HOLOS
ISSN 1807 - 1600

THE ACTION OF LIGHT ON *Saccharomyces cerevisiae* METABOLISM UNDER DIFFERENT CULTURE CONDITIONS

M.S.M. SANTOS, E.M. SILVA, C.A.L. CARDOSO, M. BATISTOTE
Universidade Estadual de Mato Grosso do Sul[1,2,3,4]
http://orcid.org/0000-0002-5343-4502[1]
maria_mascarenhas@outlook.com[1]

Submetido 17/04/2020 - Aceito 09/04/2022

DOI: 10.15628/holos.2022.10750

ABSTRACT

Although there are several studies using yeasts, little is known about the physiological behaviour of yeasts Saccharomyces cerevisiae, mainly about industrial strains that are responsible for the production of ethanol and its adaptation to different environments. Thus, this study aims to evaluate the influence of the action of white and ultraviolet light associated with other stress factors in the fermentative medium and to analyze the fermentative performance of yeast FT-858 under different growing conditions. The yeast was grown in liquid medium (2% YPD) at 30°C for 12 hours at 250 rpm. After this period, the cells were recovered by centrifugation and inoculated into the fermentation medium based on sugarcane juice and sweet sorghum at concentrations of 18, 28 and 32° Brix, which were incubated at 30 and 42°C for 10 hours at 250 rpm, under the action of white light (BR), ultraviolet (UV) and without light (S / L), promptly analysing the physiological parameters. The fermentative performance of the yeast was affected when exposed to UV light at the highest Brix concentration and high temperature.

KEYWORDS: Yeasts, Physiological response, Sweet sorghum, Sugarcane.

AÇÃO DA LUZ SOBRE O METABOLISMO DE *Saccharomyces cerevisiae* EM DIFERENTES CONDIÇÕES DE CULTIVO

RESUMO

Embora haja vários estudos utilizando leveduras pouco se conhece em relação ao comportamento fisiológico das leveduras *Saccharomyces cerevisiae*, principalmente sobre as linhagens industriais que são responsáveis pela produção de etanol e a sua adaptação a diferentes ambientes. Assim este estudo visa avaliar a influência da ação da luz branca e ultravioleta associados a outros fatores de estresse do meio fermentativo e analisar a performance fermentativa da levedura FT-858 em diferentes condições de cultivo. A levedura foi cultivada em meio líquido (YPD 2%) a 30°C por 12 horas a 250 rpm. Após este período as células foram recuperadas por centrifugação e inoculada no meio fermentativo a base de caldo de cana e sorgo sacarino nas concentrações de 18, 28 e 32°Brix que foram incubados a 30 e 42°C por 10 horas a 250 rpm, sob a ação da luz branca (BR), ultravioleta (UV) e sem luz (S/L) sendo prontamente analisados os parâmetros fisiológicos. A performance fermentativa da levedura foi afetada quando exposta a luz UV na concentração de Brix mais elevada e alta temperatura.

PALAVRAS-CHAVE: Leveduras, Resposta fisiológica, Sorgo sacarino, Cana-de-açúcar.

HOLOS
ISSN 1807 - 1600

1. INTRODUCTION

The strains of *Saccharomyces cerevisiae* used by the sugar-energy sector must be tolerant to the levels of stress in the environment and have, in addition to high ethanol production and resistance to inhibitory compounds (Hahn-Hagerdal et al., 2007). The use of more robust yeasts that can survive in industrial conditions and compete with wild yeasts and bacterial contamination, constituting a strategy to guarantee the productivity of the fermentation process (Amorim, Gryschek & Lopes, 2010; Fiedurek, Skowronek & Gromada, 2011).

Thus, according to Hirasawa et al. (2007), the good performance of the fermentation depends on the capacity that the yeasts used in the fermentation have against the countless stress factors that occur during the process. The criteria that permeate the choice of the yeast strain to be used in alcoholic fermentation are intrinsic in the responses to the conditions present in the process and its rapid adaptation (Zhao & Bai, 2009). Such factors as temperature, pH, substrate concentration that can suffer synergism, affecting yeast cells more severely, reducing the viability and directly interfering with ethanol production (Bai, Anderson & Moo-Young, 2008).

The industrial fermentation process for the production of ethanol occurs in closed vats in the absence of light, where they exposed to the numerous stressors present in this place. These conditions imposed by the fermentative medium can cause changes both ultrastructural, morphological and functional as well as acting in synergism with physical and chemical agents can cause changes in the structure of deoxyribonucleic acid - DNA (Guerrero-Beltrán & Barbosa-Cánovas, 2004). In this sense, light sources and the variation in their intensity can alter the metabolism of cells, influencing the production and accumulation of metabolites in these microorganisms (Valduga et al., 2009). Thus, according to Hirasawa et al. (2007), the good performance of the fermentation depends on the capacity that the yeasts used in the fermentation have against the countless stress factors that occur during the process. The criteria that permeate the choice of the yeast strain to use in alcoholic fermentation are intrinsic in the responses to the conditions present in the process and its rapid adaptation (Zhao & Bai, 2009). Such factors as temperature, pH, substrate concentration that can suffer synergism, affecting the yeast cells more severely, reducing the viability and directly interfering with ethanol production (Bai, Anderson & Moo-Young, 2008).

Studies by Sakaki et al. (2001), for the production of carotenoids; in which a white light of low intensity was applied in a non-pigmented yeast and in a wild strain during its growth, observed that the non-pigmented yeast was not affected, that is, there was no growth inhibition, however, in wild yeast both prevented its growth and stimulated the production of the metabolite. Light can cause changes in the metabolic pathways of yeasts, as some cells are devoid of mechanisms that promote the absorption of photon energy (Robertson, Davis & Johnson, 2013). According to Tisch & Schmoll (2010) the photon energy of visible light can cause an adjustment both in the cellular development and in the metabolism of these microorganisms.

As well as the ultraviolet irradiations between the range of 210 and 330 nm are considered to be efficient germicides; as they penetrate the cell acting directly on proteins and nucleic acids, which causes a reaction of disruption of chromosomal bases resulting in gene mutations and

HOLOS
ISSN 1807 - 1600

inactivation of enzymes leading to cell death (López-Malo & Palou, 2005). In addition, the light source and the intensity of its photons can represent a stress factor for microorganisms that do not perform photosynthesis (Bodvard et al., 2013).

Thus, despite great technological advances and vast knowledge about the metabolism and the productive potential of yeasts, there are still many unknown aspects. Mainly when it comes to the genetics and biochemistry of these microorganisms that are widely used in fermentation processes in plants and also as their physiological behaviour in the face of different levels of stress and other associated factors that can cause damage to these cells. In view of the above, this study aims to evaluate the influence of the action of white and ultraviolet light associated with fermentative stress factors and to analyze the fermentative performance of FT-858 yeast under different growing conditions.

2. METODOLOGIA

2.1. Substrate collection and preparation

The sugarcane juice was obtained directly from the process of a mill in the region of Grande Dourados and the sweet sorghum juice obtained from Embrapa Agropecuária Oeste-Dourados. The extraction was carried out by grinding and packed in sterile bottles and transported at 4 ° C to the Laboratory of Biotechnology, Biochemistry and Biotransformation of the Centro for the Study of Natural Resources-CERNA of the State University of Mato Grosso do Sul - UEMS / Dourados-MS. The material was filtered with a view to maximum removal of impurities. The total soluble solids were concentrated at 18, 28 and 32 ° Brix by evaporation using a portable refractometer. The pH was adjusted to 5.0 with 1 mol L^{-1} hydrochloric acid.

2.2. Microorganism used

The yeast used in this study was *Saccharomyces cerevisiae*: FT-858, available in the collection of microorganisms from the Laboratory of Biotechnology, Biochemistry and Biotransformation of the Centro for the Estudos of Recursos Naturais-CERNA of the Universidade Estadual de Mato Grosso do Sul - UEMS / Dourados-MS.

2.3. Pre-inoculum and fermentative condition

A pre-inoculum was made using the liquid medium YPD 2% (containing 1.0% m v^{-1} of yeast extract; 1.0% m v^{-1} of peptone and 2.0% m v^{-1} of glucose) which was sterilized at 120 ° C for 20 minutes and 0.10 grams of lyophilized yeast was inoculated. The flasks were incubated at 30 ° C for 12 hours at 250 rpm. After this period, the cells were recovered by centrifugation (800g, 20min), resuspended and washed with sterile saline solution (0.85%), resulting in a final concentration of 10 mg mL^{-1} of wet mass that was promptly inoculated in 50 mL sterilized sorghum and cane juice in concentrations of 18, 28 and 32 ° Brix in 125 mL flasks of Erlenmeyers, which were incubated for 10 hours at temperatures of 30 ° and 42 ° C at 250rpm under the influence of light sources.

HOLOS
ISSN 1807 - 1600

2.4. Action of light sources

For the analysis of the action of light sources in the development of yeast; the environments adapted with a lamp holder. Attached to the shaker that remained at 45 cm in height with an incidence of light on the samples for a period of 10 hours. The light environments used were white (BR), ultraviolet (UV) and without light (S / L). With Philips brand lamp (15W) for the action of white light and for ultraviolet light a Towalight brand lamp (15W), as well as in the absence of the light source.

2.5. Analytical methods

Cell growth analyses performed using spectrophotometric measurements at 570nm, correlated with a calibration curve according to the method of (Batistote et al., 2010). Cell viability was determined using the methylene blue counting method (Lee, Robinson & Wang, 1981) and the ethanol concentration determined in the gas chromatograph CG 3900 with flame ionization detector (Varian), according to the one described by Batistote et al. (2010).

2.6. Statistical analysis

Excel 2016 and Statística version 8.0 software used, and the results for the evaluation of the influence of light sources on yeast physiology presented by the Principal Component Analysis (PCA). All experiments carried out in triplicate.

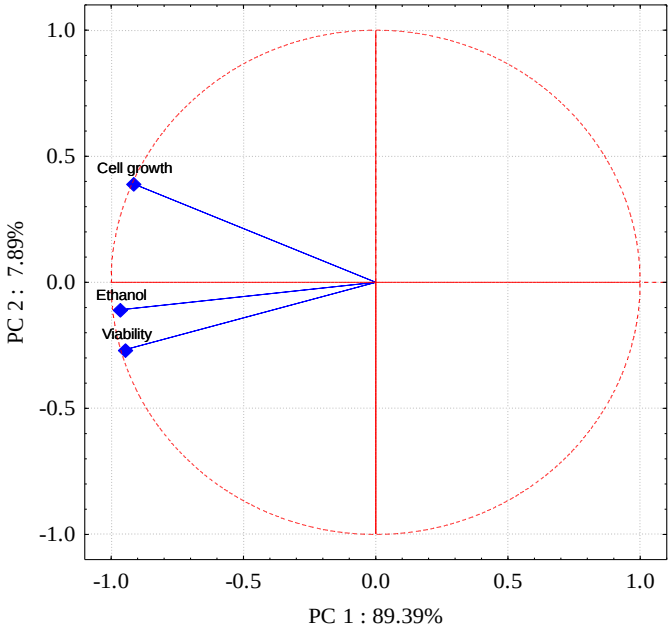
3. RESULTADOS e DISCUSSÕES

In the evaluation of the action of light, it resulted in a PCA model with two main factors that presented a total variation of 97.28%, resulting in a grouping that allowed to verify the influence of the action of light in the parameters analyzed concerning stress factors. We can see that there was a change in yeast metabolism due to the light environment and the time it was exposed (Figure 1A). The yeast FT-858 wasn't affected by the action of light in the concentrations of 18 and 28 ° Brix, when exposed to the action of white light and in the environment without light at a temperature of 30ºC. At the 32 ° Brix concentration, viability was affected, resulting in a lower ethanol concentration compared to the other environments analyzed, perhaps this was due to the high concentration of the substrate providing catabolic repression causing alteration of cellular metabolism.

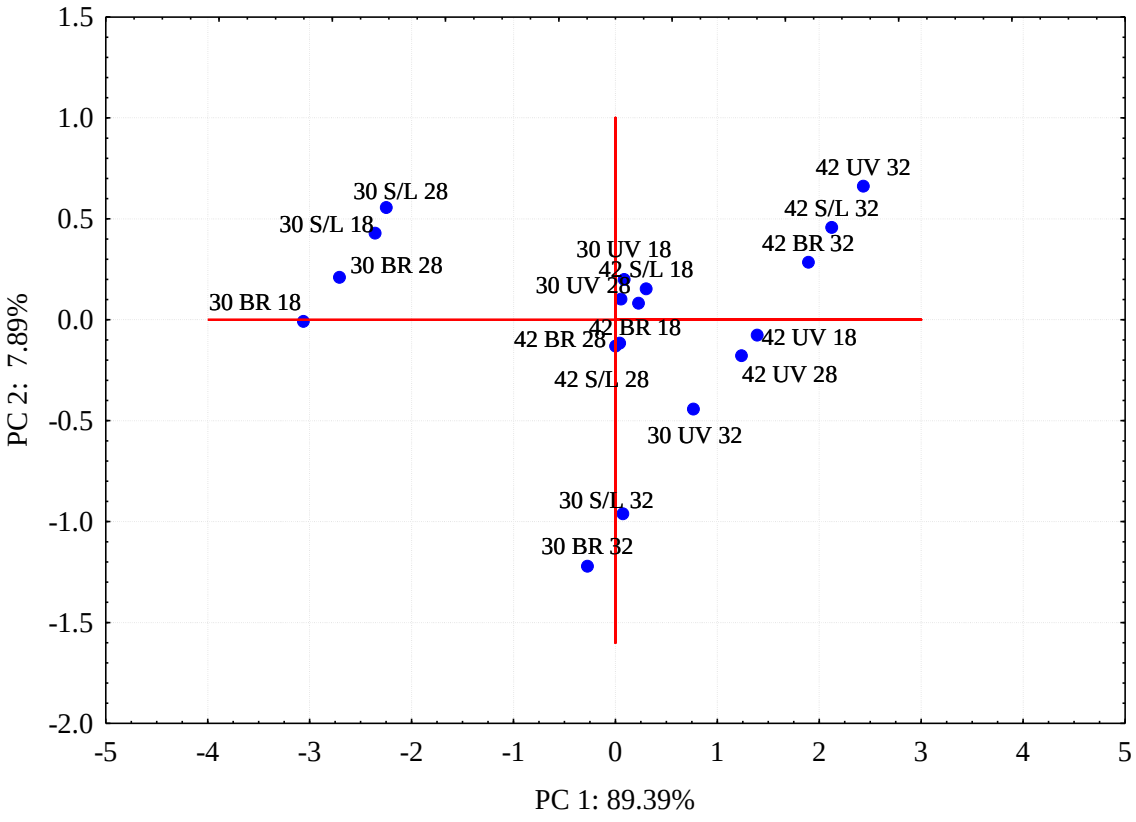
In the presence of ultraviolet light, we can observe the clustering in the opposite quadrant at all substrate concentrations and temperatures analyzed. Ethanol production important fermentative parameters (Figures 1A and 1 B).

For Mardia et al. (1979), the first two or three components of the PCA when added together should present value above 70% in the composition of the total variation of the data, considered as a suitable model. According to Gomes et al. (2017), this type of analysis allows for a clearer and more concise interpretation and explanation of the data variation, especially when submitted to the grouping.

HOLOS
ISSN 1807 - 1600



(A)



(B)

Figure 1. Principal Component Analysis (PCA) of the metabolism of industrial yeast FT-858 (A), under the action of different light sources grown in sugarcane juice at temperatures of 30 and 42 ° C (B).

According to Shima &Takagi (2009), yeasts used in industrial processes may have their fermentative capacity affected due to stress conditions and, given the intensity of the numerous stress factors combined, they can cause changes in the plasma membrane and consequently cause a budding decrease and loss in fermentative efficiency. Yeast cells have a set of mechanisms to remain integrated, thereby ensuring a balance of their functions, with which they can adapt to the environment or succumb to it (Bodvard et al., 2013).

Exposure of yeast to ultraviolet light also composes a stress factor that affects yeast directly in the viability and bioconversion parameters from glucose to ethanol. This source of radiation can break hydrogen bonds and alter nitrogen bases causing photobio - chemical changes to the nucleic acid resulting in the inactivation of cells (Billota & Daniel, 2010). *Saccharomyces cerevisiae* can respond to light stimuli with physiological and metabolic consequences, so the irradiation of light implies both the speed of growth and the formation of sprouts, interfering with the integrity of the cell wall and causing cell death (Robertson, Davis & Johnson, 2013).

Ultraviolet light is a physical agent capable of damaging deoxyribonucleic acid and when absorbed it induces changes in the DNA bases, causing injuries and distortions in the structure of this macromolecule, compromising the vital mechanisms for the cell promoting a physical block of the replication and transcription machinery of the DNA and consequently in cellular metabolism (Tornaletti, 2009).

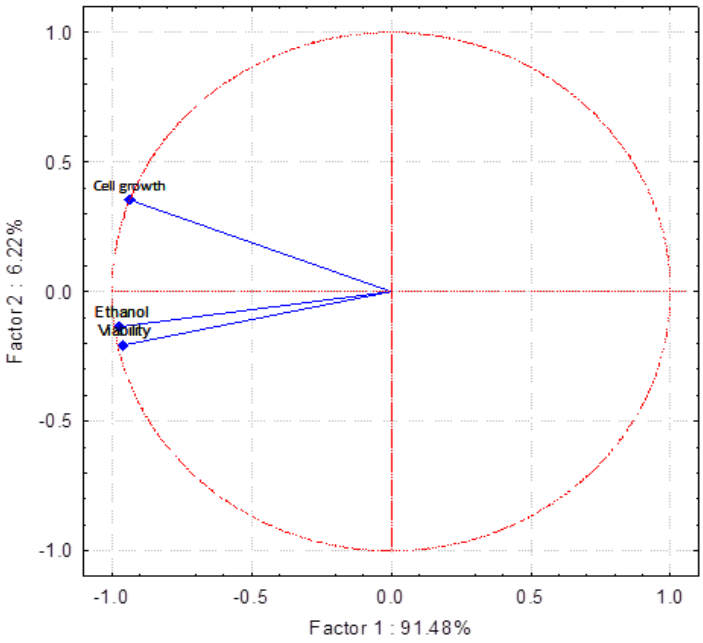
In the evaluation of FT-858 yeast grown in sorghum broth, the PCA obtained showed a total variation of 97.70% (Figure 2A). It can observe that the growth of yeast in concentrations of 18 and 28 ° Brix, when exposed in favourable environments of a light source at a temperature of 30 ° C, presented a similar metabolic profile regardless of the saccharine substrate used.

In the action of ultraviolet light, grouping occurred in the opposite quadrant at all concentrations and temperatures analyzed, this possibly has occurred due to the associated stress factors such as high substrate concentration and temperature, as well as in the action of UV light, providing the yeast to suffer more from the action of fermentative stress (Figure 2A and 2B). UV light, because it is mutagenic, may have reached deoxyribonucleic acid - DNA, causing injury and compromising genomic stability, as well as stress factors that have induced a faster response of yeast to cell stress, consequently compromising cell viability and ethanol production, interrelated parameters.

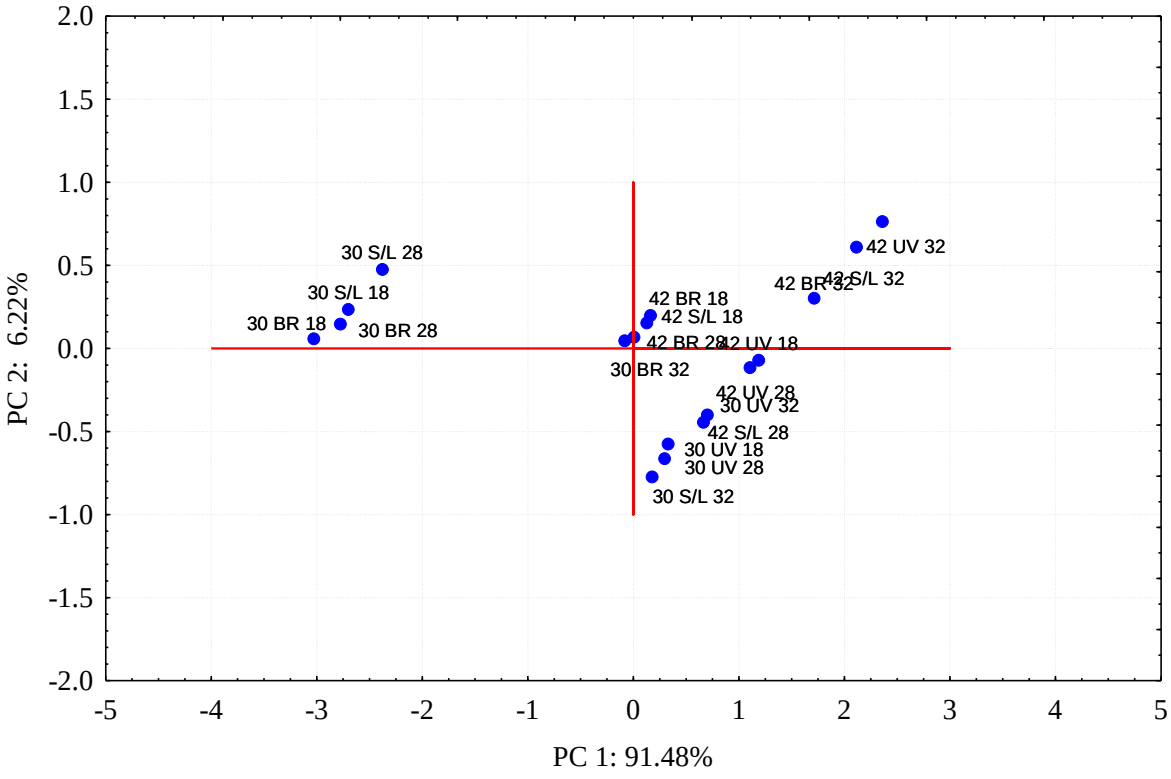
Ultraviolet light is a physical agent capable of damaging deoxyribonucleic acid and when absorbed it induces changes in the DNA bases, causing injuries and distortions in the structure of this macromolecule, compromising the vital mechanisms for the cell promoting a physical block of the replication and transcription machinery of the DNA and consequently in cellular metabolism (Tornaletti, 2009). Still, according to Cruz et al. (2015), temperature affects metabolism differently, interfering with its sprouting, thus constituting one of the factors that can interfere in the

HOLOS
ISSN 1807 - 1600

fermentation process. Yeasts present their metabolic efficiency in the temperature range between 25 ° C and 32 ° C (Sousa & Monteiro, 2011).



(A)



HOLOS
ISSN 1807 - 1600

(B)

Figure 2. Principal component analysis (PCA) in the metabolism of industrial yeast FT-858 (A). Under the action of different light sources grown in sorghum broth at temperatures of 30 and 42 ° C (B).

4. CONCLUSÃO

The present study showed that yeast FT-858, independent of saccharine substrates and the action of light, showed fermentative efficiency and was capable of producing ethanol.

In the evaluation of the action of the different light sources, the PCA presented two groups which showed physiological changes in the analysed yeast, in which under the action of white light and the environment without light did not provide cellular stress in the yeast. However, high concentrations of the substrate and the temperature associated with the action of ultraviolet light will induce yeast to respond to fermentative stress, compromising viability and ethanol production.

5. ACKNOWLEDGMENTS

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) (311975/2018-6 CALC); Programa Institucional de Bolsas aos Alunos de Pós-Graduação (PIBAP) da Universidade Estadual de Mato Grosso do Sul (UEMS); Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) –Código 001.

6. Referências bibliográficas

Amorim, H. V., Gryschek, M., & Lopes, M. L. (2010). The success and sustainability of the Brazilian sugarcane− fuel ethanol industry. In *Sustainability of the Sugar and Sugar− Ethanol Industries* (pp. 73-82). American Chemical Society.

Bai, F. W., Anderson, W. A., & Moo-Young, M. (2008). Ethanol fermentation technologies from sugar and starch feedstocks. *Biotechnology advances*, *26*(1), 89-105.

Batistote, M., Cardoso, C. A. L., Ramos, D. D., & Ernandes, J. R. (2010). 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*, 83-95.

Bilotta, P., & Daniel, L. A. (2012). Utilização de lâmpadas germicidas na desinfecção de esgoto sanitário. *Revista Ambiente & Água*, *7*(1), 120-129.

Bodvard, K., Jörhov, A., Blomberg, A., Molin, M., & Käll, M. (2013). The yeast transcription factor Crz1 is activated by light in a Ca 2+/calcineurin-dependent and PKA-independent manner. *PLoS One*, *8*(1), e53404.

Cruz, M. L., Raminho, M. L. F., Castro, A. L. M., Guidini, C. Z., RESENDE, M. D., & Ribeiro, E. J. (2015). ESTUDO DA INFLUÊNCIA DA TEMPERATURA NA RESISTÊNCIA AO ETANOL DA LEVEDURA *Saccharomyces cerevisae* Y904. *Blucher Chemical Engineering Proceedings*, *1*(2), 2260-2265.

Fiedurek, J., Skowronek, M., & Gromada, A. (2011). Selection and adaptation of *Saccharomyces cerevisae* to increased ethanol tolerance and production. *Pol J Microbiol*, *60*(1), 51-8.

Gomes, P. W. P., Reis, J. D. E., Silva, D. D. S. C., da Costa, A. P. A., Malato, B. V., Muribeca, A. D. J. B., & Gomes, P. W. P. (2017). A aplicação da técnica multivariada (PCA e HCA) em dados microbiológicos e físico-químicos de méis comercializados em Cachoeira do Arari e Salvaterra-PA. *Scientia Plena*, *13*(6).

Guerrero-Beltr· n, J. A., & Barbosa-C· novas, G. V. (2004). Advantages and limitations on processing foods by UV light. *Food science and technology international*, *10*(3), 137-147.

Hahn-Hägerdal, B., Karhumaa, K., Fonseca, C., Spencer-Martins, I., & Gorwa-Grauslund, M. F. (2007). Towards industrial pentose-fermenting yeast strains. *Applied microbiology and biotechnology*, *74*(5), 937-953.

Hirasawa, T., Yoshikawa, K., Nakakura, Y., Nagahisa, K., Furusawa, C., Katakura, Y., ... & Shioya, S. (2007). Identification of target genes conferring ethanol stress tolerance to Saccharomyces cerevisiae based on DNA microarray data analysis. *Journal of biotechnology*, *131*(1), 34-44.

Lee, S. S., Robinson, F. M., & Wang, H. Y. (1981, January). Rapid determination of yeast viability. In *Biotechnol. Bioeng. Symp.; (United States)* (Vol. 11, No. CONF-810554-). Univ. of Michigan, Ann Arbor.

Lopez-Malo, A., & Palou, E. (2005). Ultraviolet light and food preservation. *Novel food processing technologies*, 405-422.

Mardia, K. V. (1979). *Multivariate analysis* (No. 04; QA278, M3.).

Robertson, J. B., Davis, C. R., & Johnson, C. H. (2013). Visible light alters yeast metabolic rhythms by inhibiting respiration. *Proceedings of the National Academy of Sciences*, *110*(52), 21130-21135.

Sakaki, H., Nakanishi, T., Tada, A., Miki, W., & Komemushi, S. (2001). Activation of torularhodin production by *Rhodotorula glutinis* using weak white light irradiation. *Journal of bioscience and bioengineering*, *92*(3), 294-297.

Sousa, J. D., & Monteiro, R. A. B. (2012). Fatores interferentes na fermentação alcoólica para a produção de etanol. *FAZU em Revista*, (08).

Shima, J., & Takagi, H. (2009). Stress-tolerance of baker's-yeast (*Saccharomyces cerevisiae*) cells: stress-protective molecules and genes involved in stress tolerance. *Biotechnology and applied biochemistry*,*53*(3), 155-164.

Tisch, D., & Schmoll, M. (2010). Light regulation of metabolic pathways in fungi. *Applied microbiology and biotechnology*, *85*(5), 1259-1277.

Tornaletti, S. (2009). DNA repair in mammalian cells. *Cellular and Molecular Life Sciences*, *66*(6), 1010-1020.

Valduga, E., Tatsch, P. O., Tiggemann, L., Treichel, H., Toniazzo, G., Zeni, J., ... & Júnior, A. F. (2009). Produção de carotenoides: microrganismos como fonte de pigmentos naturais. *Química Nova*, *32*(9), 2429-2436.

Zhao, X. Q., & Bai, F. W. (2009). Mechanisms of yeast stress tolerance and its manipulation for efficient fuel ethanol production. *Journal of biotechnology*, *144*(1), 23-30.

COMO CITAR ESTE ARTIGO:

Mascarenhas Santos, M. do S., Silva, E. M., Cardoso, C. A. L., & Batistote, M. (2022). THE ACTION OF LIGHT ON Saccharomyces cerevisiae METABOLISM UNDER DIFFERENT CULTURE CONDITIONS. *HOLOS*, *8*. Recuperado de https://www2.ifrn.edu.br/ojs/index.php/HOLOS/article/view/10750

SOBRE OS AUTORES:

M.S.M SANTOS
Programa de Pós-Graduação em Recursos Naturais - PGRN / Centro de Estudos em Recursos Naturais - CERNA
E-mail: maria_mascarenhas@outlook.com
ORCID-ID: http://orcid.org/0000-0002-5343-4502

E.M. SILVA
Docente do Curso de Graduação em Biologia
E-mail: emiliam.silva@yahoo.com.br
ORCID-ID: https://orcid.org/0009-0007-6036-7049

C.A.L. CARDOSO
Docente do Programa de Pós-Graduação em Recursos Naturais - PGRN / Centro de Estudos em Recursos Naturais - CERNA
E-mail: claudiacardosouems1@gmail.com
ORCID-ID: http://orcid.org/0000-0002-4907-0056

M. BATISTOTE
Docente do Programa de Pós-Graduação em Recursos Naturais - PGRN / Centro de Estudos em Recursos Naturais - CERNA
E-mail: margobatistote@gmail.com
ORCID-ID: https://orcid.org/0000-0001-9865-2362

HOLOS
ISSN 1807 - 1600

Editora responsável: Francinaide de Lima Silva Nascimento

Recebido:17 de abril de 2020

Aceito: 09 de abril de 2022

Publicado: 28 de abril de 2022