Scientific Electronic Archives
Issue ID: Sci. Elec. Arch. Vol. 16 (3)
March 2023
DOI: http://dx.doi.org/10.36560/16320231670
Article link: https://sea.ufr.edu.br/SEA/article/view/1670

ISSN 2316-9281

Evaluation of stress factors in the metabolism of Pedra-2 yeast

Corresponding author
Nicole Carneiro Coertjens
Universidade Estadual de Mato Grosso do Sul
nicoleccoertjens@outlook.com

Maria do Socorro Mascarenhas
Universidade Estadual de Mato Grosso do Sul

Rebeca Fasioli Silva
Universidade Estadual de Mato Grosso do Sul

Margareth Batistote
Universidade Estadual de Mato Grosso do Sul

Abstract. The objective of this study was to evaluate the composition of the fermentation substrate as well as the effect of stress factors on the metabolism of Pedra-2 yeast. An exploratory survey was conducted for the composition of fermentable carbohydrates present in sugarcane juice. For the analyses of mineral and metal content, acid digestion of organic matter was used, determined by flame atomic absorption whit a spectroscope. The pre-inoculum was prepared whit 0.10g of the freeze-dried yeasts that were inoculated and diluted in sterile saline solution. The bioreactor with 125mL Erlenmeyer flasks, in which 50mL of sterilized sugarcane broth was added and adjusted to concentrations of 18, 25 and 32°Brix, to which the yeast colonies were inoculated with the help of a platinum loop and incubated at 30 and 40°C. Samples were collected at different times to evaluate biomass production through spectrophotometry at 570nm and cell viability by counting in a Neubauer chamber with methylene blue dye. The present study points out that the associated stress factors interfere with the studied microorganism's metabolism.
Keywords: Temperature, sugar concentration, *Saccharomyces cerevisiae*, fermentation process.

Introduction

The global energy matrix is based on non-renewable sources derived from oil, natural gas, and coal, with an average of 80% of the total energy generated according to the International Energy Agency (IEA, 2018). This high dependency rate has raised concerns due to the possible exhaustion of these sources and the high levels of pollutant emissions into the atmosphere according to the Empresa de Pesquisa Energética (EPE, 2018).

The growing need for energy and the changes aimed at minimizing the release of the gases that are the precursors of global warming has driven the search for renewable energy sources that are both environmentally friendly and economically viable. One successful global example is ethanol, a biofuel that can be produced from different biomasses or energy crops (Santos et al., 2022) and can partially or even completely replace fossil fuels.

In fact, these changes are occurring gradually, and in Brazil the production of ethanol shows itself as a strategy to reduce the emission of pollutants into the atmosphere. This country has already included ethanol in its energy matrix. At the COP21 conference, Brazil committed to reduce greenhouse gas emissions until 2025 to 37% and until 2030 to 43% (Tolmasquim et al., 2016). These rates will only be achieved by producing and using energy from renewable sources (Ali et al., 2019).

According to Silva et al. (2016), Brazil has the conditions to meet these goals, since it has abundant transformable biomass that can meet energy demands. This country is considered the largest producer of ethanol fuel based on sugarcane juice and is also the pioneer in using this biofuel on a large scale, through a consolidated and economically viable process (Lima et al., 2020).

However, to ensure significant productivity numbers, some care is needed, the main one being the choice

of the yeast strain. For this type of process, selected Saccharomyces cerevisiae yeasts are used, which must have attributes such as tolerance to stress levels in the fermentation medium (Lopes et al., 2016). According to Saini et al. (2018), there is a relationship between the ability that the yeast presents to adapt to the process and its fermentative efficiency.

Thus, the extreme conditions that are known as stress factors can interfere with the metabolism of these microorganisms and result in the loss of ethanol productivity (FAVARO et al., 2019). According to De Souza et al. (2018), the factors present in the vats act in association and corresponding to the severity trigger changes in the physiology and metabolic capacity of the yeasts.

The tolerance to different levels and conditions of stress is a key characteristic for the targeting of microorganisms for biotechnological processes. However, despite the great technological advances and the vast knowledge about the metabolism and the productive potential of yeasts, there are still many unknown aspects, especially when it comes to the genetics and biochemistry of these microorganisms and also about their physiological behavior under different levels of stress and other associated factors.

Given the above, this study aims to evaluate the fermentative substrate composition as well as the effect of stress factors on the metabolism of Pedra-2 yeast.

Materials and Methods

Place of study development

The study was developed in the Biotechnology, Biochemistry and Biotransformation Laboratory of the Centro de Estudos em Recursos Naturais-CERNA da Universidade Estadual de Mato Grosso do Sul-UEMS/Dourados-MS.

Sugarcane juice evaluation

An exploratory survey of the composition of carbohydrates present in sugarcane juice was performed. For this research, published databases related to the theme were considered. According to Araújo and Alvarenga (2011), the exploratory research contributes to the understanding of the phenomena researched, are focused on the themes in the order of importance as to their content.

Obtaining and characterization of the substrate

The sugarcane juice was collected in a mill in the region of Grande Dourados - MS. They were transported at -4°C to the laboratory, filtered in cotton and gauze for purification.

For the mineral and metal content analyses, an oxidation of the organic matter was carried out using 25mL of the sample in digestion tubes, and 20mL of nitric acid (65-70%) was added and after 30 min, the sample was placed in the digestion block and heated to 100°C ± 10°C for 1 hour. The tubes were inserted into the digestion block and heated to 100°C ± 10°C for 1 hour, then the temperature was raised to 180°C ± 10°C and 10mL of the nitroperchloric solution (3:1) was added. After volatilization another 10mL of nitroperchloric solution was added. When the mixture exhaled white smoke, the digestion was finished. The samples were diluted in 50mL of deionized water and the mineral and metal contents were determined by flame atomic absorption with a Varian 220FS spectroscope.

Fermentation conditions

The pre-inoculum was made with 0.10 g of lyophilized yeasts diluted in sterile saline solution (0.85%) and spread with the help of a swab on Petri plates previously prepared with the solid Sabouraud Dextrose Agar medium, and incubated at 30°C for 48 hours.

The sugarcane juice was prepared and the concentration of soluble solids was adjusted to concentrations of 18, 25 and 32°Brix by evaporation and checked by a portable refractometer and the pH adjusted with the aid of a pH meter to 5.0 with the addition of hydrochloric acid (1 mol L^{-1}). For the fermentation medium 50mL of sugarcane juice were added in 125mL Erlenmeyer flasks and with the help of a platinum loop, yeast colonies were inoculated in the medium and remained incubated at 250rpm at temperatures 30, 40°C and at different cultivation times samples were collected for the evaluation of fermentative parameters.

Cell growth

Cell growth analyses were performed by spectrophotometer measurements at a wavelength of 570nm (Batistote et al., 2010).

Cell viability

Viability was monitored using the methylene blue method (Lee et al., 1981). Considering that viable cells remain colorless, while non-viable cells present blue coloration.

Data analysis

The data obtained were treated and analyzed with Excel 2016 and Statistica 7 software. All experiments were performed in triplicate.

Results and discussion

The sugarcane juice is a favorable medium for the yeast development, because it presents direct fermentation carbohydrates, which favor the conversion into ethanol. It can be analyzed that the sugarcane juice presented the highest sucrose content with 20°Brix and the lowest concentrations of glucose and fructose. The highest concentration of minerals were potassium with 265.6mg/mL and sulfur with 24.9mg/mL, being iron the most abundant metal with 128.5mg/mL (Figures 1A and 1B). However, the composition of sugarcane juice varies according to the type of soil, cultivar and cultural treatments

The content of fermentable sugars as well as the presence of other compounds can range in the composition of sugarcane juice. Such molecules are important for ethanol conversion and in yeast metabolism, acting directly on fermentative efficiency (SILVA et al., 2020).

High sugar concentration fermentations (HGS) not only aim at higher ethanol production, but also provide economic, environmental and technical advantages, sucrose being an important molecule to contribute in high sugar concentration fermentations (WALKER and WALKER, 2018; RODIONOVA et al., 2021). However, there is still no fermentable substrate that is excellent in its composition and concentration, which can supply all the metabolic needs of yeast.

The presence of minerals and metals in sugarcane juice are important for the maintenance of yeast metabolism during the fermentation process. Cations such as Ca^{2+}, Mg^{2+}, K^{+}, Na^{+} and anions such as Cl^{-} and SO_4^{2-} can have severe effects on yeast growth and ethanol production.

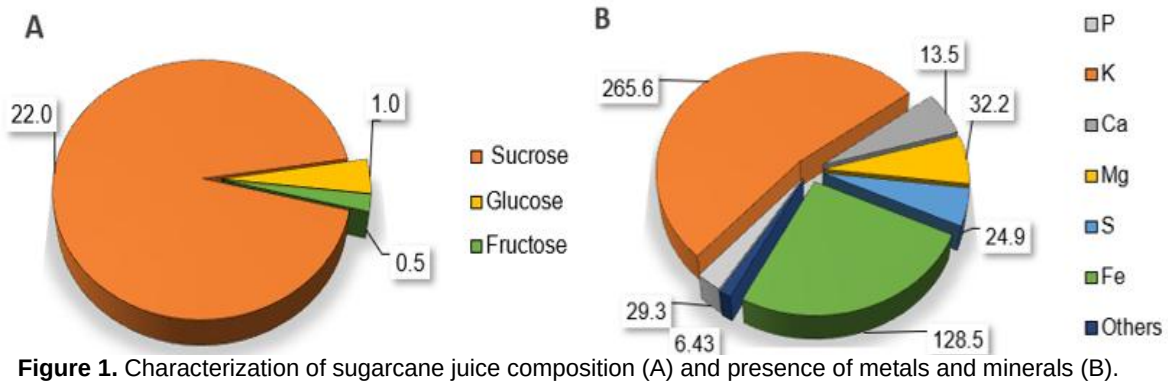

Figure 1. Characterization of sugarcane juice composition (A) and presence of metals and minerals (B).

In the evaluation of the action of thermal and osmotic stress, it can be observed that there was an alteration in the yeast physiology in the yeast Pedra-2. It was noted that at 30°C there was a better cell growth, however, at higher temperature and prolonged fermentation time, the yeast showed a sensitivity, which may be related to the synergism of the factors of stress (Figures 2A and 2B). The data suggest that the optimal growth condition for this yeast was at the concentration between 18 to 25°Brix, for the lowest temperature.

The fermentation process for ethanol production constitutes an environment in which yeast are exposed to several factors that can compromise their metabolic functions, such as the composition of the culture medium, pH, the presence of chemicals, osmotic dehydration, nutritional status and growth phase, with high temperatures being considered one of the most severe factors in this medium (SANTOS et al.,2015; TECHAPARIN et al., 2017).

S. cerevisiae yeasts used in the bioethanol production process have an optimal growth range, around 29 to 34°C (LIP et al., 2020). According to Caspeta and Nielsen (2015) higher temperatures interfere with yeast cell growth. Thus, it is inferred that the positive results of fermentation depend on the yeast's ability to withstand the numerous stress factors that occur during the fermentative process, and tolerance to heat stress is one of the essential characteristics for cell integrity.

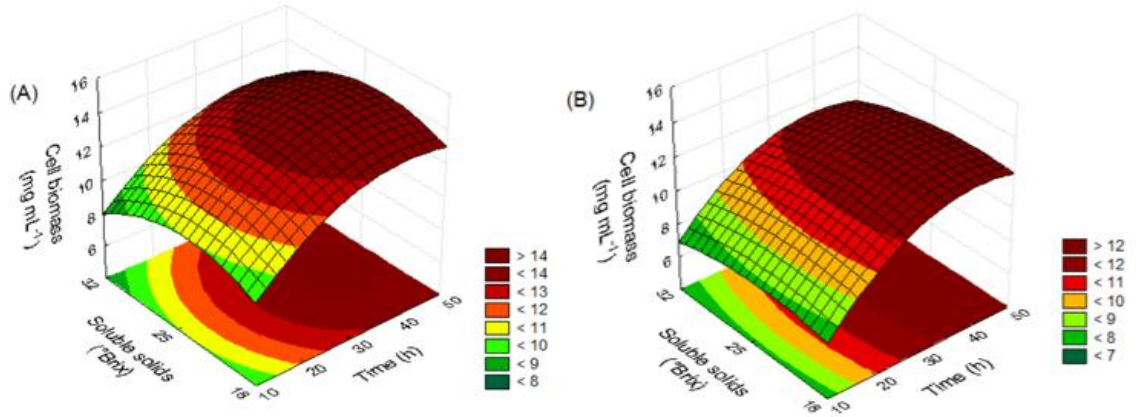

Figure 2. Evaluation of the cell growth of Pedra-2 yeast at 30ºC (A) and 40ºC (B) at different Brix concentrations and fermentation times.

In the evaluation of cell viability, it can be observed that at 30°C the best viability rate was at 25°Brix in 10 hours of fermentation, although this sugar concentration is considered high, possibly the temperature has favored the cellular maintenance of the yeast in this condition. However, at 40°C the best viability rate was at 18°Brix in 10 hours of fermentation. At higher Brix concentrations and longer fermentation times there was a decrease in the analyzed parameter (Figures 3A and 3B).

Yeasts during the fermentation process, are exposed to different perturbations that can act together resulting in cellular stress, among the factors heat stress results in the loss of fermentative efficiency of these microorganisms, and even if the temperature is being controlled oscillations can happen that are influenced by the ambient temperature, especially in summer and in tropical countries (AUESUKAREE, 2017).

For Pereira et al. (2018), cell viability is related to the adaptation of the yeast to the fermentative environment and the conditions of this environment result in a pressure on the metabolic functions of the microorganism which results in the transcriptional response that makes it get used to the effect of heat stress. Some authors consider high temperature as the major problem in fermentation vats, because as seen this stress factor causes changes in the yeast physiology and consequently in the sugar conversion metabolism (EARDLEY and TIMSON, 2020).

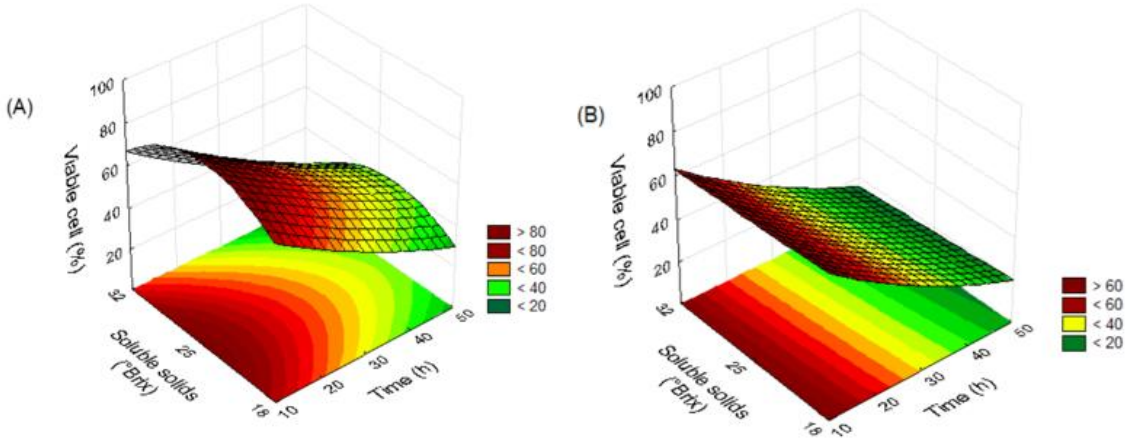

Figure 3. Evaluation of the cell viability of Pedra-2 yeast at 30ºC (A) and 40ºC (B) at different Brix concentrations and fermentation times.

For Brandão (2019), the fermentation vat is an environment, in which yeasts are under the action of numerous stress conditions, such as contamination, high pH rates, osmotic pressure, presence of acids, temperature oscillations, among others. According to the intensity and synergism of these factors the yeast can either adapt to these conditions or succumb to them.

Biochemical and physiological tests such as carbohydrate fermentation, sugar and nitrogen assimilation and growth at different temperatures are necessary for a better characterization of these microorganisms (CHIDI; BAUER and ROSSOUW, 2018).

Conclusion

The ideal condition for the development of the yeast Pedra-2, was between the concentrations 18 to 25°Brix at a temperature of 30°C. In the cell viability rate, the high concentrations and prolonged fermentation time led to a loss of viability. The present study points out that the associated stress factors interfere with the studied microorganism's metabolism.

Acknowledgment

The Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul/FUNDECT, Financiadora de Inovação e Pesquisas/FINEP, Conselho Nacional de Desenvolvimento Científico e Tecnológico/CNPq; Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil/CAPES (Código 001) for the scholarships granted to MSM and RFS.

References

ALI, Ghaffar et al. Quantitative assessment of energy conservation and renewable energy awareness among variant urban communities of Xiamen, China. Renewable and Sustainable Energy Reviews, v. 109, p. 230-238, 2019. DOI: https://doi.org/10.1016/j.rser.2019.04.028

ARAÚJO, R. F.; ALVARENGA, L. A bibliometria na pesquisa científica da pós-graduação brasileira de 1987 a 2007. Encontros Bibli: Revista Eletrônica de Biblioteconomia e Ciência da Informação, v. 16, p.51-70, 2011. DOI: https://doi.org/10.5007/1518-2924.2011v16n 31p51

AUESUKAREE, Choowong. Molecular mechanisms of the yeast adaptive response and tolerance to stresses encountered during ethanol fermentation. Journal of Bioscience and Bioengineering, v. 124, n. 2, p. 133-142, 2017. DOI: https://doi.org/10.1016/j.jbiosc.2017.03.009

BATISTOTE, Margareth et al. Desempenho de leveduras obtidas em indústria de Mato Grosso do Sul na produção de etanol em mosto a base de cana de açúcar. Ciência e Natura, v.2, p. 83-95, 2010.

BRANDÃO, Ana Carolina Tolentino. Avaliação do processo de fermentação alcoólica em condições de altas concentrações de açúcares empregando leveduras de características floculantes. 2019. 95 f. Dissertação (Mestrado em Engenharia Química) - Universidade Federal de Uberlândia, 2019. DOI http://dx.doi.org/10.14393/ufu.di.2019.2246

CASPETA, Luis; NIELSEN, Jens. Thermotolerant yeast strains adapted by laboratory evolution show trade-off at ancestral temperatures and preadaptation to other stresses. MBio, v. 6, p. 0431-15, 2015. DOI: https://doi.org/10.1128/mBio.00431-15

CHIDI, B. Silas; BAUER, F. F.; ROSSOUW, D. Organic acid metabolism and the impact of fermentation practices on wine acidity: A review. South African Journal of Enology and Viticulture, v. 39, n. 2, p. 1-15, 2018. DOI: http://dx.doi.org/10.21548/39-2-3164

DE SOUZA, Jonas Paulino et al. Improvement of Brazilian bioethanol production – Challenges and perspectives on the identification and genetic modification of new strains of Saccharomyces cerevisiae yeasts isolated during ethanol process. Fungal Biology, v. 122, p. 583-591, 2018. DOI: https://doi.org/10.1016/j.funbio.2017.12.006

EARDLEY, Joshua; TIMSON, David J. Yeast cellular stress: impacts on bioethanol production. Fermentation, v. 6, p. 109, 2020. DOI: https://doi.org/10.3390/f ermentation6040109

EPE – Empresa de Pesquisa Energética 2018. Balanço Energético Nacional. Disponível em: http://epe.gov.br-pt/publica%C3%A7%C3%B5es-dados-bertos/Publicacoes arquivos/publica%C3%A7%C3%A3o-303/t%C3%B3pico-4 19/Ben2018Int.pdf. Accessed in September 9, 2022

FAVARO, Lorenzo; JANSEN, Trudy; VAN ZYL, Willem Heber. Exploring industrial and natural Saccharomyces cerevisiae strains for the bio-based economy from biomass: the case of bioethanol. Critical Reviews in Biotechnology, v. 39, p. 800-816, 2019. DOI: https://doi.org/10.1080/07388551.2019.1619157

IEA – International Energy Agency 2018. Energy Statistics Data Browser. Disponível em: https://www.iea.org/s tatistics/efficiency/. Accessed in September 9, 2022

LEE, S. S. et al. Rapid determination of yeast viability. In: Biotechnol. Bioeng. Symp.;(United States). Univ. of Michigan, Ann Arbor, 1981.

LIMA, M. A. et al. Renewable energy in reducing greenhouse gas emissions: Reaching the goals of the Paris agreement in Brazil. Environmental Development, v. 33, p. 100504, 2020. DOI: https://doi.org/10.1016/j.envdev. 2020.100504

LIP, Ka Ying Florence et al. Selection and subsequent physiological characterization of industrial Saccharomyces cerevisiae strains during continuous growth at sub-and-supra optimal temperatures. Biotechnology Reports, v. 26, p. 0462, 2020. DOI: https://doi.org/10.1016/j.btre. 2020.e 00462

LOPES, Mario Lucio et al. Ethanol production in Brazil: a bridge between science and industry. Brazilian Journal of Microbiology, v. 47, p. 64-76, 2016. DOI: https://doi.org/10.1016/j.bjm.2016.10.003

PEREIRA, Tania. et al. Quantitative operating principles of yeast metabolism during adaptation to heat stress. Cell reports, v. 22, p. 2421-2430, 2018. DOI: https://doi.org/10.1016/j.celrep.2018.02.020

RODIONOVA, Margarita V. et al. A comprehensive review on lignocellulosic biomass biorefinery for sustainable biofuel production. International Journal of Hydrogen Energy, v. 47, p. 1481-1498 2021. https://doi.org/10.1016/j.ijhydene.2021.10.122

SAINI, Priyanka et al. Response and tolerance of yeast to changing environmental stress during ethanol fermentation. Process Biochemistry, v. 72, p. 1-12, 2018. DOI: https://doi.org/10.1016/j.procbio.2018.07.001

SANTOS, Líbia Diniz et al. Continuous ethanol fermentation in tower reactors with cell recycling using flocculent Saccharomyces cerevisiae. Process Biochemistry, v. 50, n. 11, p. 1725-1729, 2015. DOI: https://doi.org/10.1016/j.procbio.2015.07.020

SANTOS, Maria do Socorro Mascarenhas et al. Energy cultures and sustainability in biofuel production. Revista de Agricultura Neotropical, v. 9, n. 1, p. e6719-e6719, 2022. DOI: https://doi.org/10.32404/rean.v9i1.6719

SILVA, Rebeca Fasioli et al. The composition of sacarine substrates for ethanol production and the fermentative capacity Saccharomyces cerevisiae

Pedra-2. Research, Society and Development, v. 9, n. 11, p. e44891110235-e44891110235, 2020. DOI: https://doi.org/10.33448/rsd-v9i11.10235

SILVA, Wellington Costa et al. Thermal degradation of ethanolic biodiesel: Physicochemical and thermal properties evaluation. Energy, v. 114, p. 1093-1099, 2016. DOI: https://doi.org/10.1016/j.energy.2016.08.052

TECHAPARIN, Atiya. et al. High-temperature ethanol production using thermotolerant yeast newly isolated from Greater Mekong Subregion. Brazilian Journal of Microbiology, v. 48, p. 461-475, 2017. DOI: https://doi.org/10.1016/j.bjm.2017.01.006

TOLMASQUIM, M. T. et al. The Brazilian commitment to combating climate change: energy production and use. Empresa de Pesquisa Energética-EPE, Rio de Janeiro, Brazil, p. 1-96, 2016.

WALKER, Graeme M.; WALKER, Roy SK. Enhancing yeast alcoholic fermentations. Advances in Applied Microbiology, v. 105, p. 87-129, 2018. DOI: https://doi.org/10.1016/bs.aambs.2018.05.003