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Energy cultures and sustainability in biofuel production

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ABSTRACT

Energy cultures are emerging as viable renewable energy sources because they satisfy sustainability requirements. The present study involves a survey regarding the technological characteristics for sugarcane and sweet sorghum energy crops, in addition to their potential for bioethanol production. An exploratory survey was conducted regarding the agronomic and technological characteristics of cane and sorghum. Pre-inmates were produced with YPSAC 5% liquid medium, sterilized and 0.10 grams of yeasts FT858 and Pedra-2 were inoculated and incubated at 30 °C for 10 h at 250 rpm. After the production, the cells were recovered by centrifugation, at 105 rpm, resulting in a 10 mg mg^{-1} concentration of moist dough. This dough was subsequently inoculated in the fermentative medium consisting of a base of sorghum broth and cane without pH correction with a 22 °Brix. Ethanol was analysed by gas chromatography and amino acids by high-efficiency liquid chromatography. Sorghum broth presented a greater availability of serine, arginine, alanine, threonine, and tryptophan amino acids. Yeast hers presented fermentative efficiency for both substrates, but the largest ethanol production occurred in sorghum broth. The results demonstrated that sugar sorghum may be used for energy purposes.

Keywords: Biomass, *Saccharomyces cerevisiae,* Bioethanol.

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RESUMO

As culturas energéticas estão despontando como fontes renováveis de energia, pois atendem as esferas da sustentabilidade. Assim, este estudo teve como objetivo analisar as características agronômicas e tecnológicas das culturas energéticas, cana-de-açúcar e sorgo sacarino, bem como do seu potencial para a produção de bioetanol. Foi realizada uma pesquisa exploratória sobre as características agronômicas e tecnológicas da cana e do sorgo. Foram feitos pré-inóculos com o meio líquido YPSAC 5%, esterilizado e, inoculados 0,10 gramas das leveduras FT858 e Pedra-2 que foram incubados a 30 °C por 10 h a 250 rpm. Após o crescimento as células foram recuperadas por centrifugação resultando em uma concentração de 10 mg $mL-^{1}$ de massa úmida, a qual foi inoculada no meio fermentativo a base de caldo de sorgo e cana esterilizados sem correção de pH com 22 °Brix, e incubados 30 °C por 10 h a 250 rpm. O etanol foi analisado por cromatografia a gás e os aminoácidos por cromatografia líquida de alta eficiência. O caldo de sorgo apresentou maior disponibilidade dos aminoácidos serina, arginina, alanina, treonina e triptofano. As leveduras apresentaram eficiência fermentativa em ambos os substratos, e a maior produção de etanol ocorreu no caldo de sorgo. Os resultados demonstram que o sorgo sacarino pode ser empregado para fins energéticos.

Palavras-chave: Biomassa, *Saccharomyces cerevisiae,* Bioetanol.

1. Introduction

An increasing concern regarding environmental contamination and the possible scarcity of energy sources has motivated research regarding the use of renewable sources to meet energy demand while diversifying the current energy matrix (Fierro et al., 2019). With this context, energy cultures or biomasses have emerged as promising alternative energy sources. They are renewable natural resources that sustainably satisfy energy needs because they mitigate both environmental and social concerns (Acheampong et al., 2017).

Many crops are already used as biomass energy sources globally, sugarcane (*Saccharum officinarum*) being an example. Currently, it is an important part of the Brazilian energy matrix. Its production is focused on the manufacture of sugar, biofuels, and energy generation (Goldemberg, 2017). Brazil ranks second globally in ethanol production which significantly depends on sugarcane. In terms of economic cost-benefit, sugarcane-based ethanol is considered superior to ethanol alternatives (Brassolatti et al., 2016).

In economic projections, the production of Brazilian ethanol generally increases and positively influences the generation of direct and indirect jobs (Brinkman et al., 2018). However, there is a need for diversification of raw materials, which involves the participation of small farmers in this important agribusiness network (Taborda et al., 2015). In this perspective, sugar sorghum (*Sorghum bicolor* (L.) Moench), may be a satisfactory alternative to sugarcane. Sugar sorghum shares many agronomic characteristics with sugarcane, such as sugar storage in its stalks, tolerance to abiotic stresses (McCord et al., 2019), and a rapid phenological cycle. Sugar sorghum also yields a satisfactory production of green mass, forage, and grains; consequently, it is an option favouring agricultural sustainability and can be used in animal feed and clean energy production (Giacomini et al., 2013).

These sugar sorghum characteristics are promising. This implies a potential for sorghum as a complement to the sugarcane-based ethanol production. According to Umakanth et al. (2019), sorghum is a source of economically efficient renewable energy. It also has low nutritional demands and tolerates water stress. Although these plants are highly adapted to tropical and subtropical climates (Alves and Paixão, 2018), large variations may occur in their technological qualities (Miranda et al., 2020).

Energy cultures for biofuel production must demonstrate characteristics suitable for biotransformation processes, such as high curate levels in addition to protein and mineral availability in addition to nitrogen sources such as amino acids and peptides (peptides are precursors of numerous physiological processes in yeast) (Ruiz et al., 2020; Kumari et al., 2021). The composition of sugar substrates have been reported as the basis of cane and sorghum broth. These reports include the composition and presence of nutrients found in the total soluble solids. For the sugar sorghum, the reported value is 15% (Santos et al., 2018) while for sugarcane broth it is approximately 21% (Masson et al., 2015).

In fermentation processes, yeasts require nutrients and sources of carbon and nitrogen. These carbon and nitrogen sources directly influence yeast metabolic functions. Some amino acids evoke stress responses and provide resistance to cells (Santos et al., 2020). Gutiérrez-Rivera et al. (2015) indicate that the presence of free amino acids in the fermentative environment is of vital importance as it induces the synthesis of the yeast, effectively contributing to the maintenance of cellular feasibility and fermentative efficiency.

Understanding this mechanism is vital to ensure a better ethanol yield, which may guarantee the maintenance of these crops from renewable sources and environmental sustainability. The mechanism indicates that the quantity and availability of carbon and nitrogen sources in energy crop substrates employed are assimilable by yeasts (which may contribute to physiological integrity) and are fundamentally important in the production of biofuel compounds. Thus, the present study conducted a survey of the technological characteristics of sugarcane and sweet sorghum energy crops, as well as their potential to produce bioethanol.

2. Material and Methods

The study was performed at the Biotechnology, Biochemistry and Biotransformation Laboratory of the Centro de Estudos em Recursos Naturais, Universidade Estadual de Mato Grosso do Sul- CERNA- UEMS, Dourados, Mato Grosso do Sul, Brazil.

Evaluation of the saccharin substrates' agronomic and technological characteristics was performed using an exploratory survey of the sorghum sugar and sugarcane characteristics for the production of ethanol. For this research, the published databases relating to the theme were considered, and a rating of data was assigned based on relevance. For Araújo and Alvarenga (2011), exploratory research was conducted to improve understanding of the surveyed phenomena. The materials were identified in order of importance based on content.

The cane broth was produced at a mill in the Greater Dourados region, packed in sterilised flasks, and transported at low temperatures (4 °C). The broth was prepared by filtering cotton and filter paper positioned at the mill outlet to achieve maximum removal of impurities. The total soluble solids were concentrated by evaporation. The sorghum broth was produced and processed by the EMBRAPA (Dourados, Mato Grosso do Sul, Brazil) and subsequently transported to the laboratory. The broth was concentrated under the same conditions, and both substrates were calibrated to a 22 °Brix concentration using a refractometer (Lorben) and without pH correction.

The pre-inoculum preparation was performed for the YPD culture medium with the following composition: 2% of the culture contained 1.0% (p v^{-1}) of yeast extract; 1.0% (p v^{-1}) of peptone; 2.0% (p v^{-1}) glucose, sterilised at 120 °C for 20 min, 0.10 grams of inoculated lyophilised yeast FT858 and Pedra-2. The vials were incubated at 30 °C for 10 h. Then, the cells were collected by centrifugation (800 × g, 20 min), resuspended, and washed three times consecutively in sterile saline (0.85%). This resulted in a concentration of 10 mg mL^{-1} wet mass. Fermentation was performed on the saccharin substrates, sorghum broth and cane. It was subsequently sterilised, at a concentration of 22 °Brix without pH correction, in 125 ml Erlenmeyer vials containing 50 ml of the broth. Incubated aliquots were used for the analyses.

Amino acid quantification was performed on the saccharin substrates per the methodology described by Torres et al. (2018). The samples were analysed using an analytical HPLC system (LC6AD, Shimadzu, Kyoto, Japan). Free amino acids were identified by comparing retention times and the amino acid spectra to amino acid standards in the 200 to 800 nm band. The peaks were obtained from actual samples. The analyses were performed in triplicate.

The quantification of ethanol was determined with a gas chromatograph (CG-3900) using a flame ionization detector (Varian), per the methodology presented in Batistote et al. (2010). The results were analyzed using Excel version 2016 software with ActionStat supplementation. Tukey's test at 5% significance was used. The graphs were plotted with Excel version 2016. The experiments were carried out with independent repetitions for each treatment and with triplicate in the analyses.

3. Results and Discussion

The analysis of the sorghum and sugarcane’s agronomic and environmental characteristics indicated important differences between the two plants. The parameters analysed showed that saccharine sorghum has a maturation period between 3 to 4 months subsequent to planting and prior to harvest. It has a rapid vegetative cycle compared to that of sugarcane (maturation period of 12 to 18 months). Sorghum productivity is between 60 and 80 t ha^{-1}, with a maximum variation in productivity less than that of cane (60–120 t ha^{-1}). The ethanol yield was 60–70 L t^{-1} for sorghum and 70–85 L t^{-1} for cane, possibly because the highest cane yield for ethanol was related to its productivity. In addition, it can be observed that the water demands were much greater for sugarcane. Although the use of this natural resource is required for crops, water can also be an important factor in assessing a crop’s energy demands, which is a sustainability indicator. The use of these crops favours the reduction of greenhouse gases concerning fuels; such a reduction was similar in the analysed crops (Table 1).

Studies developed by Lima et al. (2011) regarding the minimum potential of ethanol generation of the sugar sorghum have reported values of approximately 58.6 L t^{-1}. Although these differences in productivity and yield in ethanol occur, it is possible to use the culture of sugar sorghum as a complement to the cane crop. Sorghum culture achieves maturation around four months; consequently, it can be planted and harvested multiple times within 12 months, the cane maturation period. However, the energy crops’ characteristics may vary according to the cultivation conditions, the variety used, the planting period, and water availability (Parazzi et al., 2018).

Albuquerque et al. (2019) presents other advantages for the use of sorghum cultures. These include seed propagation, fully mechanized cultivation, production of colms with high fermentable sugar content and the possibility of using the bagasse for energy cogeneration. According to Fernandes et al. (2014), given the short vegetation cycle, sorghum sowed from September to December can supply the mill demand from January to April, the period of entersafra. In this sense, sorghum can be utilised to produce biofuel because no adaptations are required for the plant. This increases feasibility from an industrial perspective (Oliveira, 2021).

Among energy crops, sugarcane presents better results for biofuel production despite requiring more water and a longer harvest cycle higher than most biomasses (Milanez et al., 2014). Ethanol production from this culture mitigates CO_2 emissions because CO_2 is consumed via photosynthesis (Manochio et al., 2017). In addition, the choice of an energy culture includes the consideration of cultivation characteristics, the cost of harvesting and maintenance, and the quantity and quality of raw materials, such as ethanol, electricity, or heat (Clauser et al., 2021). Increased materials’ diversification for this purpose favours a greater expansion of biofuel production.

The analysis of technological characteristics demonstrated by the substrates of energy crops, sorghum and cane saccharin, showed that the broths of these cultures have a similar composition to fermentable sugars because they are products of direct fermentation. However, some crop parameters presented differences, such as purity of 80–90% and average concentration of total soluble solids (°Brix) of 18–25, respectively. Both these values were less for sorghum. The values for reducing sugars (AIR) and total reducing sugars (ART) were different; sorghum broth contained 1–3% for air and cane broth showed a greater ART (15–2%). The cane broth had a sucrose content of 14–22%, which was greater than that in sorghum. However, the sugar sorghum broth contained fructose (0.5–2%), a monosaccharide, and glucose (0.5–1.5%) (Table 2).

Sugarcane juice and sorghum have direct fermentation carbohydrates that can be efficiently metabolised by *Saccharomyces cerevisiae* and converted to ethanol, as reported by Masson et al. (2015). Comparing the sugar sorghum broth with the cane broth, total soluble solids values were approximately 21 °Brix for the sugarcane broth, while Serna-Saldivar et al. (2012) reported values of approximately 20 °Brix for the broth. The composition of the sugar sorghum and the high content of sugars are promising for ethanol production (Albuquerque et al., 2019).

The primary technological parameters used in the industrial process are related to raw materials, such as Brix, wet cake, and purity. These parameters are used to assess the presence of reducing sugars, total sugars, and fibres and influence the choice of varieties, agronomic planning, management, and the payment made to the producer (Silva et al., 2014). Thus, variation in the presence of sugars in saccharine substrates can influence ethanol production. Such compounds are important for the fermentation process, as they directly affect the yield of ethanol, an important biotechnological product obtained from renewable natural resources and clean energy concepts. (Fiorini et al., 2016; Batista et al., 2018)

The amino acid content varied between sorghum and sugarcane saccharin substrates. The saccharine sorghum broth had greater amino acid concentrations. Species observed were mainly serine with of 78.89 ± 0.03 µg L^{-1} and arginine at 50.69 ± 0.02 µg L^{-1}. The cane broth presented the same amino acids, but at lower concentrations (Figure 1).

Studies performed by Silva et al. (2020), analysed amino acid concentrations on saccharin substrates. These concentrations varied, because sorghum broth contained serine, arginine, alanine and threonine amino acids. However, for tryptophan and isoleucine, concentrations were greater than 11.3 ± 0.08 and 6.83 ± 0.05 µg L^{-1}. The cane broth contained serine, arginine, and alanine at concentrations of 36.12 ± 0.05, 23.98 ± 0.08 and 27.44 ± 0.06 µg L^{-1}, respectively.

During the fermentative process, amino acid availability influences numerous metabolic functions in yeast, including structural components, which are directly assimilated, and actin protein formation, which is involved in various metabolic pathways. The availability of these nutrients on substrates, as well as the consumption of amino acids by yeast, is of paramount importance as they act in numerous cellular mechanisms and fermentation (Ljungdahl and Daignan-Fornier, 2012).

Table 1. Evaluation of the agronomic and environmental characteristics of energy cultures.

Parameters	Sweet sorghum	Sugarcane
Vegetative cycle (months)	3 a 4[1]	12 a 18[1]
Productivity (t ha^{-1})	60 – 80[2]	60 – 120[2]
Ethanol yield (L t^{-1})	60 –70[2]	70 – 85[2]
Water consumption (mm)/ production cycle	380 – 600[3]	1000 – 2000[4]
Reduction of Greenhouse Gas Emissions % (Ethanol X Gasoline)	40 – 62[5]	40 – 62[5]

Source: Adapted from [1]IBGE (2014); [2]Durães et al. (2012); [3]Kirchner et al. (2018); [4]Carvalho et al. (2013); [5]Wang et al. (2012a).

Table 2. Analysis of the sugars in the sugars present in the saccharin substrates.

Parameters	Cultures	
	Sweet sorghum	Sugarcane
Purity (%)[1]	60 – 75	80 – 90
Total Soluble Solids –°Brix[1]	15 – 19	18 – 25
Reducing sugars – RS (%)[1]	1 – 3	0.5 – 1
Total Reducing Sugars – TRS (%)[1]	12 – 17	15 – 24
Sucrose content (%)[2]	8 – 13	14 – 22
Glucose content (%)[2]	0.5 – 2	0.2 – 1
Fructose content (%)[2]	0.5 – 1.5	0 – 0.5

Source: Adapted from [1]Durães et al. (2012); [2]IBGE (2014);

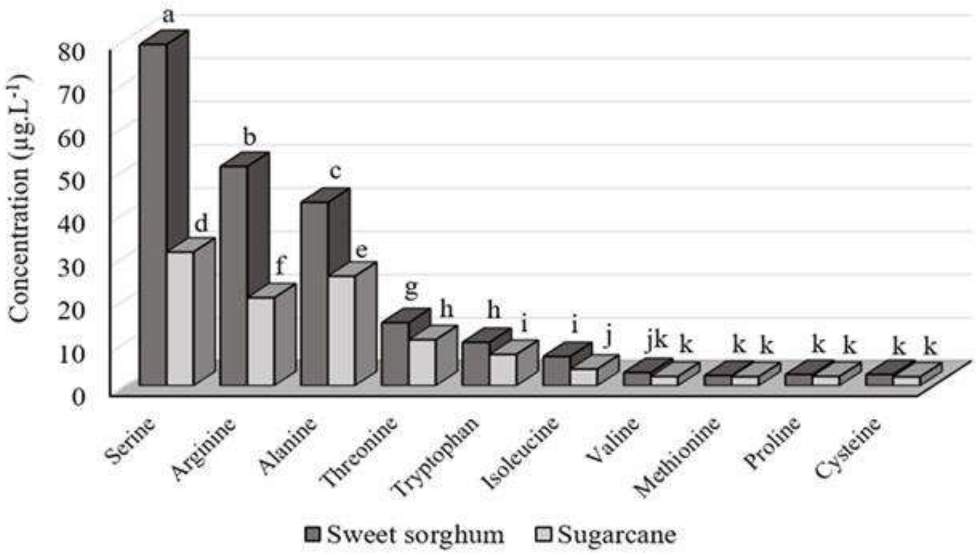

Figure 1. Quantitative evaluation of amino acids presents in saccharin substrates. The same letter in not statistically different from each other (P <0.05) by the Tukey test at 5% significance.

The presence of amino acids such as proline, tryptophan, and arginine, provides a protective action with the yeasts against ethanolic stress (Auesukaree, 2017). In this study we observed the greatest concentration of nitrogenous compounds. In the fermentative process of ethanol production, amino acids are important because they confer the yeast with nutrition that directly influences yeast metabolism. In addition, they prolong the fermentative efficiency of yeasts.

The evaluation of ethanol concentration on the saccharin substrates indicated the fermentative efficiency of yeasts (Figure 2). The yeast showed the highest concentration of ethanol when grown in the sugar sorghum broth with 8.5% (v v^{-1}) for FT858 and 7.6% (v v^{-1}) for Pedra-2 yeast. It is possible that the largest ethanol production on the sugar sorghum substrate may be related to the substrate's nutrient composition. This would be principally due to the availability of sucrose and free amino acid content. It can be inferred that energy crops have high potential for use in fermentation and can contribute to environmental sustainability because it is a renewable energy source that provides a lower global warming impact and guarantees the production of biofuel.

In Brazil, fermentation is the most used process for ethanol production (Azhar et al., 2017). It is facilitated by *S. cerevisiae* that promotes biocatalysis on the sugar substrate resulting in CO_2 release. In Brazil, CAT-1 and PE-2 strains are the most used in such a process because they present fermentative robustness and a high feasibility rate. These yeasts are responsible for 60% of total Brazilian ethanol production (Souza et al., 2018); these microorganisms populate different environments. This characteristic demonstrated by generalist organisms makes them suitable for various niches, including substrates from energy crops. (Jouhten et al., 2016)

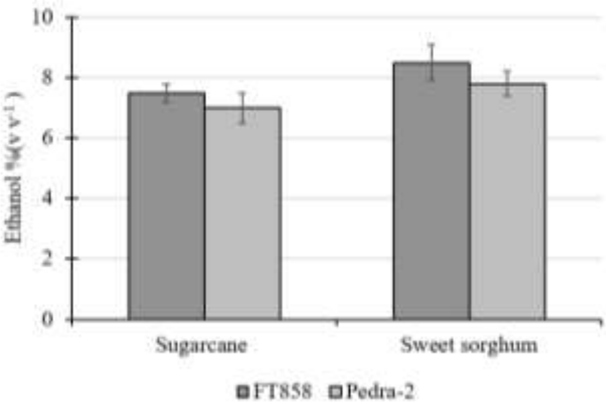

Figure 2. Analysis of ethanol concentration on saccharin substrates at 30 °C in 10 hours of fermentation. Mean followed by ± standard deviation.

The use of natural resources to diversify energy matrices has been a challenge while promising new energy resources associated with the application of new technologies. Examining these biomasses and their composition, as well as the use of selected yeast lineages and the improvement of the fermentative process, promising alternatives have emerged that may satisfy the sustainability, economic, and social chain constraints while minimising the environmental impacts by utilising energy sources.

4. Conclusions

Energy crops have distinct agronomic characteristics but have important attributes that enhance their suitability for biofuel production. The technological and environmental characteristics of substrates are based on sweet sorghum and sugarcane, which have similar compositions. Both are fermented directly, with lower production costs, resulting in a more competitive market product. Environmental indicators effectively contribute to minimising environmental impacts by reducing greenhouse gas production.

In relation to the amino acid contents in the saccharin substrates, the sorghum broth presented a greater availability of these compounds. These compounds included serine, arginine, alanine, threonine, and tryptophan. Yeast hers presented fermentative efficiency on both substrates, but the largest ethanol production occurred in sorghum broth. The evaluation of fermentable substrates from energy crops with potential biofuel production is important. The substrates indicate that the compounds present and their concentrations in the fermentation medium favour enhanced ethanol productivity.

Authors' Contribution

Maria do Socorro Mascarenhas Santos contributed to the execution of the experiment, data collection, analysis and interpretation of results, writing of the manuscript and final correction of the manuscript. Cesar Jose da Silva contributed to the writing of the manuscript. Sandra Helena da Cruz contributed to the writing of the manuscript. Margareth Batistote contributed to analysis and interpretation of results, writing of the manuscript and final correction of the manuscript. Claudia Andrea Lima Cardoso contributed to the data analysis and final correction of the manuscript.

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