

Action of Light on Metabolism of Yeast FT-858

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Abstract:

The light source can cause ultrastructural and morphological changes in yeast and affect your metabolism. Thus, this work has the objective of evaluating the influence of white and ultraviolet light on the fermentative performance of FT-858 yeast in different culture conditions. Yeast cells were grown in Yeast Extract-Peptone-Dextrose (YPD 2%) in which 0.10 g of yeast were added and incubated on a shaker for 10 h at 30 °C at 250 rpm, subsequently washed and centrifuged in solution saline. The biomass harvested was used in the fermentation experiments that were readily performed under white and ultraviolet light with temperature control. Aliquots were withdrawn for cell viability analysis and ethanol production. Yeast showed better performance under white light at 28 °Brix concentration at 30 °C. Ultraviolet light associated with high temperature and substrate concentration affected yeast physiology by directly interfering with viability and ethanol production.

Keywords: fermentation; cell stress; industrial process; ethanol

1. Introduction

Strains of *Saccharomyces cerevisiae* employed by the sugarcane sector for ethanol production, have higher levels of tolerance to stress factors and inhibitory compounds present in the fermentation medium, and still maintain high production of this biofuel [1]. For this type of process, microorganisms selected from the process itself are used, because they stand out under industrial conditions competing with wild yeasts and bacterial contamination [2, 3].

The criteria that permeate the choice of the yeast line to be used in the alcoholic fermentation are intrinsic to its physiological responses and the rapid adaptation to the process conditions [4]. Considering that factors such as temperature, pH and substrate concentration can act in synergy and affect the cells resulting in the loss of viability and directly interfering in the production of ethanol [5].

Ethanol production occurs in an anaerobic process in closed tanks in the absence of light. In this environment, yeasts are exposed to several stress conditions that can affect their performance and cause ultrastructural damages, morphological and genetic alterations, causing, depending on the interaction between these factors, changes in the structure of Deoxyribonucleic Acid [6].

According to Valduga et al. [7], the incidence of light and its intensity can cause changes in yeast metabolism, directly interfering in the production and accumulation of intracellular and extracellular metabolites compromising their basic functions. This is because some cells are devoid of photon energy absorption mechanisms [8], that is, devoid of photosynthesis mechanism [9]. *Saccharomyces cerevisiae* has no well characterized photoreceptor mechanisms [10]. Although light photon intensity can cause damage to yeast by interfering with its budding and

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developmental ability, as well as cytochrome and oxidative stress [8]. Different light sources are being studied extensively and used in biotechnological processes, given the advantages that this type of radiation presents, such as ease of application, cost and absence of waste generation at the end of the process [11,12].

Another factor considered as stress for these microorganisms is the temperature oscillation during the fermentation process. High temperatures can directly interfere with the metabolism of yeasts and consequently directly alter their cellular structures, affecting their budding cycle. According to Ancín-Azpilicueta et al. [13], yeasts do not have the ability to regulate internal heat, although they have distinct pathways that act responsibly to promote stress adaptation of the fermentation medium [14].

Thus, despite the great technological advances and the vast knowledge about the metabolism and the productive potential of the yeasts, there are still many unknown aspects, especially when it comes to the genetics and biochemistry of these microorganisms, as well as their physiological behavior in relation to the different levels of stress and other associated factors that can cause damage to these cells. Therefore, the objective of this study is to evaluate the influence of the physiological response of FT-858 yeast against the action of white and ultraviolet light in different culture conditions.

2. Results and Discussion

The FT-858 yeast showed differences in the viability rate throughout the fermentation times

and its best performance was at concentrations of 28 °Brix. At longer fermentation times and at 32 °Brix concentration, there was a gradual decrease in cell viability. Possibly, this culture condition induced catabolic repression due to the high concentration of soluble solids present in the substrate (Figure 1A). The yeast when cultured under the action of ultraviolet light, showed an increased cell proliferation (Figure 1B). It is likely that this light source has acted synergistically with high substrate concentration affecting yeast metabolism and compromising cellular viability. In the fermentation process, viability is important because the amount of viable cells is directly related to the efficient conversion of the substrate into product.

The FT-858 yeast has high performance in the first few hours of fermentation, as it has not yet been subjected to the stress of the medium and nutrient assimilation is more efficient. In addition, this yeast has a mechanism adapted to the process conditions, since it has a high budding rate in the first hours of fermentation [15].

Saccharomyces cerevisiae presents alterations in the physiological profile and the production of metabolites in response to light, altering the growth velocity and the formation of shoots, affecting the integrity of the cell wall causing cell death, as reported by Robertson, Davis and Johnson [8]. Nevertheless, according to Bodvard et al. [9], yeast cells possess a set of mechanisms to maintain their cellular integrity and ensure the balance of their functions to adapt to the environment or to succumb to it.

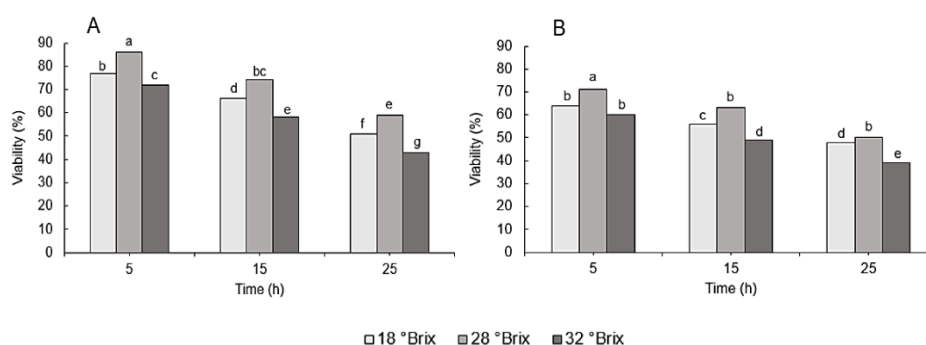


Figure 1 - Evaluation of viability of the yeast FT-858 grown on sugarcane juice at the temperature of 30 °C under action of white (A) and ultraviolet light (B). Means followed by the same letter are not statistically different by Tukey test at 5% significance.

At the temperature of 42 °C under action of white and ultraviolet light, it was observed that in longer fermentation times there was great loss in viability at concentrations of Brix studied (Figure 2A and 2B). It was observed that yeast physiology was affected, possibly the associated stress factors to which the yeast was subjected caused stress in the microorganism, corroborating Shima and Takagi [16], which describe that the intensity, frequency and numerous stress factors can affect

yeasts used in industrial processes causing changes in plasma membrane and consequently influence on budding and fermentative efficiency loss. Exposure to high temperatures, affect physiological functions of yeast and promote changes in the protein profile and their functions which are degraded leading to inhibition of cell growth, deficiency in bud separation and consequently cell death [17].

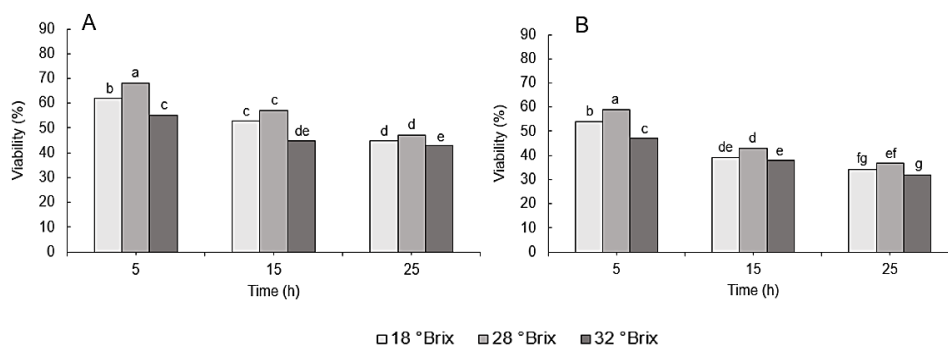


Figure 2 - Evaluation of viability of the yeast FT-858 grown on sugarcane juice at the temperature of 42 °C under action of White (A) and ultraviolet light (B). Means followed by the same letter are not statistically different by Tukey test at 5% significance.

In the ethanol concentration analysis, the FT-858 yeast presented a better yield when grown at 30 °C and the best ethanol production at 28 °Brix under the action of white light 7% (v v⁻¹). Under

ultraviolet light there was loss of this metabolite. At 42 °C the gradual loss in ethanol production was observed, as shown in Table 1.

Table 1. Analysis of the ethanol concentration (%) of FT-858 yeast grown in sugarcane.

Fermentation time (h)	18 °Brix		28 °Brix		32 °Brix	
	Environments					
	White	Uv	White	Uv	White	Uv
Temperature 30 °C						
5	2.2 ± 0.01d	1.8 ± 0.01c	2.2 ± 0.02c	1.8 ± 0.02c	1.9 ± 0.01c	1.4 ± 0.02c
15	6.0 ± 0.02a	5.4 ± 0.01a	7.0 ± 0.01a	5.6 ± 0.01a	5.0 ± 0.01a	4.5 ± 0.01a
25	3.0 ± 0.01c	3.0 ± 0.02b	3.2 ± 0.04b	3.0 ± 0.01b	2.8 ± 0.03b	2.4 ± 0.01b
Temperature 42 °C						
5	1.8 ± 0.02e	1.8 ± 0.03c	2.0 ± 0.02c	1.7 ± 0.01c	1.5 ± 0.01d	1.3 ± 0.02cd
15	3.2 ± 0.03b	2.9 ± 0.01b	3.4 ± 0.01b	3.2 ± 0.02b	2.6 ± 0.01b	2.2 ± 0.02b
25	2.0 ± 0.01e	1.9 ± 0.01c	1.8 ± 0.01c	1.6 ± 0.02c	1.6 ± 0.03cd	1.5 ± 0.01c

Values (means ± standard deviation) followed by the same letter in the column not statistically different from each other (P < 0.05) by the Tukey test at 5% significance.

The yeast during fermentation undergoes the action of stress factors and develops adaptation mechanisms for its survival [14], triggering induction and genetic repression [18, 19]. Ultraviolet light and certain chemical compounds

can cause damage to Deoxyribonucleic Acid, and such ruptures may be at the gene level, chromosomal cause changes in the processes, biochemical and cellular [20]. Perhaps, the association of stress factors together with the

action of ultraviolet light may have triggered changes at the DNA level and consequently affected the anaerobic metabolism involving the production of ethanol.

Studies by Billota and Daniel [21], applying ultraviolet light in yeasts, observed the inactivation of cells and that this is supposedly related to the action of this radiation source, which acts breaking the hydrogen bonds and promoting changes in nitrogen bases causing photobiochemical reactions in yeasts cells. Another factor that affects integrity of yeasts is temperature variation, according to Cruz et al. [22]. The ideal temperature range for *Saccharomyces cerevisiae* yeast ranges from 30-33 °C [23].

3. Material and Methods

Collection and preparation of the substrate

The sugarcane juice was obtained from an ethanol plant, was stored in sterilized flasks and transported at (4 °C) to the Biotechnology, Biochemistry and Biotransformation Laboratory of the Centro de Estudos em Recursos Natural (CERNA), at the Universidade Estadual de Mato Grosso do Sul, in Dourados, Mato Grosso do Sul. Where the material was filtered through cotton to remove impurities and subsequently through filter paper. Brix was concentrated by evaporation and was accompanied by a portable refractometer at 18, 28 and 32 °Brix. The pH was adjusted to 5.0.

Strain used

Saccharomyces cerevisiae strain FT-858 used for the fermentation process for the production of ethanol in Brazilian industries. Obtained from Fermentec located in Piracicaba, São Paulo.

Growth conditions

To develop cell biomass, a classical YPD 2% culture medium was used, containing 1.0% (w v⁻¹) yeast extract, 1.0% (w v⁻¹) peptone, and 2.0% (w v⁻¹) glucose. The medium was adjusted to pH 5.0 with 1N hydrochloric acid and sterilized in an autoclave at 120 °C for 20 min. The flasks containing the yeast cells remained in a shaker at 30 °C for 10 hours. Grown cells were collected by

centrifugation (800 g, 20 min), suspended, and washed three times in 0.85% sterile saline solution, resulting in a biomass concentration of 10 mg m L⁻¹.

Experiment fermentative

Fermentation employed sterilized sugarcane juice as the substrates, each at 18, 28, and 32 °Brix, in 125 mL Erlenmeyer flasks, where a 50 mL volume of substrate was incubated at 30 or 42 °C in a shaker at 250 rpm. At 5, 15, and 25 hours of fermentation, aliquots were collected for measurement of fermentation parameters. The experiments were carried out in triplicate.

Action of light sources

For the analysis of the action of light sources on yeast development, the environments were adapted with a lamp holder attached to the upper part of the shaker at a distance of 45 cm from the samples with direct incidence of light on them. The light environments used were white light (White) with a Philips brand lamp (15W); ultraviolet (UV-C) lamp with Towalight brand lamp (15W).

Analytical methods

Cell viability was assessed using the methylene blue dye. An aliquot was placed in a Neubauer chamber and examined under an optical microscope. Viability was determined by counting dead cells stained in blue and expressed as the percentage of viable cells in each culture [24].

Ethanol was analyzed by gas chromatography CG 3900 with flame ionization detector (Varian), using a 30m long fused silica capillary column (ZB5). The chromatographic condition used was: 1µL injection volume, 1:20 displacement ratio and 90°C oven temperature. The detector injector temperatures were 240°C. The samples were filtered in a 0.22 µm ultrafilter [25].

Statistical analysis

The results were analyzed with Excel version 2016 software with ActionStat supplementation.

The Tukey test at 5% significance and the graphs plotted with Origin 8.0.

4. Conclusions

The FT-858 yeast underwent the Uv light action and presented physiological alteration in relation to the higher concentration of Brix, high temperature and in prolonged fermentation times. Notably the synergism of stress factors affected cell viability

From the obtained results, it can be inferred that the simultaneous stress of fermentative medium in the presence of light can be a genetic factor interfering in the ethanol production.

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