Original

Influence of the use of rice bran extract as a source of nutrients on xylitol production

Influência do uso de extrato de farelo de arroz como nutriente na produção de xilitol

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Abstract

Xylose-to-xylitol bioconversion using 2.5 or 10% (v/v) rice bran extract was performed to verify the influence of this source of nutrients on *Candida guilliermondii* metabolism. Semisynthetic medium (*SM*) and sugarcane bagasse hemicellulosic hydrolysate detoxified with ion-exchange resins (*HIE*) or with alteration in pH combined with adsorption onto activated charcoal (*HAC*) were fermented in 125 mL Erlenmeyer flasks at 30 °C and 200 rpm for 72 hours. Activated charcoal supplemented with 2.5% (v/v) rice bran extract was fermented by *C. guilliermondii* in a MULTIGEN stirred tank reactor using pH 5.0 and 22.9/hour oxygen transfer volumetric coefficient. Higher values of xylitol productivity (0.70, 0.71, and 0.62 $g.Lh^{-1}$) and xylose-to-xylitol conversion yield (0.71, 0.69, and 0.63 $g.g^{-1}$) were obtained with 2.5% (v/v) rice bran in semisynthetic medium, ion-exchange resins, and activated charcoal, respectively. Moreover, during batch fermentation, the xylitol volumetric productivity and fermentation efficiency values obtained were 0.53 $g.Lh^{-1}$ and 61.1%, respectively.

Keywords: *hemicellulosic hydrolysate; sugarcane bagasse; xylose; xylitol; rice bran.*

Resumo

O estudo da bioconversão de xilose em xilitol usando 2,5 ou 10% (v/v) de extrato de farelo de arroz foi realizado para verificar a influência desse nutriente no metabolismo de *Candida guilliermondii*. Meio semi-sintético (*SM*) e hidrolisado hemicelulósico de bagaço de cana-de-açúcar purificado com resinas de troca iônica (*HIE*) ou pela alteração de pH combinado com adsorção em carvão ativo (*HAC*) foram fermentados nas condições de 30 °C e 200 rpm durante 72 horas em frascos Erlenmeyer de 125 mL. O carvão ativo suplementado com 2,5% (v/v) de farelo de arroz foi fermentado por *C. guilliermondii* em reator MULTIGEN, pH 5,0 e coeficiente volumétrico de transferência de oxigênio de 22,9/horas. Maiores valores de produtividade volumétrica em xilitol (0,70, 0,71 e 0,62 $g.Lh^{-1}$) e de conversão de xilose em xilitol (0,71, 0,69 e 0,63 $g.g^{-1}$) foram obtidos com 2,5% (v/v) de farelo de arroz em meio semissintético, resinas de troca iônica e carvão ativo. Além disso, durante a fermentação em batelada, a produtividade volumétrica de xilitol e a eficiência de fermentação foram 0,53 $g.Lh^{-1}$ e 61,1%, respectivamente.

Palavras-chave: *hidrolisado hemicelulósico; bagaço de cana-de-açúcar; xilose; xilitol; farelo de arroz.*

1 Introduction

In 2009, the production of sugarcane in Brazil was 612.2 million t, which was used to produce 34.6 million t of sugar and 25.8 billion L of alcohol (DELIÊ, 2009). For each ton of sugarcane processed, 180-280 kg bagasse are produced. The bagasse contains 41-44% cellulose, 25-27% hemicellulose, and 20-22% lignin (INSTITUTO…, 2000). Up to 80% of the pentoses present in the hemicellulosic fraction of the sugarcane bagasse can be extracted by acid hydrolysis. The hydrolysate thus obtained is composed mainly of xylose, a monosaccharide that can be used in bioconversion processes for the production of xylitol, ethanol, lactic and acetic acids, and yeast biomass (BROWNELL, NAKAS, 1991; AGUILAR et al., 2002; SAHA, 2003; MOLDES et al., 2006).

Xylitol, an alcohol with five hydroxyl groups, is a polyol used by food and pharmaceutical industries as a substitute of sucrose for people with diabetis and obesity. It also prevents dental caries and middle ear infections in young children (UHARI; KONTIOKARI; NIEMELA, 1998; MÄKINEN, 2000). Xylitol can be produced by biotechnological route using xylose and hemicellulosic hydrolysates rich in xylose (MARTÍNEZ et al., 2007, 2009; CANILHA et al., 2008).

Rice bran extract has been used as a source of nutrients in the bioconversion of the xylose contained in different hemicellulosic hydrolysates into xylitol (MARTÍNEZ et al., 2002, 2003, 2007; CANILHA; ALMEIDA E SILVA; SOLENZAL; 2004; MUSSATTO, ROBERTO, 2004; VILLARREAL et al., 2006; CANILHA et al., 2008). Rice bran is a by-product of the rice milling processing and is a source of vitamins and amino acids containing 12-15% oils, 11-12% proteins and 2.750 $kcal.kg^{-1}$ of metabolizable energy (BUTOLO, 2002).

The present study deals with the effect of the rice bran extract concentration on xylitol yield and volumetric productivity in semisynthetic medium and sugarcane bagasse hydrolysate fermentations by *Candida guilliermondii*.

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2 Materials and methods

2.1 Preparation of sugarcane bagasse hemicellulosic hydrolysate

The hemicellulosic hydrolysate was obtained by acid hydrolysis of sugarcane bagasse in a 250 L stainless steel reactor. The sugarcane bagasse was percolated with 100 mg of H_2SO_4 per gram of bagasse (dry weight) for 10 minutes at 120 °C. To reach a higher sugar concentration, the hydrolysate was concentrated in a laboratory scale evaporator under vacuum at 66 ± 4 °C.

The concentrated hydrolysate was then treated (*HAC*) prior to fermentation for removing toxic compounds formed during acid hydrolysis by using CaO to increase the pH to 7.0 and H_3PO_4 to decrease it to 5.5 afterwards. Next, it was clarified with activated charcoal (2.5%) at 200 rpm (New Brunswick Scientific Co., Inc Edison-New Jersey-USA) and 30 °C for 1 hour, according to Martínez et al. (2003). The precipitates formed during each one of these two procedures as well as the activated charcoal were removed by filtration.

In a second alternative treatment, the hydrolysate pH was increased to 5.0 using CaO, and the precipitate formed was removed by filtration. The hydrolysate (*HIE*) was treated with ion-exchange resins in the following order: A-103S weak anionic, C-150 strong cationic, A-860 strong anionic, and A-103S weak anionic. Glass columns of 59 cm lenght and 4.5 cm diameter were loaded with the resins for purification. The hydrolysate was fed to the columns by gravity at 30 °C, and the washing and regeneration operations were carried out when the resins were exhausted; the purified liquor was characterized.

The *HAC* and *HIE* treated hydrolysates were autoclaved at 100 °C for 15 minutes and aseptically supplemented with $(NH_4)_2SO_4$ (3 $g.L^{-1}$), rice bran extract (2.5 or 10% (v/v)), and $CaCl_2\ 2H_2O$ (0.1 $g.L^{-1}$) before used as the fermentation media.

2.2 Microorganism and culture conditions

The inoculum was prepared by cultivating the *Candida guilliermondii* cells in 125 mL Erlenmeyer flasks containing 50 mL medium (30 $g.L^{-1}$ xylose, 3 $g.L^{-1}$ ammonium sulphate, 0.1 $g.L^{-1}$ calcium chloride, 10% (v/v) rice bran extract). Twenty-four hour incubation was carried out under agitation (300 rpm) at 30 °C. The cells were then centrifuged at 2000 g (CU-5000 Damon/IC) for 15 minutes and resuspended in sterile water to reach the final concentration of 1 $g.L^{-1}$.

The fermentations in semisynthetic medium (*SM*), sugarcane bagasse hemicellulosic hydrolysate treated with ion-exchange resins (*HIE*) and sugarcane bagasse hemicellulosic hydrolysate treated with pH alteration and active charcoal adsorption (*HAC*) by *C. guilliermondii* were carried out in 125 mL Erlenmeyer flasks containing 50 mL media, at 30 °C and 200 rpm during 72 hours. The composition of the Semisynthetic Medium (*SM*) consisted of a mixture of synthetic (73 $g.L^{-1}$ xylose, 3 $g.L^{-1}$ ammonium sulphate and 0.1 $g.L^{-1}$ calcium chloride) and natural (2.5% (v/v) rice bran extract) compounds.

The sugarcane bagasse hemicellulosic hydrolysate treated with pH alteration plus active charcoal adsorption, supplemented with 2.5% (v/v) rice bran extract, 3 $g.L^{-1}$ ammonium sulphate and 0.1 $g.L^{-1}$ calcium chloride, was also fermented by *C. guilliermondii* in a MULTIGEN (New Brunswick Scientific Co) stirred tank reactor with 550 mL medium volume, pH 5.0, and using a value of 22.9/hours for the oxygen transfer volumetric coefficient. A double walled bottom was used for temperature control (30 °C), and the agitation (350 rpm) was performed by three-disk turbines with six blades. The composition of the sugarcane bagasse hemicellulosic hydrolysate was 6.75 $g.L^{-1}$ glucose, 68.30 $g.L^{-1}$ xylose, 4.23 $g.L^{-1}$ arabinose, and 1.90 $g.L^{-1}$ acetic acid. Fermentation tests in the stirred tank reactor were performed in triplicate.

2.3 Downstream processing of the fermented medium

The fermented media were centrifuged at 2000 g during 15 minutes in a CU-5000 Damon/IC centrifuge. The purification was then carried out in assay tubes containing 4 mL media, which were filtered after the addition of NaOH 0.1N or H_2SO_4 0.1N to correct the pH to 5.0, 6.0, 7.0, 8.0, or 9.0.

2.4 Analytical methods

Xylose, glucose, arabinose, xylitol, and acetic acid concentrations were determined by high performance liquid chromatography (Shimadzu LC-10AD, Tokyo, Japan) using a BIORAD AMINEX HPX-87H (300 × 7.8) column, RID 6A refractive index detector, using 0.01N sulphuric acid as eluent, 0.6 mL/minutes flow rate and 45 °C column temperature, detector attenuation 16×, and sample volume of 20 μL.

The oxygen transfer volumetric coefficient (k_La) was determined under standard fermentation conditions using the "gassing-out" method, as described by Bartolomew et al. (1950). After sparging the medium with nitrogen at 300 rpm and 30 °C, the oxygen concentration was shown to increase with time according to the following Equation 1:

$$\ln(C^* - C) = \ln(C^* - Co) - k_L a\, t \quad (1)$$

where C* is the dissolved oxygen concentration at saturation; C is the dissolved oxygen concentration in the culture medium; and Co is the initial oxygen concentration.

The cellular concentrations were determined from absorbance measurements in a BECKMAN DU 640B spectrophotometer (Fullerton, CA) by comparing the optical density of a cell suspension with a standard curve that relates absorbance at 600 nm as a function of cell dry weight.

The concentration of total proteins was determined using a modification of the Lowry method, according to Lucarini and Kilikian (1999).

3 Results and discussion

3.1 Preparation of sugarcane bagasse hemicellulosic hydrolysate

Table 1 presents the composition and pH of the original, concentrated, and treated hydrolysates. During the concentration

stage, 4.6 and 4-fold increases in the arabinose and xylose and glucose concentrations were verified. This behavior was not observed for the acetic acid concentration (increase of 2.4 times) since its boiling point was 63 °C when the pressure exceed to 100 mmHg under vacuum.

The hydrolysate was submitted to a detoxification with the use of ion-exchange resins and combining alteration of pH and adsorption with activated charcoal aiming to decrease the concentration of the compounds that can inhibit the cell metabolism during fermentation. During this stage, there were decreases of 39.75 and 29.06% in the acetic acid concentrations of the hydrolysates treated with resins or activated charcoal, respectively. The detoxification of brewery's spent grain hydrolysate with Dowex MWA1 anionic resin was reported by Carvalheiro et al. (2005). According to these authors, the hydrolysate treatment without previous pH adjustment was the method that removed the largest quantity of inhibitory compounds. This detoxification method allowed removal of 48% acetic acid, 52% formic acid, 100% levulinic acid, 64% furfural, and 44% hydroxymethylfurfural (HMF). Chandel et al. (2007) performed the treatment of a sugarcane bagasse hemicellulosic hydrolysate with ion-exchange resins, activated charcoal, overliming, and laccase. Ion-exchange was a more efficient treatment than activated charcoal removing 64.3 and 38.7% furans, 75.8 and 57% total phenolics and 85.2 and 46.8% acetic acid, repectively. The overliming and laccase-based treatments did not caused any effect on the acetic acid level. A decrease of 100% was verified in the concentration of acetic acid in the sugarcane bagasse hemicellulosic hydrolysate after treatment with resins in the following order: MN-150, A-860, C-155, and A-103 (MARTÍNEZ et al., 2007). According to Villarreal et al. (2006), acetic acid was totally or almost totally removed during treatment of eucalyptus hemicellulosic hydrolysate with ion-exchange resins in the following sequence: cationic Applexion, anionic Purolite A-860, cationic Purolite C-150, and anionic Applexion. Meanwhile, the concentration of this acid was not largely affected (0-32%) by treatment with activated charcoal.

The detoxification method with ion-exchange resins had a little effect on xylose recovery. Similar results were reported by Villarreal et al. (2006) and Martínez et al. (2007) during treatment of eucalyptus and sugarcane hemicellulosic hydrolysates with ion-exchange resins, respectively.

Regarding sugar concentrations, there were decreases of 19, 18, and 18 % in the concentrations of glucose, xylose, and arabinose after treatment with activated charcoal. Similar results were reported by different authors testing treatment with charcoal (MARTÍNEZ et al., 2002, 2003, 2007; VILLARREAL et al., 2006).

Table 1. Composition and pH of the hemicellulosic hydrolysate: original (H_o), concentrated (H_1), treated with ion-exchange resins (*HIE*) and with activated charcoal (*HAC*).

Parameters	H_o	H_1	*HIE*	*HAC*
D-glucose ($g.L^{-1}$)	1.18	4.79	2.75	3.88
D-xylose ($g.L^{-1}$)	19.79	91.59	89.97	75.01
L-arabinose ($g.L^{-1}$)	0.94	4.34	2.87	3.55
Acetic acid ($g.L^{-1}$)	2.31	5.61	3.38	3.98
pH	1.82	2.52	9.97	5.50

3.2 Fermentations in erlenmeyer flasks

The xylitol production from synthetic medium and sugarcane bagasse hemicellulosic hydrolysate treated with resins or activated charcoal by *Candida guilliermondii* was evaluated using 2.5 or 10% (v/v) of rice bran extract. Figure 1 presents the profiles of glucose, xylose, arabinose and acetic acid consumptions, xylitol production, and cellular growth during the fermentations.

As can be seen, the use of different concentrations of rice bran extract did not produce significant changes in the fermentation of synthetic medium and sugarcane hydrolysate treated with ion-exchange resins. A xylose consumption of

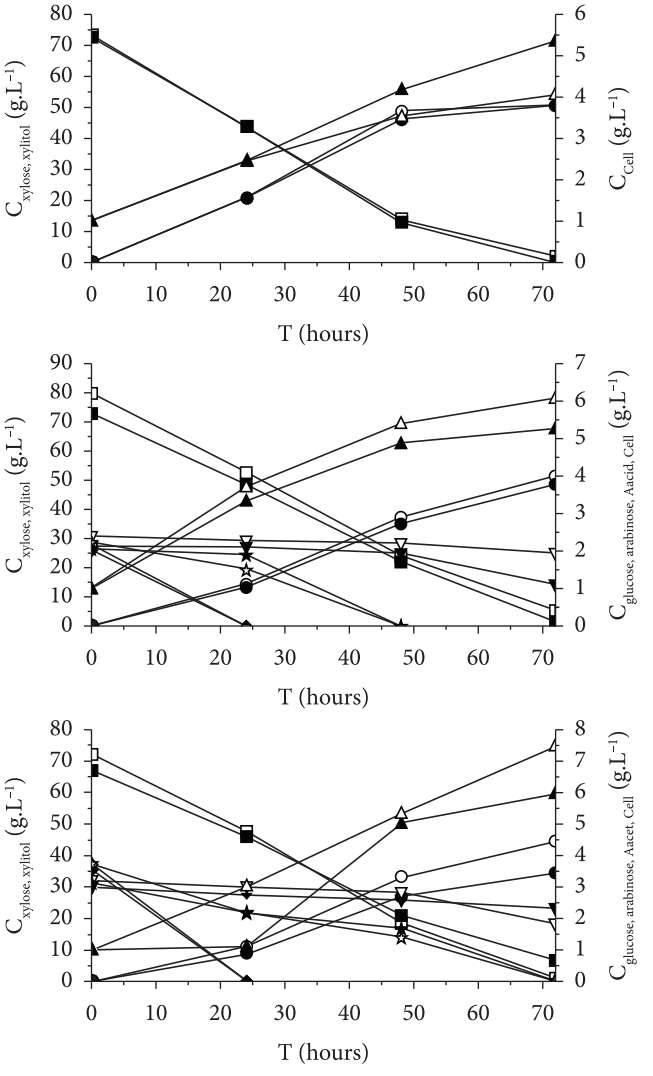

Figure 1. Concentration profiles of glucose (◇,◆), xylose (□,■), arabinose (▽,▼), xylitol (○,●), acetic acid (☆,★) and cell (Δ,▲) a) throughout fermentation of synthetic medium; b) sugarcane hemicelullosic hydrolysate treated with resins; and c) activated charcoal using 2.5 and 10% (v/v) rice bran extract respectively.

97.9-100% was verified in the synthetic medium fermentation, while a xylose consumption of 93.23-97.1% was observed in the hydrolysate fermentation. Meanwhile, xylitol production (51 $g.L^{-1}$) was almost identical in both media. On the other hand, there was a lower cell growth in synthetic medium (4-5.35 $g.L^{-1}$) than in hydrolysate (5.27-6.1 $g.L^{-1}$).

With regard to fermentation of the hydrolysate treated with activated charcoal, higher xylose consumption (98.7%), xylitol production (44.5 $g.L^{-1}$), and cell growth (7.04 $g.L^{-1}$) were verified with the use of 2.5% (v/v) rice bran extract. Decreases of 8.7, 22.6, and 28.1% were verified in xylose consumption, xylitol production, and cell growth, respectively, with the use of the higher concentration of rice bran extract (10% (v/v)).

The results of xylitol volumetric productivity, xylose-to-xylitol bioconversion yield and fermentation efficiency obtained in each medium are shown in Table 2. The increase in the rice bran extract concentration from 2.5 to 10% (v/v) decreased the xylitol productivity, yield factor, and fermentation efficiency, regardless of the medium used. This effect was more significant in the hydrolysate treated with activated charcoal. In this hydrolysate, the increase of rice bran concentration produced a decrease from 0.62 to 0.48 $g.Lh^{-1}$, 0.63 to 0.57 g/g, and 68.3 to 62.2% in the aforementioned fermentative parameters, respectively. Bernardi et al. (2006) studied the use of rice bran extract (2.5, 5, 10, 20, and 30 $g.L^{-1}$) as a supplement for a hemicellulosic hydrolysate of oats spelts in the xylose-to-xylitol bioconversion with the same yeast. According to these authors, maximum activities of the enzymes xylose reductase (0.5077 U/mg_{prot}) and xylitol dehydrogenase (0.9624 U/mg_{prot}) were achieved with a higher concentration of rice bran. Meanwhile, higher xylose-to-xylitol bioconversion factor (0.89 g/g) was obtained using 10 $g.L^{-1}$ rice bran extract, which corresponded to lower values of xylose reductase and xylitol deshydrogenase activities (0.2908 and 0.244 U/mg_{prot}, respectively). In addition, the increase in the rice bran concentration from 10 to 30 $g.L^{-1}$ increased the cell growth. Villarreal et al. (2006) studied the purification process of eucalyptus hemicellulosic hydrolysate using activated charcoal and ion-exchange resins for xylitol production. These authors reported that the better xylitol volumetric productivities obtained (0.65 and 0.68 $g.Lh^{-1}$) were achieved after treatment with ion-exchange resins. According to Canilha et al. (2008), 0.37 $g.Lh^{-1}$ xylitol volumetric productivity and 0.65 g/g xylitol yield were obtained during xylose-to-xylitol bioconversion by *C. guilliermondii* in wheat straw hemicellulosic hydrolysate treated with active charcoal.

Taking these results into account, the next step was to evaluate the fermentation using a lower concentration of rice bran extract in a stirred tank reactor.

3.3 Fermentation in stirred tank reactor

The progress of the *HAC* fermentation in the fermentor is shown in Figure 2. The oxygen transfer rate corresponding to a k_La of 22.9 h^{-1} promoted 99.21% xylose assimilation and resulted in 37.85 $g.L^{-1}$ xylitol production and 8.99 $g.L^{-1}$ cell growth after 72 hours of fermentation. According to these results, it was concluded that the xylose metabolism was directed towards

Table 2. Effect of the concentration of rice bran extract on xylitol volumetric productivity (Q_p), xylitol bioconversion factor ($Y_{p/s}$) and fermentation efficiency (ε) obtained by fermentation of synthetic medium (SM), sugarcane hydrolysate treated with resins (HIE) and treated with activated charcoal (HAC).

Medium	$C_{rice\ bran}$ ($g.L^{-1}$)	Q_p ($g.Lh^{-1}$)	$Y_{p/s}$ (g/g)	ε (%)
SM	5	0.703	0.7107	77.50
	20	0.702	0.6922	75.92
HIE	5	0.715	0.6916	75.42
	20	0.677	0.6819	74.43
HAC	5	0.618	0.6263	68.30
	20	0.478	0.5706	62.23

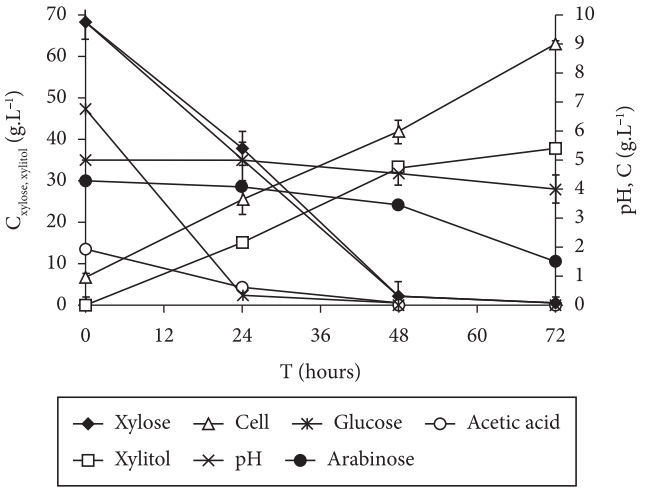

Figure 2. Concentration profiles of glucose, xylose, arabinose and acetic acid consumption, xylitol production, cellular growth and pH during the discontinuous process of xylitol production from sugarcane hemicellulosic hydrolysate treated with activated charcoal by *Candida guilliermondii*.

xylitol production (0.56 g/g yield) instead of towards cell growth (0.12 g/g yield). A probable explanation for this behavior is that the dissolved oxygen concentration in the medium was relatively low, and the cells were under oxygen limitation.

Besides xylose, small amounts of glucose (6.75 $g.L^{-1}$) and arabinose (4.23 $g.L^{-1}$) were also present in the hydrolysate treated with activated charcoal. The hexose was not detected after 24 hours of fermentation. To the contrary, arabinose was assimilated (64.54%) only after 48 hours of fermentation, which coincided with the xylose exhaustion. Affleck (2000) reported that 40% arabinose was consumed after 170 hours fermentation by *Candida tropicalis* in a 1 L reactor. Martínez et al. (2007) reported that 52.41% arabinose was consumed after 130 hours fermentation by *Candida guilliermondii* in a 15 L reactor.

The acetic acid (1.90 $g.L^{-1}$) present in the hemicellulosic hydrolysate was completely consumed after 48 hours of fermentation. Canilha et al. (2003) also reported a similar behavior for *Candida guilliermondii* cultivated in wheat straw hemicellulosic hydrolysate. A possible explanation for acetic

acid consumption is that this compound directly enters the Krebs cycle via acetyl-CoA and is used to produce energy.

During this fermentation, 0.526 $g.Lh^{-1}$ xylitol volumetric productivity and 61.07% fermentation efficiency were obtained. Martinez et al. (2003) reported that, at pH 5.5 and with 20 h^{-1} oxygen transfer coefficient, higher xylitol volumetric productivity (0.70 $g.Lh^{-1}$) was achieved in a study on continuous fermentation process for xylitol production from sugarcane bagasse hydrolysate. Canilha, Almeida e Silva and Solenzal (2004) performed studies on eucalyptus hydrolysate detoxification with pH adjustment combined with activated charcoal adsorption (CA, 70.91 $g.L^{-1}$ xylose) or ion-exchange resins (IE, 60.92 $g.L^{-1}$ xylose) and their fermentation by *Candida guilliermondii* using 2 $g.L^{-1}$ ammonium sulphate and 5 $g.L^{-1}$ rice bran extract as nutritional supplements. These authors reported lower xylitol volumetric productivity (0.30 $g.Lh^{-1}$) and fermentation efficiency (44%) during fermentation of the hydrolysate CA. On the other hand, higher xylitol volumetric productivity (0.68 $g.Lh^{-1}$) and fermentation efficiency (83%) were verified during the fermentation in the IE hydrolysate. Meanwhile, Martínez et al. (2007) reported lower xylitol volumetric productivity (0.478 $g.Lh^{-1}$) and higher fermentation efficiency (81.81%) during the fermentation of sugarcane hydrolysate treated with ion-exchange resins by *Candida guilliermondii* using 3 $g.L^{-1}$ ammonium sulphate, 0.1 $g.L^{-1}$ calcium chloride, and 20 $g.L^{-1}$ rice bran extract as nutrients. Martínez et al. (2009), in a study on batch fermentation process of xylitol from semisynthetic medium using 20 $g.L^{-1}$ rice bran extract reported that 0.66 $g.Lh^{-1}$ xylitol volumetric productivity and 0.70 g/g xylitol yield were obtained from 96% xylose consumed after 92 hours of fermentation. Carvalho, Canilha and Silva (2007) studied the effect of nutritional supplementation by adding or not ammonium sulphate (30 $g.L^{-1}$) and/or rice bran extract (10% v/v) to the fermentation medium during the semi-continuous xylitol bioproduction in sugarcane bagasse hydrolysate. According to these authors, in the absence of nutrients the xylitol production, productivity, and yield did not exceed 12.1 $g.L^{-1}$, 0.13 $g.Lh^{-1}$, and 0.30 g/g, respectively. On the other hand, supplementation of the hemicellulosic hydrolysate with ammonium sulphate and rice bran extract considerably improved the xylose-to-xylitol bioconversion. Silva and Roberto (1999), however, did not see any improvement on the xylose-to-xylitol bioconversion by the same yeast strain in single-batch fermentation due to the supplementation of rice straw hemicellulosic hydrolysate with ammonium sulfate and rice bran extract.

Table 3. Concentration of total proteins (C_p) and values of pH after centrifugation (pH_a).

pH	C_p ($mg.L^{-1}$)	pH_a
3.64 (Standard)	2706.8	3.60
5.00	2675.7	4.95
6.00	2616.5	5.86
7.00	1991.7	6.66
8.00	2104.9	7.30

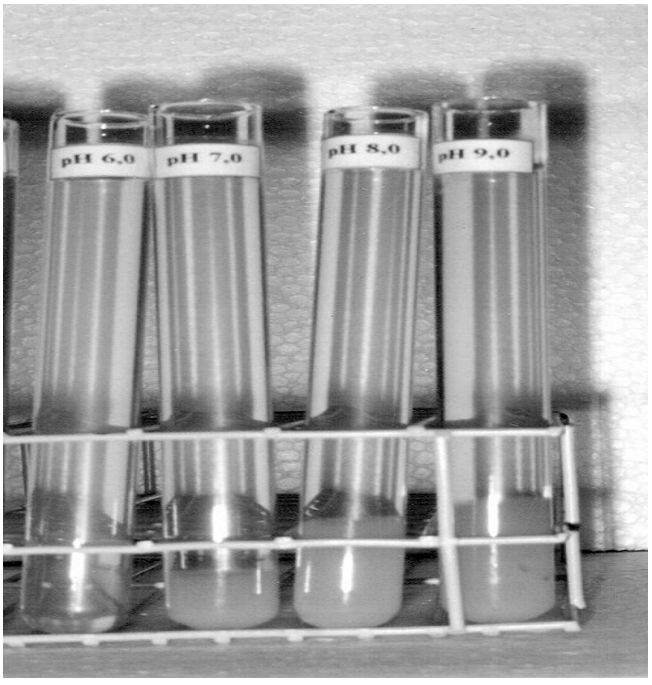

Figure 3. Formation of precipitates in the fermented media when the pH was adjusted among 6.0 and 9.0.

3.4 Downstream processing of the fermented medium

The xylitol-rich fermented liquor contained yeast and fragments of yeast as well as other chemicals such as ingredients of cultivation medium, residual substrates, and fermentation by-products. The medium was initially subjected to a centrifugation step. No precipitates were formed in pH 5.0 or 6.0, regardless the type of fermented medium. On the other hand, when the pH was adjusted to 7.0, 8.0, and 9.0, there was a cinsiderable formation of precipitates in all fermentation media supplemented with 20 $g.L^{-1}$ of rice bran. Figure 3 shows the obtained results in pH among 6.0 and 9.0. The precipitation was faster at pH 7.0.

Table 3 presents the concentration of total proteins and the values of pH after centrifugation. When pH was adjusted to 7.0 and 8.0, a higher removal of proteins corresponding to 26.4 and 22.2% was obtained and the pH of the fermented medium changed (no data were determined at pH 9.0). Conversely, the removal of protein was not affected by pH adjustment to 5.0 or 6.0, reaching values of 1.15 and 3.35%, respectively, and the pH values did not exhibit significant changes.

A synthetic medium containing all the supplements used in the fermentation was later prepared and submitted to centrifugation. A precipitate with the same characteristics of color and odor was observed, which allowed us to conclude that the rice bran extract was the component hindering the downstream processing of the fermented medium.

4 Conclusions

The use of a lower concentration of rice bran extract (2.5% (v/v)) favored the downstream processing of the xylitol contained in the fermented medium eliminating the necessity

of performing a second centrifugation step after pH adjustment, which precipitated rice bran-derived impurities.

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