5%, containing (1.0% (w/v) yeast extract, 1.0% (w/v) peptone, 5.0% (w/v) sucrose), with pH adjusted to 5.0 with hydrochloric acid (1N) and sterilized in an autoclave at 120ºC for 20 minutes. The flasks containing yeast cells were incubated in "shaker" type CT-712R, 30ºC for 24h. After growth cells were collected by centrifugation (800g, 20 min.), suspended and washed three consecutive times in saline (0.85%) sterile, at a concentration of 10 mg mL^{-1}.

Experiment fermentative

The fermentation was carried out on the substrates saccharine sorghum broth and cane sterilized at the concentration of 18 and 32°C without pH correction, in flasks of 125 mL erlenmeyers, containing 50 mL of the substrates and incubated at temperatures of 30 ºC in "Shaker" tipe CT-712R at 250rpm. At determined fermentation times (5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 hours), aliquots were removed for analysis of the fermentation parameters. The experiments were carried out in triplicate.

Analytical methods

Cell density was measured by spectrophotometric at 570 nm and correlated to a dry weight/OD calibration curve [16].

Cell viability was determined by methylene blue staining [17].

Ethanol was analyzed by gas chromatography CG 3900 with flame ionization detector (Varian), using a 30m long fused silica capillary column (ZB-5). The chromatographic condition used was: 1μL injection volume, 1:20 displacement ratio and 90ºC oven temperature. The detector injector temperatures were 240°C. The samples were filtered in a 0.22 μm ultrafilter.

Statistical analysis

The results were analyzed with the Excel software version 2016 and the average was followed by the standard deviation and Origin 8.0.

3. RESULTS AND DISCUSSION

In the analysis of the technological characteristics of the substrates, it is possible to observe that both the broth of sugarcane and saccharine sorghum had similar composition in relation to sugars, which are the basis for the fermentation process. However, they showed differences in these carbohydrates concentration, the broth of sugarcane presented sucrose content of 14 to 22%, greater than that found in sorghum, 8 to 13%. However, the percentage of monosaccharides present in the broth of sorghum was higher, fructose 0.5-2% and glucose 0.5-1.5%, when compared to the sugarcane broth. The mean concentration of Brix degrees was greater for sugarcane broth 18 to 25. The analysis of pH was similar for both saccharin substrates. Regarding the average productivity of each biomass in tonnes per hectare, it was observed that the productivity of sugarcane is superior to that of sorghum, around 60-120 and 60-80 t ha^{-1}, respectively. The analysis of ethanol conversion yield showed that sugarcane has larger yield, 7046 L ha^{-1} compared with ethanol yield of sorghum, 6442 L ha^{-1} (Table 1).

According to Pitarelo et al. and Yu et al. [18,19], the biomasses, sugarcane and sorghum, have carbohydrates of direct fermentation in their composition, which can be metabolized by yeasts, Saccharomyces cerevisiae, converting them into ethanol [20]. Studies developed by Masson et al. [21], comparing broth of saccharine sorghum with broth of sugarcane, found higher Brix values in sugarcane broth, 21.2%. Serna-Saldívar et al. [22], presented in their studies values of total soluble solids of 20% for saccharine sorghum broth. The pH values between 4.5

to 6.5, according to Amorim [14], are appropriate for fermentation process, because it provides adequate conditions for growth and development of yeasts.

Sorghum has rapid growth cycle, according to Souza et al. [23], being ready for harvest into 130 days, and requires little availability of water and nutrients from the soil. This crop can be used in addition to sugarcane in the off-season production of fuel ethanol [24]. Studies by Borges et al. [25], performing the yield ratio of ethanol using sorghum as a substrate, found values between 50 and 65 liters of ethanol per tonne with a projection of 4,544 to 6,636 L ha^{-1}, however, in terms of productivity per planted area, sugarcane is higher, since it is already consolidated as a raw material for ethanol in Brazil, with an average production of between 5000 and 7000 L ha^{-1} [25, 26]

Table 1. Evaluation of the productivity of direct fermentation biomass.

Parameters	Substrates	
	Sugarcane	Saccharine sorghum
°Brix	18 – 25[1]	15 – 19[1]
pH	5	5,4
Productivity (t ha^{-1})	60-120[2]	60 – 80[3]
Ethanol yield (L ha^{-1})	7046[3]	6442[3]
Sucrose content (%)	14 – 22[1]	8 – 13[1]
Glucose content (%)	0,2 – 1[1]	0,5 – 2[1]
Fructose content (%)	0 – 0,5[1]	0,5 – 1,5[1]

Source: Adapted from [1][27]; [2][28]; [3][29].

In the analysis of fermentation test, performed on saccharin substrates using Saccharomyces cerevisiae FT-858, the yeast presented ability to metabolize these carbon sources in different Brix concentrations, since there was evidence of fermentation by the presence of foam and bubbles in the Duhran tubes (Table 2).

In the analysis of fermentative capacity, using the industrial yeasts Pedra-2, Catanduva-1, Red Star and Ragi Instan on sugarcane substrate in the concentrations of (12, 15, 24 and 30 °Brix) at 30°C for 24 hours, the data showed that the yeasts have fermentative capacity in all °Brix concentrations, except at 30 [30]. Studying the yeasts response of in relation to the peculiarities of the fermentation medium results in choosing a strain that suits the process thus resulting in an efficient substrate conversion into final product [31].

Table 2. Analysis of fermentative capacity of FT-858 industrial yeast on saccharine substrates.

	Sugarcane		Saccharine sorghum	
	Concentration of °Brix			
Yeast	18°	32°	18°	32°
FT- 858	+	+	+	+

Source: Authors

Biomass production of yeast FT-858 reached maximum at the time of 30 hours of fermentation in the substrates analyzed. In the sugarcane broth at 18 °Brix, the yeast presented cell growth of 13 mg mL^{-1} and at 32 °Brix concentration, 12 mg mL^{-1} (Figure 3A). In the sorghum broth at 18°Brix the biomass concentration presented was 12 mg mL^{-1} and, at 32°Brix, 11 mg mL^{-1} (Figure 3B). The data showed that the yeast FT-858 is capable of efficiently metabolize the saccharin substrates.

The yeast FT-858 features, high fermentative performance, resistance to low pH, high index of budding, tolerance to high alcohol content, high viability during fermentation recycles, low foaming, non-flocculent, speed of fermentation with high yield in final product and low residual sugar according to Amorim [32]. Studies by Masson et al. [21], using direct fermentation substrates, observed that the budding index presented by FT-858 yeast in sugarcane must was 14.46%, while in sorghum it was 6.84%.

The cell viability rate showed a greater number of viable cells until 30 hours of fermentation. In sugarcane broth at the concentration of 18°Brix the yeast presented 50% viability, however at 32°Brix it can be observed a 10% loss (Figure 4A). In sorghum the concentration of 18°C the viability was 47%, at 32°Brix this rate was 36%. It is observed that in longer fermentation times there was a great fall in viability, comparing the Brix degrees and substrates (Figure 4B). Probably, regardless of the substrate, the yeast FT-858 suffered the osmotic pressure of the fermentation medium, resulting in a drop in cell viability in high levels of sugars.

In the assessment of fermentative performance of the industrial yeast FT-858 in broth of sugarcane and sorghum at the concentration of 16 Brix at 30°C using an inoculum of 30 g L^{-1}, the viability rate of the yeast on sugarcane broth was 92.36%, and 89.48% on sorghum, yet, according to the authors, this yeast presented a budding rate efficiency during 10 hours of fermentation [22].

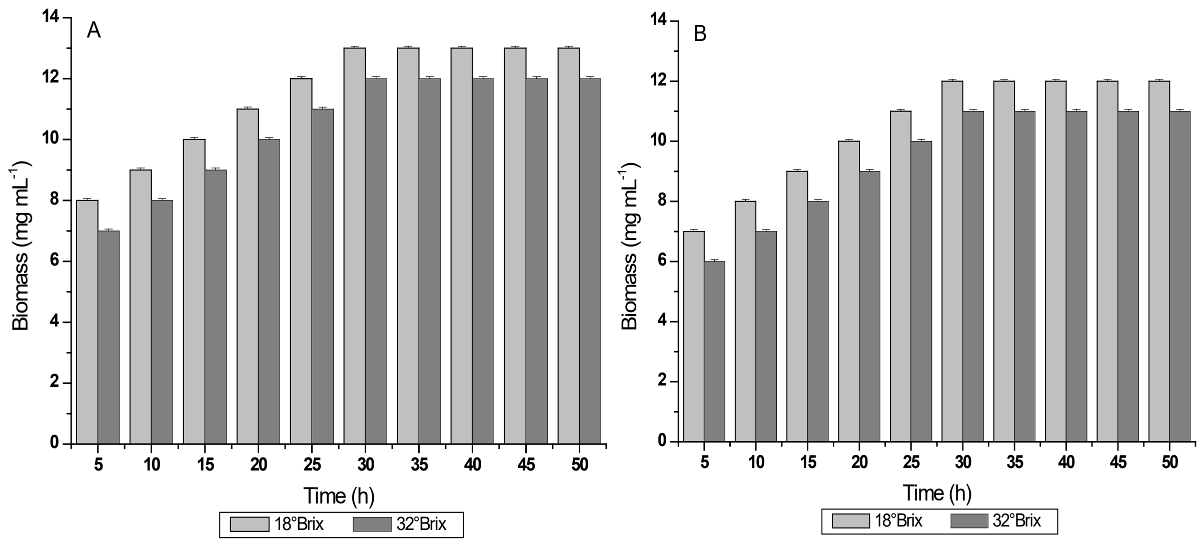

Figure 3. Evaluation of the biomass production of industrial yeast FT-858, in the substrates: sugar cane (A) and sorghum (B) at 30°C in different concentrations of Brix. Average of three readings followed by ± standard deviation of the samples.

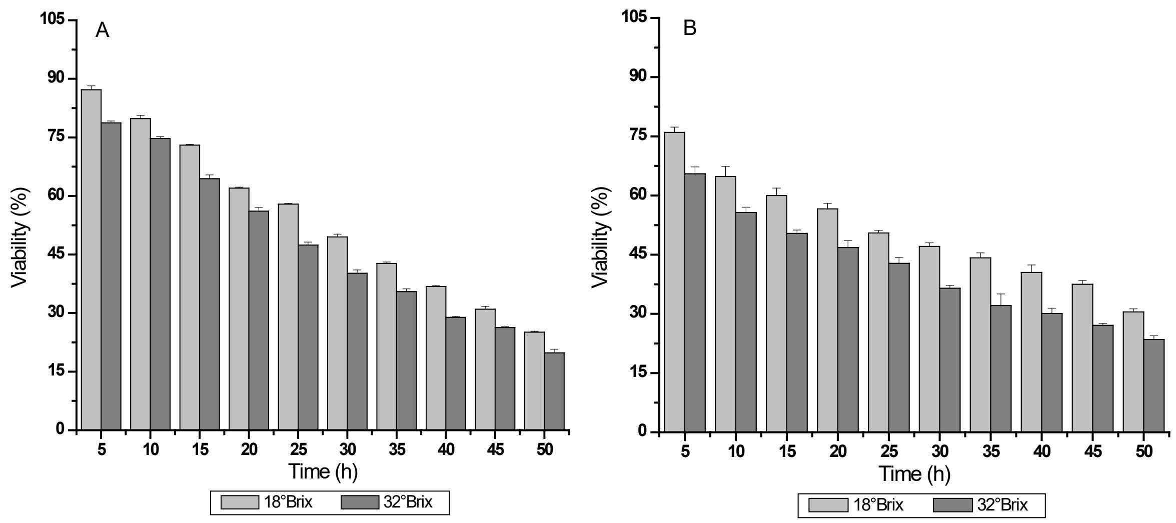

Figure 4. Evaluation of the cellular viability of the industrial yeast FT-858, in the substrates: sugar cane (A) and sorghum (B) at 30°C in different concentrations of Brix. Average of three readings followed by ± standard deviation of the samples.

The yeast FT-858 presented the greatest concentration of ethanol in saccharine substrates at 10 hours of fermentation. In the sugarcane broth at 18 °Brix, the ethanol production was 7.5% (v/v), showing loss of 1.5% (v/v) in ethanol concentration at 32°Brix (Figure 5A). In sorghum at 18°Brix, the ethanol concentration was 7.0% (v/v), and it was observed that in high sugar content there was a loss of 1.0% (v/v) in ethanol production (Figure 5B). It is likely that the loss of fermentative capacity of the yeast FT-858 is related to the high concentration of sugar in saccharin substrates, which consequently caused a great loss in ethanol production in the longer fermentation times. *Saccharomyces cerevisiae* regardless of the saccharine substrates of direct fermentation presents its best fermentative performance for ethanol production at the concentrations of 18° to 32º Brix for 10 to 15 hours of fermentation. The physiological behavior of industrial yeasts for ethanol production, this important biotechnological product, is directly correlated to the conduction factors during the fermentation process.

Studies by Moreira et al. [30], with different Saccharomyces cerevisiae industrial strains in cane juice based at 12,15, 24 and 30°Brix at 30°C for 8 hours of fermentation, it was observed that both the production of biomass as the ethanol concentration were higher at 15°Brix. However, cell viability was more expressive at 12 °Brix. The physiological behavior of the yeasts during the fermentation process is related to the availability of nutrients present in the substrate and that these influence in the multiplication and cell growth and in the transformation efficiency of the sugar in alcohol [33].

Masson et al. [21] evaluating the fermentative yield of the FT-858 yeast, the ethanol production cultivated in sugar cane and sorghum at 16°Brix at 30°C for 10 hours of fermentation, found yield values of 87.51% for sugarcane and 81.38% for sorghum with ethanol production around 7.32% cane and 6.33% sorghum respectively. In studies related to economic growth in the sugarcane sector in industries located in Dourados/MS, which addressed the main steps for ethanol production, the authors reported that the main raw material for ethanol production is sugarcane, the main yeast used in the fermentation process were Cat-1 and Pedra-2, which show efficient concentration of ethanol at 18º Brix at temperatures between 30°C and 35°C for 8 to 10 hours of fermentation. It can be observed in this study that, regardless of substrate, the highest ethanol production occurred between 10 to 15 hours of fermentation at 18ºBrix at 30ºC [34].

In the evaluation of fermentation parameters using industrial yeasts: Catanduva-1, Pedra-2, Red Star and Ragi Instan cultivated in must at the concentrations (12, 15, 24 and 30°Brix), at 30ºC, it was observed that the highest ethanol production was 8.5 (v/v) in 8 hours of fermentation [31]. Ribeiro Filho et al. [35] found 5.9% (v/v) of ethanol in experiments using sorghum as raw material for fermentation and Meneguetti et al. [36], values between 7 and 10% (v/v) for fermentation based on sugarcane.

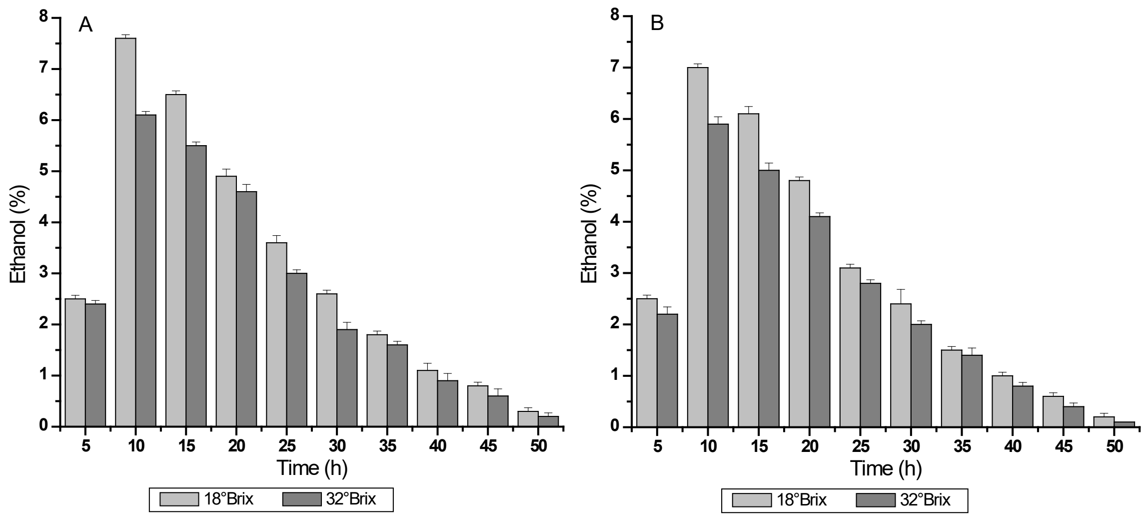

Figure 5. Evaluation of the ethanol concentration of the industrial yeast FT-858, in the substrates: sugar cane (A) and sorghum (B) at 30°C in different concentration of Brix. Average of three readings followed by ± standard deviation of the samples.

4. CONCLUSION

The technological parameters of the saccharine substrates analyzed showed potential for ethanol production. The yeast FT-858 presented fermentative capacity in the different substrates and concentrations tested.

The industrial yeast cultivated in saccharine substrates presented better fermentative robustness broth of sugarcane. In the evaluation of biomass production and cell viability, the highest Brix concentrations in the substrates analyzed affected the fermentative performance of the yeast leading to a great loss of viability and ethanol concentration. The highest concentrations of ethanol occurred at 10 and 15 hours of fermentation for both substrates, however, in prolonged times there was loss of this important biotechnological product.

5. ACKNOWLEDGMENTS

The authors thank Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul (FUNDECT), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (CALC. grant number 310801/2015-0), CAPES for the scholarship of research and the Programa de Pós-Graduação em Recursos Naturais - PGRN.

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