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Synergistic action of thermal and ethanolic stress in industrial *Saccharomyces cerevisiae* strains

Ação do sinergismo do estresse térmico e etanólico em linhagens de *Saccharomyces cerevisiae* industriais

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RESUMO

Saccharomyces cerevisiae é amplamente utilizada para a produção de alimentos e biocombustíveis, devido à sua eficiência na conversão de substratos. No entanto, fatores de estresse podem ser tóxicos, prejudicando o metabolismo das leveduras. Assim, este estudo visou analisar os impactos do estresse térmico e etanólico em *S. cerevisiae*, Fleischmann® e Pedra-2. Foi feito um pré-inóculo adicionando 0,10 g das leveduras liofilizadas a 1 mL de solução salina estéril (0,85%), e 400 µL foram inoculados em placas de Petri, preparadas com Agar Sabouraud e incubadas a 30°C. As colônias crescidas foram transferidas para meio fermentativo de caldo de cana, a 22 °Brix e pH 5.0, contendo álcool etílico (PA 99,5%) nas concentrações de 0, 8 e 16% ($v.v^{-1}$). Os frascos foram mantidos a 250 rpm nas temperaturas de 30 e 40°C. Amostras foram coletadas em 8 e 16 horas para as análises. A produção de biomassa e viabilidade celular diminuiu com o aumento do etanol e do tempo de fermentação. A linhagem Fleischmann® mostrou maior sensibilidade ao estresse do que Pedra-2. Altas concentrações de etanol e temperaturas prejudicaram o crescimento e a viabilidade das leveduras. O estudo destaca a importância de selecionar leveduras adaptáveis para melhorar a eficiência da fermentação industrial.

Palavras-chave: Leveduras. Fermentação. Condições de estresse. Bioetanol

ABSTRACT

Saccharomyces cerevisiae is widely used for the production of food and biofuels, due to its efficiency in converting substrates. However, stress factors can be toxic, damaging yeast metabolism. Therefore, this study aimed to analyze the impacts of thermal and ethanolic stress on S. cerevisiae, Fleischmann® and Pedra-2. A pre-inoculum was made by adding 0.10 g of lyophilized yeast to 1 mL of sterile saline solution (0.85%), and 400 µL were inoculated into Petri dishes prepared with Sabouraud Agar and incubated at 30°C. The grown colonies were transferred to sugarcane juice fermentation medium, at 22 °Brix and pH 5.0, containing ethyl alcohol (PA 99.5%) at concentrations of 0, 8 and 16% (v.v-1). The flasks were kept at 250 rpm at temperatures of 30 and 40°C. Samples were collected at 8 and 16 hours for analysis. Biomass production and cell viability decreased with increasing ethanol and fermentation time. The Fleischmann® strain showed greater sensitivity to stress than Pedra-2. High concentrations of ethanol and temperatures impaired yeast growth and viability. The study highlights the importance of selecting adaptable yeasts to improve the efficiency of industrial fermentation.

Keywords: Yeasts. Fermentation. Stress conditions. Bioethanol

1. INTRODUCTION

Saccharomyces cerevisiae is among the main microorganisms used in biotechnological processes due to its versatile metabolic profile and adaptable growth conditions. These unique characteristics make this yeast a promising tool for production, ranging from food production to biofuels, among others Batistote and Santos (2020), highlight that these microorganisms are excellent converters of fermentable substrates in bioethanol production processes and can be used to produce secondary compounds, since they have distinct metabolic routes. According to Baptista et al. (2021), these yeasts have stood out and are being used in new processes, becoming the focus of technological innovations, demonstrating its ability to stimulate advances in various areas of knowledge, contributing to the promotion of sustainability and the development of a sustainable green economy.

These microorganisms have an important role, as since ancient times they have been used by humans in various industrial processes due to their efficient ability to bio convert sugars in the production of beverages, bakery, ethanol, among others (AZHAR et al., 2017). In general, the fermentative route is unquestionably the most efficient way to obtain ethanol on a large scale, due to its low cost and high productivity (STAMENKOVIĆ et al., 2020), however, even though industrial fermentations result in a high alcohol yield, this process creates an unfavorable environment for these microorganisms (WALKER and BASSO, 2020), as they are subjected to numerous stress factors. The ability of these microorganisms to deal with these challenges is essential for the efficiency and success of the industrial process, especially on a larger scale such as the production of biofuels (SHARMA et al., 2022).

In the fermentative environment, selective pressures occur, such as high concentrations of ethanol (LAIRÓN-PERIS et al., 2021), osmotic stress, thermal instability, pH variations, contaminating agents, among others, that intersperse and cause changes in the metabolism and physiology of cells, influencing the formation of undesirable metabolites and fermentative efficiency (COERTJENS et al., 2023). Paradoxically, even though ethanol is a product of yeast metabolism, high concentrations in the medium are toxic to these microorganisms, causing changes in metabolic pathways and cellular compartments, limiting their biotechnological potential (MUELLER et al., 2020).

High concentrations of ethanol can interfere with the functional metabolism yeast, according to Jin et al. (2022), ethanol has the ability to cross the cell membrane, resulting in

increased fluidity and permeability, triggering negative consequences, such as loss of cell integrity, which interferes with growth, loss of cell viability and even death (BRANDT et al., 2019). Once inside the cell, ethanol is retained in the hydrophobic region of the phospholipid bilayer and interacts with unsaturated fatty acids and proteins present in this region (CHETTY et al., 2022), restricting the movement of these compounds, increasing polarity and hindering the free exchange of polar molecules into the intracellular environment (BERTRAND et al., 2020; RIBEIRO; BOURBON-MELO and SÁ-CORREIA, 2022), triggering structural changes in the properties of the plasma membrane, which directly affect the positioning of proteins in the phospholipid bilayer, interfering with the ability of cells to maintain the concentration gradient of compounds across the cytoplasmic membrane, inhibiting glucose metabolism (FRALLICCIARDI et al., 2022).

Stress factors trigger numerous response mechanisms in yeast, causing cascading effects. Temperature, for example, compromises cell viability, metabolic capacity of these microorganisms, cellular stability and also protein synthesis. However, osmotic pressure induces the production of glycerol to the detriment of ethanol (DA SILVA SANTOS et al., 2018; POSTARU et al., 2023). Furthermore, these stress factors can induce changes in the genetic material, deoxyribonucleic acid-DNA, such as single and double strand breaks, nucleotide modifications and protein-DNA interactions, leading to genetic instability.

Selected yeasts have the ability to withstand an anoxic environment, high temperatures and a high alcohol content generated during fermentation, overcoming wild yeasts in these aspects, which can be considered a selective advantage. However, it is important to highlight that high concentrations of ethanol in the fermentation medium can be potentially prejudicial (ZHANG et al., 2023). The concentration of this metabolite normally varies between 8% and 10% in the fermentation medium. Associated with other stress factors, this high concentration of ethanol can lead to cell death, reduced viability rate and loss of fermentative capacity, being classic examples of the harmful effects caused by toxic agents, as highlighted by Mueller et al. (2019).

The quest to understand the action of synergism related to high concentrations of ethanol and temperatures in yeast during the fermentation process is crucial to maintaining the integrity and vitality cells, aiming to ensure the efficiency of ethanol production. In this sense, evaluating cytotoxic damage related to high concentrations of ethanol and temperature may prove to be an important strategy in preventing harmful interference in industrial environments. This preventative approach is essential to understand the

physiological mechanisms of yeasts during the adverse conditions of fermentative environment, ensuring the success and sustainability of large-scale ethanol production. Therefore, this study aims to analyze the synergic effect of thermal and ethanolic stress on industrial yeas strains of *Saccharomyces cerevisiae*.

2. MATERIAL AND METHODS

The study was developed in the Biotechnology, Biochemistry and Biotransformation Laboratory of the Centro de Estudo em Recursos Naturais – CERNA, Universidade Estadual do Mato Grosso do Sul, Dourados/MS. The microorganisms used in this study were the yeasts *Saccharomyces cerevisiae* Fleischmann® and Pedra-2, available at the Biotechnology, Biochemistry, and Biotransformation Laboratory of the Natural Resources Study Center (CERNA), State University of Mato Grosso do Sul, Dourados/MS.

For the pre-inoculum, 0.10 g of lyophilized yeast was used and added to 1 mL of sterile saline solution (0.85%). The solution was homogenized and, with the aid of an automatic micropipette, 400 µL were collected and inoculated into previously prepared Petri dishes containing Sabouraud Agar solid medium, which were autoclaved at 120 °C for 20 min, and spread with the aid of a swab. The plates were incubated in an oven at 30 °C for 36 hours or until colonies grew. The colonies were collected with the aid of a Platinum loop and inoculated in a fermentative medium based on sugarcane juice adjusted, with the aid of a portable refractometer, to a concentration of 22 °Brix at pH 5.0, which was sterilized at 120 °C for 20 min. For ethanolic stress, ethyl alcohol 99,5% PA was added at concentrations of 0, 8 and 16% ($v.v^{-1}$). The flasks were kept at 250 rpm at temperatures of 30 and 40 °C. At pre-defined times of 8 and 16 hours, aliquots were collected for analysis.

For the analysis of biomass production, 300 µL of samples were collected and added to 10 mL of distilled water in test tubes. The analyzes were carried out using spectrophotometric measurements, at 570 nm, according to the methodology of Batistote et al. (2010). To evaluate cell viability, 10 µL of samples were collected and added to methylene blue dye. Cells were counted in a Neubauer chamber with the aid of an optical microscope, according to the method of Lee et al. (1981).

At different times of cultivation in the fermentative environment, in ethanol concentrations (0,8 and 16 $v.v^{-1}$), 5 µL of samples were collected with the aid of a micropipette and dripped into previously prepared Petri dishes containing sterile Potato Agar solid medium. The plates were incubated in an oven for 24 hours at 30 °C. After the

incubation period, a qualitative analysis of colony growth was carried out, following the methodology adapted by Mueller et al. (2020). Data analysis was carried out using Excel 2019 software and consisted of presenting the mean and standard deviation. The graphs were plotted with the same software.

3. RESULTS AND DISCUSSION

The evaluation of biomass production and cell viability of the Fleischmann® and Pedra-2 strains in relation to temperature, fermentation time and ethanol concentration demonstrated changes in the analyzed parameters (Table 1). With the addition of ethanol and increased fermentation time, both strains showed a reduction in biomass production and cell viability, with Fleischmann® being more sensitive to the effects of stress, presenting a biomass production of only 4.13 $mg.mL^{-1}$ and cell viability of 39.88% in 16% ethanol concentration ($v.v^{-1}$) in 16 hours of fermentation. Regarding temperature, when exposed to 40 °C for 8 hours, this same strain reached 7.91 $mg.mL^{-1}$ of biomass and 78% viability, while Pedra-2 showed cell growth of 9.86 $mg.mL^{-1}$ and 90.05% viability. Again, Fleischamnn® demonstrated lower tolerance to stress factor synergisms, which resulted in lower biomass production 3.70 $mg.mL^{-1}$ and 15.25% cell viability in the presence of 16% ($v.v^{-1}$) ethanol in 16 hours of fermentation at 40 °C. According to the results, the importance of considering the interaction between different environmental factors in fermentation and how this can affect the growth and viability of yeast is highlighted. Furthermore, they highlight the need to carry out detailed monitoring of the fermentation process and the need to choose the yeasts that best adapt to the process conditions, enabling better efficiency of the fermentation process.

Brazil is the second largest producer of ethanol, a product resulting from alcoholic fermentation, based on sugarcane juice and selected yeasts. However, during the fermentation process, yeasts are subjected to an extremely hostile environment, which leads to the appearance of stress at the cellular level. As the fermentation process progresses, the concentration of ethanol in the medium increases, resulting in a harmful condition for yeast cells, such as blockage and cell proliferation, affecting cell viability (NGUYET et al.2022).

The data highlights the negative impact of ethanol and high temperature on the cell, demonstrating a decline in cell development and viability of the yeasts analyzed. According to (EARDLEY and TIMSON, 2020), a well-known effect of ethanol on yeast is its inhibition

of growth and viability during fermentation. Yeast, when subjected to ethanol stress, initially struggles to maintain energy production. Although stress is often perceived as harmful, which it often does in industrial fermentations, these challenges and stress becomes a selective way and can improve evolutionary fitness and drive selection for more robust traits, as can be observed in the responses among the yeasts mentioned in the table 1.

Table 1. Assessment of biomass production and viability of Fleischmann®, Pedra-2 strains on the synergism of the action of thermal and ethanolic stress.

			Fleischmann®		Pedra-2	
Temperature (ºC)	**Time (h)**	**Ethanol Concentration (%)**	**Biomass ($mg.mL^{-1}$)**	**Viability (%)**	**Biomass ($mg.mL^{-1}$)**	**Viability (%)**
30 ºC	8	0	5.50 ± 0.01	96.00± 0.03	6.34 ± 0.09	99.01 ± 1.11
		8	4.43 ± 0.09	67.22 ± 4.90	5.59 ± 0.03	90.04 ± 1.10
		16	4.13 ± 0.01	39.88 ± 9.03	4.61 ± 0.01	69.74 ± 2.84
	16	0	10.85 ± 0.01	90.00 ± 1.49	9.64 ±0.03	95.00 ± 0.02
		8	3.75 ± 0.04	44.19 ± 0.50	5.06 ± 0.04	82.38 ± 2.37
		16	3.28 ± 0.02	28.05 ± 0.51	4.71 ± 0.02	61.03 ± 2.52
40 ºC	8	0	7.91 ± 0.02	78.00 ± 0.03	9.86 ± 0.09	90.05 ± 0.03
		8	4.00 ± 0.01	54.15 ± 3.73	5.00 ± 0.02	69.51 ± 0.89
		16	3.90 ± 0.01	22.14 ± 2.78	4.16 ± 0.01	43.25 ± 0.17
	16	0	7.86 ± 0.02	61.00 ± 0.02	10.75 ± 0.03	85.50 ± 2.35
		8	3.90 ± 0.01	39.91 ± 2.37	4.44 ± 0.03	60.55 ± 3.92
		16	3.70 ± 0.07	15.25 ± 0.71	4.11 ± 0.02	40.56 ± 3.42

Source: Prepared by the authors (2024).

Ethanol is one of the main normal metabolites of yeast cells but also one of the most important stressors of yeast (SOSTARIC et al., 2021). Mainly when yeasts were used for fermentation of high concentration of ethanol, which causes stress to yeast cells, negatively affecting cellular physiological activity, decreasing fermentation performance and ethanol yield (SAMAKKARN et al.,2021). Currently, obtaining maximum ethanol production continues to be the challenge to be overcome (EARDLEY and TIMSON, 2020).

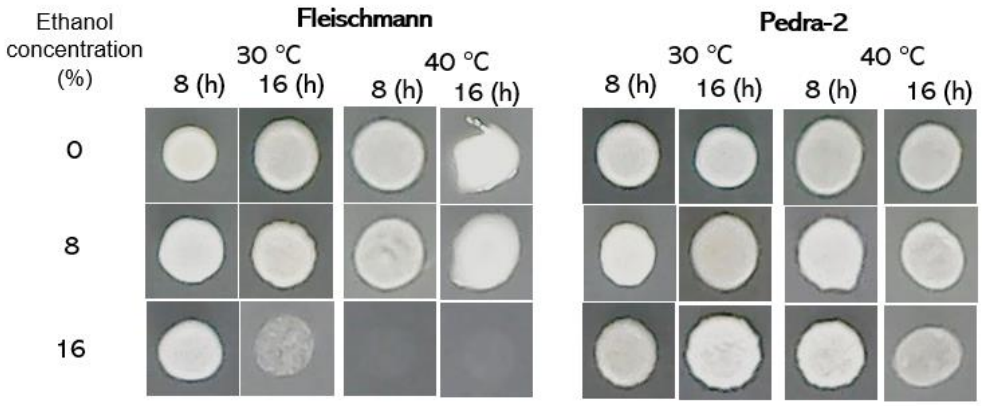
The data in Figure 1 highlights the negative impact caused by temperature in conjunction with the increase ethanol concentration in the medium, at a temperature of 30°C during 8 hours of fermentation and 0% ethanol, excellent colony growth was obtained for

Fleischmann® and Pedra-2 yeast, even during a period of 8 hours at the same temperature at a concentration 16% ethanol, Fleischmann® yeast presented a deformity in its colony and Pedra-2 visibly maintained its growth. When subjected to the same temperature, but for a period of 16 hours of fermentation at a concentration of 16% ethanol, the colony presented a deformity for Fleischmann®, and for Pedra-2, at the same temperature and fermentation time, it remained denser, showing greater resistance to ethanol concentration.

At a temperature of 40 °C, 8 hours of fermentation and an ethanol concentration of 0%, Fleischmann® and Pedra-2 yeast recorded good growth in the colonies, however, within 8 hours, Fleishmann® yeast already showed less formation in colonies and Pedra-2 remained. At ethanol concentration of 16% there was no colony growth for Fleishmann® and for Pedra-2 it grew in a weak manner.

Temperature is a critical environmental factor that can significantly impact the physiology of yeast. Elevated temperatures compromise cell viability by destabilizing the integrity of cellular structures and functions (EIGENFELD; KERPES and BECKER, 2021). The main effects include protein denaturation and destabilization of cell membranes; metabolic capacity, as the metabolic processes of yeast cells are highly temperature-sensitive, and extreme temperatures can inhibit enzymatic activities, slowing down or halting metabolic pathways; cellular stability, where structural components of the cell, such as membranes and cytoskeletal elements, can become destabilized under thermal stress, leading to loss of cellular integrity; and protein synthesis, which can be impaired by high temperatures, affecting the function of ribosomes and other components of the protein synthesis machinery (SILVA et al., 2023;TIMIRA et al., 2024).

Figure 1. Qualitative evaluation of the toxic action of different concentrations of ethanol associated with temperature on the development of Fleischmann® and Pedra-2 colonies.



Source: Prepared by the authors (2024).

Among the changes caused by ethanol, yeast morphology served as a sensitive reading and reflected several cellular events, so that the maintenance of cellular morphology was crucial for the physiological metabolism of yeast (ITTO-NAKAMA et al., 2022). Therefore, it is a great challenge for cells to maintain the integrity of the cell wall by resisting external stress. The cell wall is the target of attack by ethanol, as it is the barrier that separates cells from the external environment, in addition to being an important channel for transporting material and exchanging information between cells and the outside world.

4. FINAL CONSIDERATIONS

In the fermentation process, yeast cells are continuously and simultaneously subjected to different types of cellular stress, which determines constant cellular adaptation and classification of more robust strains. The data in the article highlights the response of cells to thermal and ethanolic stress, highlighting the negative impact on cell viability and development. Both at high temperature and ethanol concentration there was a significant drop in results. Under the action of stressors, yeast cells can modify their morphological colony growth architecture.

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REFERENCES

AZHAR, Siti Hajar Mohd et al. Yeasts in sustainable bioethanol production: A review. **Biochemistry and Biophysics Reports**, v. 10, p. 52-61, 2017.

BAPTISTA, Sara L. et al. Metabolic engineering of *Saccharomyces cerevisiae* for the production of top value chemicals from biorefinery carbohydrates. **Biotechnology Advances**, v. 47, p. 107697, 2021.

BATISTOTE, Margareth et al. Desempenho de leveduras obtidas em indústrias de Mato Grosso do Sul na produção de etanol em mosto a base de cana de açúcar. **Ciência e**

Natura, v. 32, p. 83-95, 2010.

BATISTOTE, Margareth; SANTOS, Maria do Socorro Mascarenhas. Analysis of fermentative parameters and the importance of *Saccharomyces cerevisiae* in the development of goods and services. **Research, Society and Development**, v. 9, p. e93691110586-e93691110586, 2020.

BERTRAND, Brandt et al. Biophysical characterization of the insertion of two potent antimicrobial peptides-Pin2 and its variant Pin2 [GVG] in biological model membranes. **Biochimica et Biophysica Acta (BBA)-Biomembranes**, v. 1862, p. 183105, 2020.

BRANDT, Bianca A. et al. Overcoming lignocellulose-derived microbial inhibitors: advancing the *Saccharomyces cerevisiae* resistance toolbox. **Biofuels, Bioproducts and Biorefining**, v. 13, p. 1520-1536, 2019.

CHETTY, Bronwyn Jean et al. Improvement of cell-tethered cellulase activity in recombinant strains of *Saccharomyces cerevisiae*. **Applied Microbiology and Biotechnology**, v. 106, p. 6347-6361, 2022.

COERTJENS, Nicole C. et al. Evaluation of stress factors in the metabolism of Pedra-2 yeast. **Scientific Electronic Archives**, v. 16, 2023.

CRAY, Jonathan A. et al. Chaotropicity: a key factor in product tolerance of biofuel-producing microorganisms. **Current Opinion in Biotechnology**, v. 33, p. 228-259, 2015.

DA SILVA SANTOS, Ane Francielly et al. Perfil de produção de etanol e trealose em *Saccharomyces cerevisiae* cultivadas em mosto a base de caldo de cana. **Scientia Plena**, v. 14, 2018.

EARDLEY, Joshua; TIMSON, David J. Yeast cellular stress: impacts on bioethanol production. **Fermentation**, v. 6, n. 4, p. 109, 2020.

EIGENFELD, Marco; KERPES, Roland; BECKER, Thomas. Understanding the impact of industrial stress conditions on replicative aging in *Saccharomyces cerevisiae*. **Frontiers in Fungal Biology**, v. 2, p. 665490, 2021.

FRALLICCIARDI, Jacopo et al. Membrane thickness, lipid phase and sterol type are determining factors in the permeability of membranes to small solutes. **Nature Communications**, v. 13, p. 1605, 2022.

ITTO-NAKAMA, Kaori, et al. AI-based forecasting of ethanol fermentation using yeast morphological data. **Bioscience, Biotechnology, and Biochemistry**, v. 86, p. 125-134, 2022.

JIN, Xiaofan et al. Wheat gluten peptides enhance ethanol stress tolerance by regulating the membrane lipid composition in yeast. **Journal of Agricultural and Food Chemistry**, v. 70, p. 5057-5065, 2022.

LAIRÓN-PERIS, María et al. Lipid composition analysis reveals mechanisms of ethanol tolerance in the model yeast *Saccharomyces cerevisiae*. **Applied and Environmental Microbiology**, v. 87, p. e00440-21, 2021.

LEE, S. S; ROBINSON, F. M; WANG, H. Y. Rapid determination of yeast viability. **Biotechnology Bioeng. Symp**, v. 11; p. 641-649, 1981.

MUELLER, Larissa Pires et al. O potencial associado das fontes renováveis e *Saccharomyces cerevisiae* para produção de bioetanol. **Educação Ambiental em Ação**, v. 18, n. 68, 2019.

MUELLER, Larissa Pires et al. The effects of thermal and ethanolic stress in industrial strains of *Saccharomyces cerevisiae*. **Research, Society and Development**, v. 9, p. e6819109091-e6819109091, 2020.

NGUYET, Vo Thi Anh, et al. Acquired resistance to severe ethanol stress-induced inhibition of proteasomal proteolysis in *Saccharomyces cerevisiae*. **Biochimica Et Biophysica Acta (BBA)-General Subjects,** v. 1866, p. 130241, 2022

POSTARU, Madalina et al. Cellular Stress Impact on Yeast Activity in Biotechnological Processes—A Short Overview. **Microorganisms**, v. 11, n. 10, p. 2522, 2023.

RIBEIRO, Ricardo A.; BOURBON-MELO, Nuno; SÁ-CORREIA, Isabel. The cell wall and the response and tolerance to stresses of biotechnological relevance in yeasts**. Frontiers in Microbiology**, v. 13, p. 953479, 2022.

SAMAKKARN, Wiwan; RATANAKHANOKCHAI, Khanok; SOONTORNGUN, Nitnipa. Reprogramming of the ethanol stress response in *Saccharomyces cerevisiae* by the transcription factor Znf1 and its effect on the biosynthesis of glycerol and ethanol. **Applied and Environmental Microbiology**, v. 87, p. e00588-21, 2021.

SHARMA, Juhi et al. Engineering of *Saccharomyces cerevisiae* as a consolidated bioprocessing host to produce cellulosic ethanol: Recent advancements and current challenges. **Biotechnology Advances**, v. 56, p. 107925, 2022.

SILVA, Rebeca Fasioli et al. Proteínas de choque térmico e perfil proteico em *Saccharomyces cerevisiae*. **Observatório De La Economía Latinoamericana**, v. 21, n. 9, p. 13403-13420, 2023.

STAMENKOVIĆ, Olivera S. et al. Production of biofuels from sorghum. **Renewable and Sustainable Energy Reviews**, v. 124, p. 109769, 2020.

TIMIRA, Vaileth, et al. Potential use of yeast protein in terms of biorefinery, functionality, and sustainability in food industry. **Comprehensive Reviews in Food Science and Food Safety**, v. 23, p. e13326, 2024,

WALKER, Graeme M.; BASSO, Thiago O. Mitigating stress in industrial yeasts. **Fungal Biology**, v. 124, p. 387-397, 2020.

ZABED, Hossain et al. Bioethanol production from fermentable sugar juice. **The Scientific World Journal**, v. 2014, 2019.

ZHANG, Liang et al. Medium-chain fatty acid production from thermal hydrolysed sludge without external electron donor supplementation. **Bioresource Technology**, v. 374, p. 128805, 2023.