

Maria do Socorro Mascarenhas Santos ¹ Margareth Batistote ²

ABSTRACT

Brazil has edaphoclimatic conditions to produce a diversity of crops with energetic potential. Thus, the study aims to correlate the technological parameters of biomasses suitable for fermentation, quantify metabolites produced by FT-858 yeast, as well as assess the yield and fermentative efficiency for bioethanol production. It was performed studies of technological qualities of the biomasses and fermentative capacity testing with inverted Durhan tubes. For metabolites production, the FT-858 yeast was pre-grown in liquid medium (YPD 2%), recovered by centrifugation and inoculated in the substrates, in definite times aliquots were collected for analysis of ethanol concentration performed by gas chromatography and glycerol accumulation by enzymatic kit of triglycerides. The yield and fermentative efficiency were assessed by consumption of sugar using DNS method, and ethanol density using digital densimeter. According to the results, the yeast presented better performance in sweet sorghum, which also presented more expressive values of fermentative efficiency and yield. Sweet sorghum has great bioenergetic potential and can be used as complement to sugarcane to increase ethanol production.

Keywords: Sugarcane; Sweet sorghum; Saccharomyces cerevisiae

¹ Mestre em Recursos Naturais. Doutorado em andamento no Programa de Pós-graduação em Recursos Naturais pela Universidade Estadual de Mato Grosso do Sul, UEMS, Unidade de Dourados, Mato Grosso do Sul, Brasil maria_mascarenhas@outlook.com

² Doutora em Biotecnologia. Docente do Programa de Pós-graduação em Recursos Naturais pela Universidade Estadual de Mato Grosso do Sul, UEMS, Unidade de Dourados, Mato Grosso do Sul, Brasil margareth@uems.br

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he replacement of fossil fuels by alternative energy sources has been presented as a promising way to reduce greenhouse gases emission (GHG) from anthropogenic activities (Moreira 2011). The reduction of these gases is essential to reduce emissions by 50 - 85% by 2050, providing stabilization of these gases atmospheric concentrations at levels that enable mitigation of climate change effects on the environment (Edenhofer et al. 2012), promoting research on alternative renewable

sources that can be employed for power generation, a good example are plant biomasses.

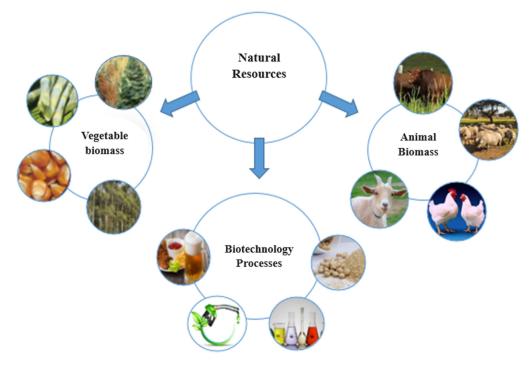
Brazil stands out as a great biomass producer. The biomass supply in 2005 was 558 million tons, with growth projection of 1402 million tons by 2030 (Moraes et al. 2017). Due to its great biodiversity, Brazil has a wide variety of biomass types in its territory, but only a few can be used for power generation, due to the availability of raw materials in a particular region. The country is at the forefront of energy production from biomass, the main parts of the biomass can be converted into biogas and biofuels by different technologies, which can be modified into thermal, mechanical or electrical energy and biofuels, being used primarily as a renewable and sustainable energy source (Barbosa & Langer 2011).

In this context, firstly it is necessary to understand the concept of Biomass and its features. Thus, biomass is any material that can be decomposed by chemical and biological agents, characterized as a renewable resource that comes from the total mass of organic matter that accumulates in a place, and can be of plant or animal origin, or even their waste. Biomass can be found in several forms and the best known are wood and waste generated by agricultural crops, agricultural industries and industries in general, livestock, energy forests and municipal solid waste according to Fernandes (2012).

Society, in general, is already familiar with biomass importance and how these natural resources can be used as raw materials sources in industrial processes, providing benefits to the environment. Such processes of biomass transformation have the ability to produce within the limits of renewable resources being: (a) plant biomass (b) animal biomass and (c) biotechnological processes, as shown in (Figure 01).

Among the main sources of renewable energy in Brazil are the biomasses representing 24% of the internal energy supply in the country, highlighting the use of sugarcane (Empresa de Pesquisa Energética 2015). However, biomass conversion into several products with added value and less environmental impact still depends on development and implementation of sustainable processes at economically viable levels.

Figure 01. The transformation of renewable sources from value added generation in industrial processes.



Source: The Authors

Among the main biomasses cultivated in Brazil, sugarcane and sweet sorghum are suitable to be used for sugar, ethanol and bio-energy production, considering that after juice extraction it is purified being sensitive to the action of fermentative agents, thus classified as direct fermentation substrates. Sweet sorghum *licolor* (L.) Moench), as reported by Albuquerque et al. (2012), can be employed in fuel ethanol production because it presents high-yield and fermentable sugars, and represent an economic gain since there are no required changes in the plant of sugarcane production, according to Anandan et al. (2012), this biomass can be processed in the same way as sugarcane. Another advantage of this crop is the easy adaptation to different environments and high capacity of CO_2 conversion into carbon source (Pin et al. 2011).

In the United States, the production of ethanol has maize as the raw material, however in this process enzymes are used that break the starch (Bortoletto & Alcarde 2015). In European countries the biofuel production process is based on sugar beet (*Beta vulgaris*) with a process similar to that of ethanol from sugarcane, as the beet has a high percentage of sucrose (Oliveira et al. 2012). With this it can be observed that the Brazil is in a privileged position because it is the largest exporter and presents

advantages in relation to the technologies applied to the production of this fuel according to the União de Produtores de Cana-de-açúcar (Única 2012).

However, it is necessary to understand how the chemical compounds present in these crops might affect the yeasts during the fermentation process, as well as the stressing factors associated with bioethanol production process. In the fermentation processes of the plants the appropriate and used temperature is 30 to 32°C, with a concentration of soluble solids between 18-22° Brix as recommended by Ceballos-Schiavone (2009). The fermentation time should not exceed 10 to 12 hours, as longer times may favor contamination by wild bacteria and yeast (Mongelo 2012).

Biotechnological processes can play an important role in transformation of biomass into energy, because the efficiency of the fermentation process and its yield result from a biotransformation process, in which the agent responsible for converting sugar into alcohol is a living being, the yeast *Saccharomyces cerevisiae* (Badotti et al. 2008). In the fermentation process productivity and yield parameters are essential factors that should be analyzed in order to measure the losses and process efficiency, as well as raw material condition is directly related to ethanol quality and quantity (Santos et al. 2013).

Biofuel production can be obtained from raw materials such as: starches (cassava, sweet potato, corn and other grains), cellulosic (wood and agricultural residues, among which sugarcane bagasse stands out) and saccharines (sugarcane, sweet sorghum, and sugar beet) (Sivasakthivelan et al. 2014). Through different technologies employed in their production process, and can be juice first and second generation (Pitarelo et al. 2012).

According to the Companhia Nacional de Abastecimento (Conab 2017), in the 2016/2017 harvest, 27.80 billion of fuel ethanol were produced, with 11.07 billion of anhydrous ethanol and 16.73 billion of hydrated. However, it is necessary to understand how the chemical compounds present in these crops might affect the yeasts during the fermentation process, as well as the stressing factors associated with bioethanol production process. In this context, the study aims to correlate the technological parameters of biomasses suitable for fermentation and quantify metabolites produced by yeast FT-858, as well as assess the yield and fermentative efficiency for production of bioethanol.

MATERIALS AND METHODS

JUICE EXTRACTION FROM BIOMASS

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Sugarcane juice was obtained from an ethanol plant in the region and stored in sterile bottles. Sorghum was obtained by extraction by milling, Embrapa Agropecuária Oeste and transported at (4 °C) to the Biotechnology, Biochemistry and Biotransformation Laboratory of the Centro de Estudos em Recursos Natural (CERNA), at the Universidade Estadual de Mato Grosso do Sul, in Dourados, Mato Grosso do Sul. Where the material was filtered through cotton to remove impurities and subsequently through filter paper. For Brix degrees concentration, evaporation was performed in thermostated plate accompanied by a portable refractometer; both substrates were calibrated at concentrations of 18 and 22° Brix. The pH was adjusted to 5.0 with (1mol L⁻¹) hydrochloric acid.

TECHNOLOGICAL PARAMETERS OF SACCHARINE SUBSTRATES

Assessment of technological parameters of saccharine substrates, was performed in a qualitative exploratory way through a comparative bibliographical study of the results found in this study regarding the technological characteristics of the saccharine substrates.

BIOTECHNOLOGICAL PROCESS

YEAST FERMENTATIVE CAPACITY TRIAL ON SACCHARINE SUBSTRATES

For the assessment of fermentative capacity, industrial yeast FT-858 were used, which are widely used in fuel ethanol production. It was performed plating using solid Sabouraud Agar Dextrose medium in Petri dishes of 90 mm diameter, consisting in the preparation of 0.10 g of yeast samples in sterile saline solution (0.85%), by serial dilution from 1.10^{-1} to 1.10^{-4} , of which aliquots of 100 µL were withdrawn and scraped with a Drigalski handle. Plates remained incubated by for 24h at 30 °C. Substrates were calibrated with the aid of a portable refractometer at different concentrations of total soluble solids (18 and 22 °Brix), and with a graduated pipette 10.0 mL of the juice were added in test tubes containing inverted Durham tubes, the set was autoclaved at 120 °C for 20 minutes. Then, a yeast colony was inoculated into each tube that remained incubated for 24h and subsequently analyzed regarding formation of CO₂ bubble retained in the Durham tubes in addition to foam presence, indicative of fermentation.

PRE-INOCULUM AND FERMENTATION

For the preparation of pre-inoculum, it was used classical liquid cultivation medium YPD 2%, containing 1.0% (w v⁻¹) yeast extract; 1.0% (w v⁻¹) peptone; 2.0% (w v⁻¹) glucose, autoclaved at 120°C for 20 minutes, 0.10 g of lyophilized yeast FT-858 was inoculated. Flasks containing the inoculum were

incubated in shaker model CT-712R, for 10h at 30°C and 250rpm. After growth cells were collected by centrifugation (800g, 20min), suspended and washed three consecutive times in sterile saline solution (0.85%), resulting in a concentration of 10 mg mL⁻¹ of wet mass that was used for the fermentative experiments. Fermentation was performed on the saccharine substrates sorghum and sugarcane juice sterilized at the concentration of 18 and 22° Brix, in Erlenmeyer of 125 mL, containing 50 mL of juice and incubated at temperatures of 30 and 40°C at 250rpm. At different times, aliquots of 04 mL were removed and centrifuged (800 g, 20min), and the supernatant was used to assess extracellular glycerol and ethanol concentration. All experiments were performed in triplicate.

EXTRACTION OF METABOLITES

Secondary metabolites were obtained from the fermentation medium in juice saccharine substrates. Extracellular glycerol was quantified by supernatant analysis and determined using an enzymatic kit for analysis of triglycerides (Laborlab®), correlating with a standard curve of glycerol obtained at concentrations ranging from 0.05 to 0.80 g L^{-1} . In the times (15, 25 and 45 hours) of fermentation and samples were centrifuged (800 g) and the supernatant was used for analysis of alcohol content, distillation was performed using a microdistiller and subsequently the alcohol content was determined by the alcohol density obtained in Anton Paar DMA4100M digital densimeter according to Amorim (1997).

INVESTIGATION OF BIOMASSES WITH POTENTIAL FOR BIOFUEL PRODUCTION

Biofuel was produced using the sugarcane and sorghum juice extracted from biomasses with high content of fermentable sugars, for 10 hours of fermentation and samples were centrifuged (800 g) and the supernatant was used for analysis of alcohol content, distillation was performed using a microdistiller and subsequently the alcohol content was determined by the alcohol density obtained in Anton Paar DMA4100M digital densimeter according to Amorim (1997). The determination of fermentative efficiency based on stoichiometric calculation of Gay-Lussac. Fermentative yield was performed based on ethanol produced by the consumption of sugars (Aquarone et al. 1983). The consumption of total reducing sugars (TRS) was determined by the method 3.5 – dinitrosalicylic – DNS described by Miller (1959).

STATISTICAL ANALYSIS

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The results were analyzed with Excel version 2016 software with ActionStat supplementation with means followed by standard deviation and the graphs plotted with Origin 8.0.

RESULTS AND DISCUSSION

Technological parameters

In the analysis of technological characteristics of substrates, it can be observed that both the juice of sugarcane as the sweet sorghum have similar composition regarding sugars, which are the basis for the fermentation process. However, there were differences in these carbohydrates' concentration, with sugarcane juice presenting sucrose content from 14 to 22%, greater than that found in sorghum, 8 to 13%. Regarding the average productivity of each biomass in tons per hectare, the productivity of sugarcane is greater than sorghum, around 60-120 and 60-80 (t ha⁻¹), respectively. The analysis of yield in ethanol conversion shows that sugarcane has a greater yield, 7046 (L ha⁻¹) compared with ethanol yield of sorghum, 6442 (L ha⁻¹), according to the Table 01.

Danamatana	Substrates		
Parameters	Sugarcane	Sweet sorghum	
Productivity (t ha ⁻¹)	$60-120^2$	$60 - 80^3$	
Ethanol yield (L ha ⁻¹)	7046 ³	6442^{3}	
Sucrose content (%)	$14 - 22^{1}$	$8 - 13^{1}$	
Suitable for harvesting (months)	12 - 18	3 – 4	

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Source: Adapted from 1(Almodares & Hadi 2009), (IBGE 2014); 2(Taborda et al. 2015); 3(Moreira et al. 2015).

In the 2016/2017 harvest, approximately 9.1 million hectares were collected, an increase of 5.3% compared to the 2015/2016 harvest according to data from the Companhia Nacional de Abastecimento (Conab 2016). This position is due to advancements and improvements of production techniques which were partially directed to genetic improvement of sugarcane, with development of varieties better adapted to environmental and agroclimatic conditions aiming to contribute and provide raw materials with high productivity, high sucrose content and good biomass production (Morais et al. 2015).

The biomasses, sugarcane and sorghum, present carbohydrates of direct fermentation in their composition, according to Nuanpeng et al. (2018), which can be metabolized by yeasts, Saccharomyces

cerevisiae, converting them into ethanol (Almodares & Hadi, 2009). Studies developed by Masson et al. (2015), comparing sweet sorghum juice with sugarcane juice, found higher Brix concentration values in the sugarcane juice, 21.2%. Serna-Saldívar et al. (2012) presented in their studies values of total soluble solids of 20% for sweet sorghum juice.

Sorghum has a rapid growth cycle, according to Souza et al. (2005), being suitable for cutting by 130 days, being undemanding regarding water and nutrients availability from the soil. This crop can be used as a complement to sugarcane during off-season for fuel ethanol production (Tavian et al. 2014). Studies by Borges et al. (2010), relating ethanol yield using sorghum as a substrate, found values between 50 and 65 liters of ethanol per ton with a projection from 4544 to 6636 (L ha-1). However, in terms of production per area planted, sugarcane is higher, since it already is consolidated as a raw material for ethanol in Brazil, with mean production between 5000 and 7000 (L ha-1).

In the analysis of the fermentative capacity test in saccharin substrates with yeast FT-858, demonstrated that this strain is able to bioconvert this carbon source and efficiently, since there was presence of foam and bubbles in the Duhran tube under different fermentation conditions (Table 02).

Yeast	Temperature (°C)	°Brix	Sacch	Saccharine substrates		
			Sugarcane	Sweet sorghum		
	30	18	+	+		
FT-858 <u>40</u> 30 40	40	10	+	+		
	30	22	+	+		
	40		+	+		

Table 02. Analysis of fermentative capacity of FT-858 industrial yeast on saccharine substrates.

Source: authors.

To study the physiological response of yeast in concerning substrate bioconversion and understanding fermentation peculiarities may result in the choice of a yeast strain that best suits the process, resulting in greater efficiency in the conversion of the final product, bioethanol (Pacheco 2010). It can be suggested that these cultivars are economically advantageous for ethanol production and provide a favorable environment for yeast metabolism.

Sorghum is a perennial crop and has agronomic characteristics conducive to biofuel production, easy to handle and adapt to edaphoclimatic conditions according to Chan-u-tit et al. (2013), with

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management of accessible technologies to most producers, and containing high sugar concentrations similar to sugarcane (Yu et al. 2014). This crop has great energetic potential as renewable energy sources and given its characteristics of precocity and having short cycle and tolerance, it is promising to be cultivated as second crop (safrinha) (Giacomini et al. 2013).

Metabolites production

The strain FT-858 cultivated in sugarcane juice substrate, in the evaluation of glycerol accumulation, showed a slight variation in relation to the total concentration of soluble solids (°Brix). However, analyzing the accumulation as a function of temperature, it can be observed that there was no change in the accumulation of this metabolite in the fermentation medium at 30°C and in the substrate concentrations of ° Brix. At 40°C, the accumulation was 0.50 g L⁻¹ in sorghum. Analysis of the ethanol concentration of FT-858 strain grown in sugarcane juice showed that the best performance was at 30°C at 15 hours of fermentation at 22° Brix concentration with 7.0% (v v⁻¹) ethanol. However, it was in the sorghum juice that the best ethanol production 7.5% (v v⁻¹) occurred under the analyzed condition. At the temperature of 40°C, the fermentative performance was affected, because ethanol concentration was around 3.5% (v v⁻¹) at 22° Brix (Table 03). It is likely that longer fermentation times along with high temperature induced the yeast to stress, given the physiological response that this microorganism showed, especially in relation to ethanol production.

In longer times there was a decline in the assessed parameters. Maybe the high temperature and longer period of fermentation may have stressed the yeast interfering directly in the metabolic pathways of the microorganism. Metabolite production in yeast is related to the synthesis mechanism. Ethanol production originates from the same glycerol biosynthesis pathway. Thus, under favorable conditions yeast produces ethanol, however under stress conditions glycerol accumulation occurs. This metabolite is an inhibitor of ethanol production according to Basso et al. (2008) and Vázques-Lima et al. (2014).

		18°Brix		22°H	22°Brix	
Substrates	Fermentation Time (h)	Glycerol (g L ⁻¹)	Ethanol % (v v ⁻¹)	Glycerol (g L ⁻¹)	Ethanol % (v v ⁻¹)	
l'emperature de 3	30°C					
Sugarcane	15	0.38 ± 0.01	6.0 ± 0.01	0.40 ± 0.01	7.0 ± 0.01	
	25	0.36 ± 0.00	3.0 ± 0.00	0.38 ± 0.00	3.7 ± 0.00	
	45	0.32 ± 0.01	0.7 ± 0.01	0.35 ± 0.01	0.7 ± 0.01	
Sweet sorghum	15	0.40 ± 0.00	6.5 ± 0.00	0.43 ± 0.00	7.5 ± 0.00	

Table 03. Metabolites assessment of yeast FT-858 of biotechnological interest produced using saccharine substrates at different concentrations and temperatures.

	25 45	0.42 ± 0.01 0.36 ± 0.01	3.0 ± 0.01 0.7 ± 0.01	0.46 ± 0.01 0.43 ± 0.01	3.6 ± 0.01 0.9 ± 0.01
Temperature de 40°C	43	0.30 ± 0.01	0.7 ± 0.01	0.45 ± 0.01	0.9 ± 0.01
S	15	0.42 ± 0.03	3.0 ± 0.03	0.45 ± 0.00	3.3 ± 0.00
Sugarcane	25	0.42 ± 0.00	1.7 ± 0.00	0.42 ± 0.01	2.0 ± 0.01
	45	0.43 ± 0.03	0.2 ± 0.02	0.36 ± 0.01	0.3 ± 0.01
Sweet sorghum	15	0.43 ± 0.00	3.0 ± 0.00	0.50 ± 0.02	3.5 ± 0.02
	25	0.45 ± 0.00	2.0 ± 0.01	0.50 ± 0.00	2.7 ± 0.00
	45	0.45 ± 0.00	0.3 ± 0.00	0.50 ± 0.00	0.4 ± 0.00

Mean of three samples readings followed (\pm) by standard deviation. Source: authors

In studies on the physiology of industrial yeast strains performed by Moreira et al. (2015), growing strains on sugarcane juice substrate at 12, 15, 24 and 30° Brix and temperature of 30°C. The authors observed that the best rates were obtained at the time of 08 hours of fermentation at 15° Brix, with cell viability of 96% and ethanol concentration of 8.5% (v v⁻¹). Studies by Ramos et al. (2013), with 66 *Saccharomyces cerevisiae* strains isolated both from industrial processes and fruits, subjected to stressing conditions at a temperature range between 30 and 42°C, with osmolarity of 0.5, 0.7 and 1 mol L⁻¹ NaCl, and adding ethanol of 06, 12 and 18% (v v⁻¹). Obtained as response two different groups of yeasts, one composed of 21 yeasts that were more resistant to the stress levels, and the others showed sensitivity at 42°C with osmolarity concentrations and presence of ethanol, 1 mol L⁻¹ NaCl in 12% (v v⁻¹).

According to Masson et al. (2015), comparing the fermentative process in sugarcane and sweet sorghum at 16° Brix concentration for 10 hours of fermentation, assessing cell viability of an industrial yeast strain in these substrates, the cell number was similar for both substrates. The data from this study differ from those described in the literature. However, fermentative conditions interfere with the production of matabolites. In this sense, it can be inferred that each yeast strain presents different response mechanisms to the adverse conditions of the fermentative medium even to the substrate concentration for ethanol production.

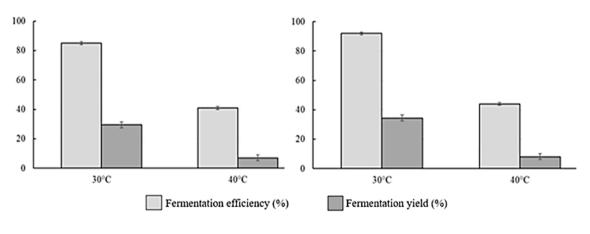
The amount of glycerol produced during fermentation can affect the final yield of ethanol, therefore, the selection of yeast strains that present greater tolerance to stress levels are required since they may present low amount of glycerol production (Basso et al. 2015). It is important to emphasize that glycerol production is one of several mechanisms for adaptation of yeast cells to stressing factors that occurs during fermentation (Aslankoohi et al. 2015). Strains of *Saccharomyces cerevisiae* employed by the sucroenergetic sector must be tolerant to stress levels of the medium in addition to present high ethanol production and resistance to different compounds that can be

inhibitory for their metabolism (Hahn-Hagerdal et al. 2007). Ethanol produced via fermentation is the result of a biotechnological process, once the agent responsible for the transformation of sugar into alcohol is a microorganism, the yeast, the most used for biofuel production (Pereira et al. 2015).

Biomass potential for biofuel production

The analysis of fermentative efficiency and yield using the yeast FT-858 in sugarcane juice substrate at the temperature of 30°C showed that although fermentative efficiency was of 85%, the yield was around 29%. At the temperature of 40°C, the fermentative efficiency was of 41% and yield 7%. In sweet sorghum juice at 30°C, the fermentative efficiency was of 92% and yield of 34%. At the temperature of 40 °C, the percentages were 44% and 8% for fermentative efficiency and yield respectively, due to thermal stress, the yeast showed sensitivity to this variable, which may have compromised its fermentative efficiency and ethanol yield (Figures 02A and 02B).

Figure 02. Yield and fermentative efficiency of FT-858 yeast grown in juice of sugarcane (A) and sweet sorghum (B) at different temperatures.



Source: Authors

Studies by Davila-Gomez et al. (2011), using the juice of five varieties of sweet sorghum and *Saccharomyces cerevisiae* (ATCC 24858), obtained fermentative efficiency ranging from 79.9 to 89.7%. While Ratnavathi et al. (2010) observed fermentative efficiency values ranging from 86.5 to 94.7% for sweet sorghum, using the yeast CFTR 01. Already Lima et al. (2007), using 16 yeast isolates observed that only one isolate showed 45.8% fermentative yield and 85.1% fermentative efficiency in sugarcane juice.

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In our studies the yield an fermentative efficiency values were 92% and 34% in sweet sorghum, thus the yeast FT-858 presented better efficiency, and the yield was similar to the reported by Dornelles and Rodrigues (2006) of 37.59%. In the study performed by Masson et al. (2015), assessing fermentative yield in saccharine substrates, the results presented 87.5% for sugarcane juice and 81.3% for sorghum, respectively.

CONCLUSION

In the analysis of the technological parameters, there was a small variation between the saccharin substrates. Glycerol accumulation was more pronounced at 40°C. Ethanol production was affected at higher temperatures and longer fermentation times. Considering the few studies with yeast FT-858 in different concentrations of Brix and saccharin substrates, this study shows the potential of this microorganism in biotechnological processes.

The substrate that presented the best yield and fermentative efficiency was the sweet sorghum juice at a concentration of 22 °Brix and 30 °C. Therefore, it can be inferred that, given the characteristics of this biomass, it can be used in biofuel production and supply of sugarcane out of season.

The results highlight the importance of the exploitation of renewable sources, as they can contribute to the increase of biofuel production, besides providing knowledge in the search for new compounds of biotechnological interest.

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Fontes Renováveis e Suas Aplicações em Processos Biotecnológicos

RESUMO

O Brasil possui condições edafoclimáticas para produzir uma diversidade de culturas com potencial energético. Assim, o estudo tem como objetivo correlacionar os parâmetros tecnológicos de biomassa adequados para fermentação, quantificar metabólitos produzidos pela levedura FT-858, bem como avaliar o rendimento e a eficiência fermentativa da produção de bioetanol. Foram realizados estudos das qualidades tecnológicas da biomassa e teste de capacidade fermentativa com tubos de Durhan invertidos. Para a produção de metabólitos, a levedura FT-858 foi pré-cultivada em meio líquido (YPD 2%), recuperada por centrifugação e inoculada nos substratos, em tempos definidos foram colhidas alíquotas para análise da concentração de etanol realizada por cromatografia gasosa e acumulação de glicerol por kit enzimático de triglicerídeos. O rendimento e a eficiência fermentativa foram avaliados pelo consumo de açúcar pelo método DNS e a densidade do etanol pelo densímetro digital. De acordo com os resultados, a levedura apresentou melhor desempenho no sorgo sacarino, que também apresentou valores mais expressivos de eficiência e rendimento fermentativo. O sorgo sacarino possui grande potencial bioenergético e pode ser utilizado como complemento da cana-de-açúcar para aumentar a produção de etanol.

Palavras-Chave: Cana-de-açúcar; Sorgo sacarino; Saccharomyces cerevisiae

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