

Analysis of fermentative parameters and the importance of *Saccharomyces cerevisiae* in the development of goods and services

Análise de parâmetros fermentativos e a importância de *Saccharomyces cerevisiae* no desenvolvimento de bens e serviços

Análisis de parámetros fermentativos y la importancia de *Saccharomyces cerevisiae* en el desarrollo de bienes y servicios

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Abstract

In the fermentation process, yeasts need to adapt to the environmental changes that occur during the production process. Responses to these adjustments can alter biochemical routes and the amount of metabolites produced. Thus, the objective was to analyze the fermentative parameters of industrial yeast strains in different growing conditions, as well as to evaluate the its applicability in different sectors of goods and services. A pre-inoculum was performed with the YPSAC 5% medium for the activation of the yeasts Catanduva-1 and Fleischmann that remained incubated for 24 hours at 30 °C at 250 rpm. After the cells were recovered by centrifugation and inoculated in the fermentation medium based on sugarcane juice at 15 °Brix at temperatures of 30 and 40 °C. Aliquots were removed for the analysis of the fermentative parameters. Concomitantly, a survey was carried out regarding the use of yeasts in the process of preparing goods and services. The data show that the best yeast fermentation performance occurred at 30 °C in 10 hours. In addition, yeasts have the ability to produce, under ideal conditions, metabolites that can be used in different biotechnological processes.

Keywords: Metabolites; Fermentation process; Biotechnology.

Resumo

No processo de fermentação, as leveduras precisam se adaptar às mudanças ambientais que ocorrem durante o processo de produção. As respostas a esses ajustes podem alterar as rotas bioquímicas e a quantidade de metabólitos produzidos. Assim, o objetivo foi analisar os parâmetros fermentativos de cepas de leveduras industriais em diferentes condições de cultivo, bem como avaliar sua aplicabilidade em diferentes setores de bens e serviços. Um pré-inóculo foi realizado com o meio YPSAC 5% para ativação das leveduras Catanduva-1 e Fleischmann que permaneceram incubadas por 24 horas a 30 °C a 250 rpm. Após as células foram recuperadas por centrifugação e inoculadas no meio de fermentação a base de caldo de cana a 15 °Brix nas temperaturas de 30 e 40 °C. Alíquotas foram removidas para análise dos parâmetros fermentativos. Concomitantemente, foi realizado levantamento sobre a utilização de leveduras no processo de preparação de bens e serviços. Os dados mostram que o melhor desempenho de fermentação da levedura ocorreu a 30 °C em 10 horas. Além disso, as leveduras têm a capacidade de produzir, em condições ideais, metabólitos que podem ser utilizados em diversos processos biotecnológicos.

Palavras-chave: Metabólitos; Processo de fermentação; Biotecnologia.

Resumen

En el proceso de fermentación, las levaduras necesitan adaptarse a los cambios ambientales que ocurren durante el proceso de producción. Las respuestas a estos ajustes pueden alterar las rutas bioquímicas y la cantidad de metabolitos producidos. Así, el objetivo fue analizar los parámetros fermentativos de cepas de levadura industrial en diferentes condiciones de cultivo, así como evaluar su aplicabilidad en diferentes sectores de bienes y servicios. Se realizó un preinóculo con medio YPSAC al 5% para la activación de las levaduras Catanduva-1 y Fleischmann que permanecieron incubadas durante 24 horas a 30 °C a 250 rpm. Posteriormente las células fueron recuperadas por centrifugación e inoculadas en el medio de fermentación a base de jugo de caña de azúcar a 15 °Brix a temperaturas de 30 y 40 °C. Se retiraron alícuotas para análisis de los parámetros fermentativos. Paralelamente, se realizó una encuesta sobre el uso de levaduras en el proceso de elaboración de bienes y servicios. Los datos muestran que el mejor rendimiento de fermentación de la levadura se produjo a 30 °C en 10 horas. Además, las levaduras tienen la capacidad de producir, en condiciones ideales, metabolitos que pueden utilizarse en diversos procesos biotecnológicos.

Palabras clave: Metabolitos; Proceso de fermentación; Biotecnología.

1. Introduction

The fermentation process uses selected yeast strains resulting in significant gains in productivity and efficiency, due to the characteristics of the microorganisms such as fermentation stresses tolerance, ability of deployment in distillery and stoichiometry favorable to high ethanol production (Basso et al., 2008). It may also be benefited by other technological advances such as the fermentation with high sugar content, resulting in economic, social and environmental benefits (Basso et al., 2011).

Thus, changes in the profile of intracellular metabolites produced by *Saccharomyces cerevisiae* Meyen ex EC Hansen may occur, which may be related to adaptive mechanisms that admit that they provide yeast cells with better adaptation and survival in the face of stress factors present in the fermentation process (Pereira et al., 2011). In addition, according to Santos et al., (2010), such metabolites are considered substrates for fermentation and may be dispersed in the medium or accumulated inside the yeasts. Thus, strains that are capable of fermenting in media with a high concentration of sugars accumulate high concentrations of metabolites (Barbosa, 2013).

In the last decade, research on the use of these yeasts in relation to molecular and genetic modifications has been intensified for the efficient and viable production of ethanol using five-carbon sugars as a substrate (Pereira et al., 2011; Zhang et al., 2015), in addition to several other processes such as the production of vitamins, proteins, enzymes, heterologous proteins and in products of pharmaceutical interest through manipulation with new metabolic pathways that are possible through genetic improvements, since its genome has already been completely sequenced (Badotti et al., 2008).

Some strains can also be used as raw material converters in the processes of obtaining biofuels, in addition to other applications such as the production of secondary compounds with a focus on biorefineries. Still in the area of metabolites, there is the prospecting of yeasts and the study of physiological routes for the production of different compounds that can be used in different biotechnological processes.

However, as yeasts are considered an essential component of fermentation processes, it is necessary to understand the fermentative performance in relation to temperature and the prolonged fermentation time to better understand the different physiological mechanisms of these microorganisms. Thus, the objective was to analyze the fermentative parameters of industrial yeast strains in different cultivation conditions, as well as to evaluate the applicability of this microorganism in different sectors of goods and services.

2. Materials and Methods

2.1 Yeast strains

The studied strains Catanduva-1 (Cat-1) and Fleischmann (Fle) were obtained through the LNF Company, Latin American Applied Biotechnology, located on Fioravante Pozza Street, 198 - Bento Gonçalves, RS, Brazil.

2.2 Fermentation experiment

The medium for yeast fermentations contained YPSac 5% (1.0% (w v⁻¹) yeast extract; 1.0% (w v⁻¹) of peptone; 5.0% (w v⁻¹) sucrose), with pH adjusted to 5.0 with hydrochloric acid (1N) and sterilized in an autoclave at 120 °C for 20 minutes. The flasks containing yeast cells were incubated in shaker type CT-712R, 30 °C for 24h at 250 rpm. After growth, cells were collected by centrifugation (800g, 20 min.), suspended and washed three consecutive times in saline (0.85%) sterile, and biomass obtained was used in the fermentation test.

Growth was carried out in 125 mL flasks with 50 mL of wort of sugarcane broth with 15 °Brix without pH correction. The flasks were incubated in shaker type CT-712R, at temperatures of 30 and 40 °C at 250 rpm. At specified times of (10, 20 and 40 hours), during the fermentation, samples were removed for analysis of fermentation parameters.

2.3 Analytical methods

The biomass production was measured by turbidity readings at 570 nm and correlated to a dry weight/optical density calibration curve. The cell viability was determined by methylene blue staining (Lee et al., 1981).

The concentration of glycerol was determined by enzymatic kit for the analysis of triglycerides. 10µL of the sample and 1.0 mL of the enzymatic reagent were added in the test tube and incubated in a water bath at 37 °C for 10 min. The samples were determined at 505 nm in a spectrophotometer, correlated with a curve. The total reducing sugars was determined by 3,5-dinitrosalicylic acid-DNS method (Miller, 1959).

2.4 Applicability of *Saccharomyces cerevisiae* to obtain biotechnological products

An exploratory descriptive research was carried out, in which documents such as articles and other published works evaluated the use of *Saccharomyces cerevisiae* and the use in different areas of production. This study also presents a qualitative interpretation of the data obtained, which according to Prodanov & Freitas, (2013) such a quantitative approach can be used to categorize and interpret the information.

3. Results and Discussion

The study of fermentative parameters in industrial yeasts grown in must based on sugarcane juice is extremely important. Such parameters can be monitored during the fermentation process allowing a greater production of ethanol. In the analysis of the fermentation parameters of industrial yeasts grown based on sugarcane juice at a concentration of 15 °Brix, at a temperature of 30 °C, and different fermentation times are shown in Table 1.

It was observed that the yeast Fleischmann showed a viability rate of 77% in 10 hours of fermentation. The yeast Catanduva-1 showed an index of living cells in this fermentation period of 90%, showing that it is more adapted to the industrial fermentation process. However, in the longer periods, 20 and 40 hours, there was a sharp drop in the rate of living cells for both yeasts. In the evaluation of biomass production at 30 °C, the best performance was in the time of 20 hours of fermentation for both strains analyzed (Table 1).

At 40 °C there was a retraction of the values of the biomass and viability fermentative parameters demonstrating that the temperature and the prolonged fermentation time acted as stress factors in the yeast physiology. However, the yeast Catanduva-1 was more resistant to these disorders, since there was a small change in the accumulation of glycerol by this strain. In this study, it can be seen that the total residual sugar was considerably high for both strains in the 10 hour time, decreasing in the subsequent times. The analysis of the quantification of glycerol showed that there was no variation in the production of this metabolite as shown in Table 1.

Yeasts adapted to the industrial process respond efficiently when exposed to ideal fermentation conditions, such as a temperature of 30 °C, considered ideal for maintaining their physiological pathways. Fermentation time is also considered by some authors as a stress factor, as there may be an accumulation of ethanol in the fermentation medium causing

changes in the metabolic routes and even in cell viability.

Table 1. Analysis of fermentative parameters and glycerol quantification in *Saccharomyces cerevisiae* grown in must with cane juice at 30 and 40 °C.

Yeasts	Time (h)	Biomass (mg mL ⁻¹)	Cell Viability (%)	Total Residual Sugar – ATR (%)	Glycerol (g L ⁻¹)
Temperature 30°C					
Fleischmann	10	14.0 ± 1.77	77	70	0.50 ± 0.01
	20	12.0 ± 1.65	58	60	0.52 ± 0.00
	40	12.0 ± 1.64	38	48	0.55 ± 0.04
Catanduva-1	10	12.0 ± 1.66	90	60	0.40 ± 0.01
	20	13.2 ± 1.87	88	40	0.41 ± 0.00
	40	13.8 ± 1.88	85	20	0.43 ± 0.00
Temperature 40°C					
Fleischmann	10	14.0 ± 1.77	65	78	0.53 ± 0.01
	20	12.0 ± 1.65	48	65	0.56 ± 0.01
	40	12.0 ± 1.64	29	53	0.57 ± 0.03
Catanduva-1	10	9.5 ± 1.70	80	76	0.42 ± 0.03
	20	11.7 ± 1.62	76	46	0.43 ± 0.00
	40	12.8 ± 1.65	68	30	0.45 ± 0.01

Source: Authors.

Exposure of yeast cells to high temperatures causes increased membrane permeability, while lower temperatures result in decreased permeability (Fairbairn, 2012). The rate of cell viability can be influenced by relatively low concentrations of ethanol, which inhibits the growth of yeasts, by directly interfering in cell division, thus decreasing the production of biomass. However, at higher concentrations of ethanol, cell viability is lost and, consequently, cell death (Stanley et al., 2010).

Studies using must based on sugarcane juice are important, since this substrate has sucrose as a fundamental component, this being the main raw material used by the industries in the fermentation process, thus, evaluating the possible interference of the accumulation of compounds like ethanol and glycerol from yeasts and their possible implications are important to prevent losses in the ethanol production process.

Yeasts are important because the production of ethanol depends on its integrity and remains in the fermentation medium. Therefore, determining the influence of temperature and fermentation time on industrial yeasts can provide parameters to help understand your metabolism and ensure more efficient ethanol production.

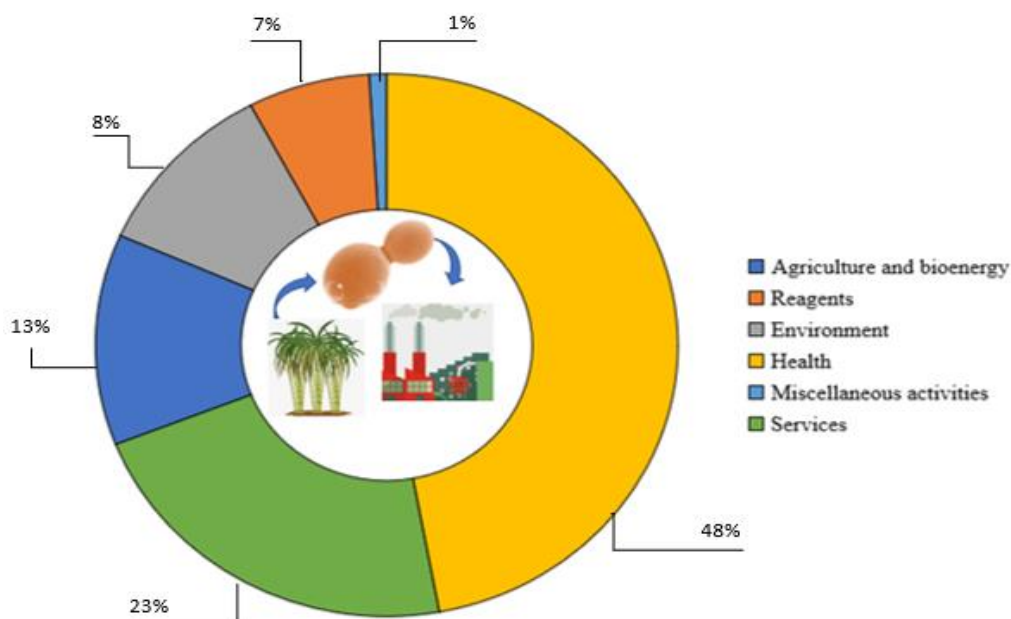
Studies developed by Batistote et al. (2010), in the 2008/2009 harvest, in industries located in the State of Mato Grosso do Sul - MS, showed that the industrial strains Catantuva-

1, Pedra-2, Barra Grande and Fleischmann were the most used for ethanol production in thus confirming its applicability in fermentation processes due to its high capacity to convert substrate into ethanol, an important biotechnological product.

Industrial yeasts have mechanisms to respond to stress conditions, and adapt quickly to these adverse external factors, adjusting their metabolism to avoid loss of viability and, possibly, changes in the accumulation of carbohydrates. (Zhao & Bai, 2009). In doing so, some metabolites are accumulated shortly before cells enter the logarithmic growth phase, suggesting that this is the metabolic response to stress caused by high concentrations of ethanol, pH, temperature fluctuation and prolonged fermentation time, as described by François et al., (2012).

The application of yeasts in biotechnological processes has proved to be an important responsible tool in the production of goods and services in the most diverse sectors of modern society, which has benefited from these processes. The search for renewable sources of energy has generated biomass, which can be transformed through the wide use of fermentative processes, which allow the production and release of countless compounds. In this context, some compounds such as glycerol, which can be used in the explosives industry, pharmaceuticals, in the production of ethanol, butanol and fusel oil applied in the cosmetic, agricultural, food, as well as service industries, bioprocesses have aroused worldwide interest, but in different areas of the known (Figure 1).

Figure 1. The use of *Saccharomyces cerevisiae* in biotechnological processes.



Source: Authors.

In this perspective, biotechnological processes need to be improved, a good example is the Brazilian fermentation process, which uses genetically improved sugarcane varieties, in addition to selected and personalized *S. cerevisiae* yeasts, such as CAT-1, PE -2, BG-1, SA-1, Y904 and FT858, these yeasts leveraged national agribusiness, placing Brazil at the forefront as the world's second largest producer of ethanol.

Yeasts can be used to obtain products that range from therapeutic proteins and industrial enzymes and various metabolites, just give conditions to the yeasts for biosynthesis to occur, as stated by Knudsen et al. (2015), when using *S. cerevisiae*, nutrients and an assimilable carbon source are needed to obtain a specific product such as ethanol or in a spectrum of products such as ethanol and by-products such as glycerol and other commercially valuable alcohols (Nandy & Srivastava , 2018).

It is still possible, through genetic engineering, to direct the metabolism of these yeasts and alter their physiological response, obtaining metabolites such as glycerol in greater quantity, in addition to several other processes such as the production of vitamins, proteins, enzymes, heterologous proteins and products of pharmaceutical interest. through manipulation with new metabolic pathways that are possible through genetic improvements and secondary compounds focusing on biorefineries according to Naghshbandi et al. (2019) and Kawai, et al. (2019).

4. Conclusions

The data show differences in the fermentative parameters analyzed in the cultivated industrial lines, as the studied yeasts were sensitive to prolonged fermentation times and higher temperatures.

Biotechnological studies using different strains of *Saccharomyces cerevisiae* demonstrate that such microorganisms have metabolic routes with a high capacity for the production of numerous compounds, which contribute to the development of various goods and services.

The *Saccharomyces cerevisiae* have a high capacity for the production of other compounds, admitting that this microorganism may be susceptible to genetic modifications and have its metabolic routes altered in order to produce compounds for use in different biotechnological areas. In this way, studies focusing on the use of microorganisms as well as new technological advances may contribute to increase the production of numerous

compounds also aiming at their quality, since such metabolites can be used in different areas of knowledge as well as in industrial processes distinct.

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