

PRELIMINARY STUDY OF THE ASSIMILATION CAPACITY OF SOYBEAN HULLS HYDROLYZATE BY RHODOTORULA MUCILAGINOSA CTC 7688

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Resumo:

Microorganisms are today a consolidated source of value-added products. They can produce everything from lipids to antibiotics that are fundamental to human health. Yeasts, for example, have been studied for their ability to assimilate agroindustrial residues. The objective of this work was to study the ability of the yeast *Rhodotorula mucilaginosa* to assimilate a hydrolysate of soybean hulls for the production of lipids. Cultures were carried out on the main carbohydrates present in the hydrolysates of soybean hulls (arabinose, glucose and xylose) in order to verify the behavior of the yeast during the assimilation of each of these carbohydrates. During the cultivation the biomass concentration and the pH were evaluated. At the end of the culture, the lipid content was determined. The results showed that there was considerable biomass production when the hydrolysate was used ($9.11 \pm$

Palavras-chave: Hydrolysis; soybean hulls; microbiology.

Modalidade de Participação: Pós-Graduação

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1 INTRODUCTION

Currently there is a great interest in the production of lipids by microbial route, either by the potential scale expansion of production or possibility of cultivation under controlled conditions, which reduces the possibility of losses. In microbial route the lipids are accumulated in the cells as triglycerides of long chain fatty acids. These fatty acids are predominantly composed of esters with a composition of 16 to 18 carbons, similar to those found in vegetable oils (such as soybean oil, for example), making it possible to use them as raw material in the biodiesel production (BENTO, 2017).

The source of lipids for the production of biodiesel is basically soybean oil. The production of large-scale soybeans for the biodiesel sector along with the food industry generates a large quantity of hulls that are considered low value and are basically used for animal feed. For each ton of harvested soybeans approximately 50 kg of hulls are obtained, which represents approximately 5% of the initial mass (BLASI et al., 2000). These soybean hulls are rich in lignocellulosic materials, which after hydrolysis processes provide carbohydrates generated by the breakdown of the cellulose and hemicellulose contained in the hulls. Cassales (2010) determined that the hydrolysates of soybean hulls under the conditions studied contained 39.7% glucose, 19.6% xylose and 5.9% arabinose, confirming the potential of using the hydrolysate of soybean hulls as a source of carbohydrates for microbial cultures.

According to Ageitos et al. (2011) a microorganism is considered a lipid producer when it can accumulate at least 20% of its own biomass as lipids. Among the microorganisms capable of accumulating at least 20% of lipids, the yeasts stand out. Yeast are single-celled microorganisms with asexual reproduction that are classified as members of the fungi kingdom. Some yeasts are grown for use in the food industry, but others grow naturally in soils and plants all over the world. They grow in any medium that contains carbohydrate because they contain enzymes that are responsible for the transformation of carbohydrates into protein biomass, alcohol or even lipids (BENTO, 2017).

Some types of yeast are good producers of lipids, but each species assimilates a same carbohydrate in different ways. Some species assimilate a particular carbohydrate very well, while another species may not assimilate the same carbohydrate. Knowing the ability of a microorganism to assimilate a carbohydrate is essential to apply it industrially.

The objective of this study was to analyze the ability of the oleaginous yeast *Rhodotorula mucilaginosa* to assimilate carbohydrates present in the hydrolysate of soybean hulls.

2 METHODOLOGY

2.1 Microorganism

In the cultures the wild yeast *Rhodotorula mucilaginosa* was used. This yeast was isolated by the team of the Bioprocess Engineering Laboratory of the Federal University of Rio Grande in a previous study (OTERO, 2011) and deposited in the André Tosello Foundation under number 7688.

2.2 Reactivation, pre-inoculum and inoculum

The yeast was reactivated in agar slant tubes containing Yeast Malt (YM) agar, composed of (g/L): yeast extract (3), malt extract (3), peptone (5), glucose (10) and bacteriological agar (20). The tubes were placed in an incubator (Quimis, Q-316 MA, Brazil) at 25 °C for 48 h.

Ten milliliters of YM broth were added to each tube and the microbial colonies were scrapped with a nickel-chromium loop. The suspension was then poured into the sterile tubes. These tubes were then placed in an incubator at 25 °C for 48 h. YM broth composition was (g/L): yeast extract (3), malt extract (3), peptone (5), glucose (10).

After 48 h of pre-inoculum, the contents of 2 pre-inoculum tubes were poured under aseptic conditions to each Erlenmeyer flask containing 180 mL of YM broth, reaching the final volume of 200 mL. The Erlenmeyer flasks were placed in a rotary shaker (Tecnal TE-424, Brazil) at 180 rpm and 25 °C. The number of cells was estimated through the Neubauer chamber until reaching a minimum of 1×10^8 cells/mL (SANTOS et al., 2012). The experiment was performed in triplicate.

2.3 Shake flask cultivation

The culture medium used was the one proposed by Evans and Ratledge (1983), composed of (g/L): NH_4Cl , 0.5; KH_2PO_4 , 7.0; Na_2HPO_4 , 2.0; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1.5; yeast extract, 1.5; $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$, 0.1; $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 0.008; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.0001. The sources of carbon (carbohydrates) used were soybean hulls hydrolysate, arabinose, glucose and xylose, all with an initial concentration of 30 g/L. Cultures were carried out in a rotary shaker at 180 rpm and 25 °C. All experiments were conducted in triplicate. The initial cell concentration of the microorganism was 1×10^7 for all experiments (SANTOS et al., 2012). The final volume considering the medium plus the inoculum was 200 mL in all cases.

The hydrolysates of soybean hulls were obtained following the methodology of Cassales (2010). The conditions for the hydrolysis were: 118 °C, 2.8 mmol $\text{H}_2\text{SO}_4 \text{ g}^{-1}$ (amount of acid per gram of hulls) and 8.8 g.g⁻¹ (solid:liquid ratio).

2.4 Biomass, pH and lipids determination

Every 24 h, aliquots of 5 mL of each Erlenmeyer flask were collected under sterile conditions for the determination of biomass and pH. Each aliquot was centrifuged (Daiki 80-2B, Brazil) at 3000 rpm for 15 minutes. The supernatant was used for pH determination on a digital pH meter (Marte MB-10, Brazil). The precipitate was used to determine the biomass concentration according to the methodology proposed by Choi and Park (2003). The lipid content was determined using the method of Bligh and Dyer (1959) adapted by Manirakiza et al. (2001).

3 RESULTS AND DISCUSSION

Hydrolysate and arabinose culture were monitored for 240 h while cultures with glucose and xylose were monitored for 192 h. The end of the culture was determined by applying a test of analysis of variance in the data of the last three samples and verifying that there was no statistical difference at 95% confidence level. Cultures using the pure carbohydrates were made to study the behavior of the microorganism during the assimilation of each of them individually. In Figure 1 are shown the profiles for pH and biomass concentration throughout the cultivation.

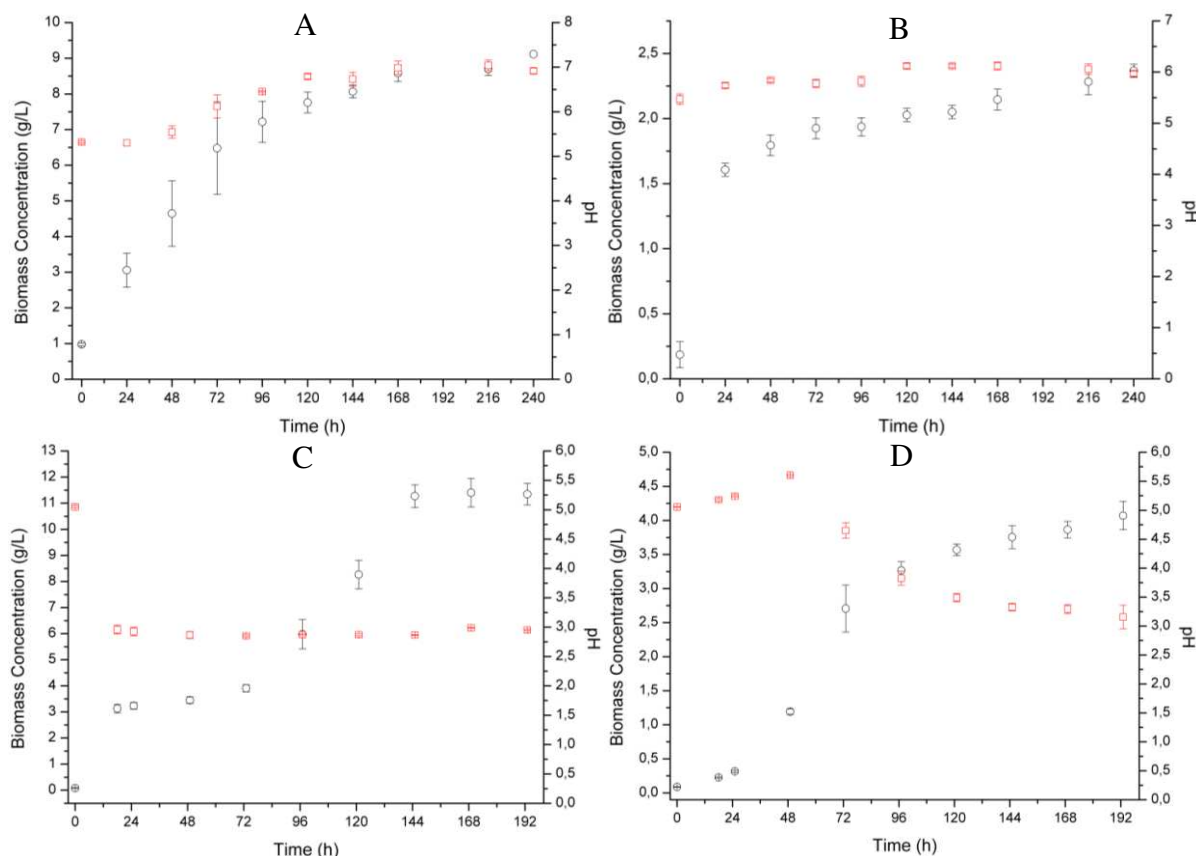
For arabinose, the exponential phase occurred in the first 24 h of culture, while the stationary one started with 168 h. The maximum biomass produced was 2.36 ± 0.05 g/L when reached 240 h of cultivation. The initial pH was 5.47 ± 0.10 , with a small increase along the culture, when in 240 h 5.97 ± 0.05 was recorded.

For glucose, the exponential phase occurred after 72 h of cultivation while the stationary one started with 144 h. The maximum biomass produced was $11.34 \text{ g/L} \pm 0.41$ in 144 h. The initial pH was 5.05 ± 0.01 , but within 24 h of cultivation it declined to 2.92 ± 0.07 and it remained practically stable until the end of cultivation.

For xylose, the exponential phase occurred after 24 h of cultivation while the stationary one started with 96 h. The maximum biomass was reached in 192 h (4.07 ± 0.20 g/L). The initial pH was 5.05 ± 0.01 , dropping along the culture reaching 3.15 ± 0.20 in 193 h.

The cultivation using the hydrolysate reached maximum biomass production in 240 h ($9.11 \text{ g/L} \pm < 0.01$). The pH increased slightly during the cultivation reaching 6.91 ± 0.06 in 240 h. The exponential phase occurred between first hours until 96 h of cultivation.

Figure 1 - Profile of pH and biomass concentration throughout the cultivation using (A) hydrolysate of soybean hulls, (B) arabinose, (C) glucose and (D) xylose as carbon source.



Source: author, 2018.

(□) pH profile; (○) biomass concentration profile. Whiskers indicate standard deviation between triplicate.

In Table 1 are shown the results for the final concentration of biomass, lipids and kinetic parameters of the cultures using carbohydrates and soybeans hull hydrolysate. Cultivation using glucose produced significant biomass concentration as well as total lipids, considering that it is a non-optimized medium. However, the same did not happen with the other cultivations. Consequently, the low lipid content affected the total lipid parameter resulting in low values for them.

Table 1 - Parameters of culture and lipid determination.

Carbon source	Biomass (g/L)	Total lipids (g/L)	Lipid content (%)	P _x (g/L.h)	μ _{max} (h ⁻¹)
Hydrolysate	9.11 ± < 0.01	0.48 ± 0.06	5.30 ± 0.68	0.033 ± < 0.001	0.025 ± 0.003
Arabinose	2.36 ± 0.05	0.06 ± < 0.01	2.57 ± 0.32	0.009 ± < 0.001	0.047 ± < 0.001
Glucose	11.34 ± 0.41	3.67 ± 0.56	32.26 ± 3.86	0.058 ± 0.002	0.158 ± 0.009
Xylose	4.07 ± 0.20	0.14 ± 0.03	3.49 ± 0.99	0.020 ± < 0.001	0.047 ± 0.002

Source: author, 2018.

Mean values ± standard deviation for triplicate; P_x = productivity; μ_{max} = maximum specific growth rate.

Gomes et al. (2002) and Jimoh et al. (2012) conducted studies using different strains of *Rhodotorula mucilaginosa* on different carbon sources with arabinose being one of them. The authors observed that most strains were found to be incapable or weakly able to assimilate arabinose. The strain used in the present study was also weak in assimilating arabinose in the way it was tested.

Ahmad et al. (2015) using 30 g/L xylose as a carbon source in the cultivation of *Rhodotorula mucilaginosa* obtained biomass, total lipids and lipid content equal to 10.78 g/L, 1.5 g/L and 14.41%, respectively. These values are higher than those determined in the present study. The same authors using glucose 30 g/L as carbon source obtained biomass, total lipids and lipid content equal to 16.79 g/L, 3.61 g/L and 21.55%, respectively. These values are relatively close to those determined in the present study.

4 FINAL CONSIDERATIONS

The yeast *Rhodotorula mucilaginosa* showed to be able to assimilate the carbohydrates present in the hydrolysate of the soybean hulls. For a culture medium not optimized, there was a significant production of biomass but with a low lipid content.

The cultivation in the pure carbohydrates showed that although the glucose had presented a relevant result, arabinose and xylose were not well assimilated by the *Rhodotorula mucilaginosa* suggesting that the same difficulty of assimilation might happen in the hydrolysate that contains the same carbohydrates.

It is suggested to optimize the assimilation of the carbohydrates in the hydrolysate aiming to increase the biomass production as well as the lipid content. Variations in the carbon nitrogen ratio and the initial carbohydrate concentration may be factors to be tested in order to achieve this goal.

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