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Cassava hydrolysates used as substrates for the production of second-generation ethanol by *Saccharomyces cerevisiae* ATCC 26602 immobilized in sodium alginate spheres

Hidrolisados de mandioca utilizados como substratos para produção de etanol de segunda geração por *Saccharomyces cerevisiae* ATCC 26602 imobilizada em esferas de alginato de sódio

Hidrolizados de yuca utilizados como sustratos para la producción de etanol de segunda generación por *Saccharomyces cerevisiae* ATCC 26602 inmovilizado sobre esferas de alginato de sodio

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Abstract
Cassava has a prominent position in the Brazilian agriculture and economy. During the production of flour, a very large volume of residue is generated. One way to use these residues is through the production of ethanol. In this context, the present research aimed to use cassava residues as raw material for ethanol production. For this, sulfuric acid was used at concentrations of 1.0 to 5.0% during 5, 10 and 15 minutes of heating in an autoclave. The fermentation was carried out by *Saccharomyces cerevisiae* ATCC 26602, and to achieve a better performance, it was immobilized in sodium alginate spheres. Ethanol production was also estimated using a synthetic medium added with glucose that served as a comparison standard. The results of the experiment showed that the highest concentration of reducing sugars obtained by the hydrolysis of the residue was with the use of 2.0% of H_2SO_4 with 15 min. heating at 121 ºC, releasing 56.26 g/L of reducing sugars. The parameters used that led to the highest production of ethanol by the yeast, 7.85 g/L of ethanol, were: pH of 6.5, growth temperature of 30 °C, without agitation. The results showed that cassava residues can be used as a potential substrate for ethanol production. Thus, this work presents data on the most suitable conditions for the use of these industrial wastes in order to generate less polluting fuel energy, an increasingly attractive feature in a world where economic and environmental concerns grow every day.
Keywords: Fermentation; Yeast; Celullosic ethanol; Acid hydrolysis; Residue.

Resumo
A mandioca apresenta colocação de destaque na agricultura e na economia brasileira. Durante a produção da farinha é gerado um volume muito grande de resíduos. Uma forma de aproveitamento desses resíduos é através da produção de etanol. Neste âmbito, a presente pesquisa teve como objetivo utilizar resíduos da mandioca como matéria-prima para a produção de etanol. Para isso, utilizou-se ácido sulfúrico nas concentrações de 1,0 a 5,0% durante 5, 10 e 15 minutos de aquecimento em autoclave. A fermentação foi realizada por *Saccharomyces cerevisiae* ATCC 26602, e para alcançar um melhor desempenho, foi imobilizada em esferas de alginato de sódio, a partir disso. A produção de etanol também foi estimada utilizando meio sintético adicionado de glicose que serviu como padrão de comparação. Os resultados do experimento mostraram que a maior concentração de açúcares redutores obtida pela hidrólise do resíduo foi com a utilização de 2,0% de H_2SO_4 com 15 min. de aquecimento a 121 ºC, liberando 56,26 g/L de açúcares redutores. Os parâmetros utilizados que levaram à maior produção de etanol pela levedura, 7,85 g/L de etanol, foram: pH de 6,5, temperatura de crescimento de 30 °C, sem agitação. Os resultados mostraram que resíduos de mandioca podem ser utilizados como um substrato potencial para a produção de etanol. Assim, este trabalho apresenta dados sobre as condições mais adequadas para o aproveitamento destes rejeitos industriais tendo em vista a geração de

energia combustível menos poluente, característica cada vez mais atrativa em um mundo onde as preocupações económicas e ambientais crescem a cada dia.
Palavras-chave: Fermentação; Levedura; Etanol celulósico; Hidrólise ácida; Resíduo.

Resumen
La yuca ocupa una posición destacada en la agricultura y la economía brasileñas. Durante la producción de harina se genera un volumen muy grande de residuos. Una forma de aprovechar estos residuos es mediante la producción de etanol. En este contexto, la presente investigación tuvo como objetivo utilizar residuos de yuca como materia prima para la producción de etanol. Para ello se utilizó ácido sulfúrico en concentraciones de 1,0 a 5,0% durante 5, 10 y 15 minutos de calentamiento en autoclave. La fermentación se realizó con *Saccharomyces cerevisiae* ATCC 26602, y para lograr un mejor rendimiento se inmovilizó en esferas de alginato de sodio. La producción de etanol también se estimó utilizando un medio sintético al que se le añadió glucosa que sirvió como estándar de comparación. Los resultados del experimento mostraron que la mayor concentración de azúcares reductores obtenida por la hidrólisis del residuo fue con el uso de 2.0% de H2SO4 con 15 min. calentando a 121 ºC, liberando 56,26 g/L de azúcares reductores. Los parámetros utilizados que llevaron a la mayor producción de etanol por parte de la levadura, 7,85 g/L de etanol, fueron: pH de 6,5, temperatura de crecimiento de 30 °C, sin agitación. Los resultados mostraron que los residuos de yuca se pueden utilizar como sustrato potencial para la producción de etanol. Así, este trabajo presenta datos sobre las condiciones más adecuadas para el aprovechamiento de estos residuos industriales con el fin de generar energía combustible menos contaminante, una característica cada vez más atractiva en un mundo donde las preocupaciones económicas y ambientales crecen cada día.
Palabras clave: Fermentación; Levadura; Etanol celulósico; Hidrólisis ácida; Residuo.

1. Introduction

Cassava is one of the main energetic foods in developing countries. Brazil is the second largest producer of cassava with 10% of the world production producing between 22 and 25 tons. More than 100 countries are producers (Otekunrin; Sawicka, 2019).

Among the cassava products, flour has a prominent place, being among the most produced agricultural products in the country, its production has been increasingly intensified and with that the residues generated during its production also intensify and end up causing environmental damage (Polachini et al., 2020).

The average yield of cassava is 25 to 30% for flour production, the rest is classified as waste divided into: liquid and solid parts. When discarded incorrectly, they generate river and soil pollution, generating toxic materials and bad smells, attracting insects and rodents. In addition, the excess of organic matter dumped in rivers is responsible for the eutrophication of its waters (Correia et al., 2018).

A possible solution for these residues generated during the production of cassava flour is the production of ethanol from its solid residues, studies show that for the production of ethanol cassava has great potential being similar to that of sugar cane.

Fuels such as those described above have been gaining ground in the market, competing with those of fossil origin because they are cheaper and generate less environmental damage, a large part of the ethanol fuel in Brazil comes from the fermentation of sugar cane, however, several other raw materials can be used for this, from cellulosic based to those with a large amount of starch such as cassava.

The production of second-generation bioethanol or cellulosic ethanol is presented as an advantage in relation to environmental issues, as it is obtained from fermentable sugars from the breakage of the cellulose and hemicellulose chains in the biomass of industrial, agricultural and forestry residues, not competing, thus, with the arable areas used for food production, since the product of interest has already been extracted. In addition, other crops that are not used for human consumption can be used. To achieve this, new technologies are being developed to increase the production of this fuel, combining sustainability and economic viability in obtaining it (Ofori-Boateng & Lee, 2014).

In the same way, research for the production, for the increase of yield and improvement in the efficiency of the

process of second generation fuels contribute to the development of an integrated and diversified technology in the production of fuels, chemical products, energy and other materials of interest to the industrial market, aiming at the minimum generation of greenhouse gases and residues from renewable energy sources and that encompass the concept of biorefinery as applied to petroleum derivatives.

Saccharomyces cerevisiae is one of the most used yeasts in fermentation processes for the production of bioethanol, due to its easy isolation, obtaining and few nutritional requirements. On the other hand, the immobilization technique is defined as a physical barrier where microorganisms are confined and acquire the ease of adaptation, allowing their reuse in various fermentation processes, due to the protection generated by the immobilization matrix, which makes this method advantageous in the production of ethanol (Toogood & Scrutton, 2018).

In view of this, the objective of this research was to analyze which acid hydrolysis process would cause the greatest release of sugars present in discarded cassava residues to later analyze the effect of the immobilization of *Saccharomyces cerevisiae* ATCC 26602 in sodium alginate spheres in the production of second-generation ethanol.

2. Methodology

Cassava industrial waste was supplied by the flour industry Moreá Alimentos Ltda., located in the municipality of Monte Alegre de Minas, Brazil.

2.1 Cassava Industrial Residue

The industrial cassava residue, consisting of cassava husks, intershells and tips, was washed and dried in the sun for 48 hours, until it reached approximately 12% humidity. Subsequently, the shells were ground to increase the contact surface and stored in plastic containers. Particle size was homogenized to < 0.64 mm using a Produtest brand atomizer.

2.1.1 Hydrolysis

The hydrolysis was carried out with sulfuric acid (H_2SO_4) at diluted concentrations of 1.0; 2.0; 3.0; 4.0 and 5.0% (v/v), in triplicate. For this, 10g of cassava residues were weighed in 250 mL Erlenmeyers and 100 mL of the diluted acid was added. The heat-treated hydrolysates were maintained in an autoclave at 121 °C/1 kgf/cm2 for 5, 10 and 15 minutes.

At the end of each hydrolysis, they were left at room temperature until cooling, and then the pH was adjusted to 6.5 and 7.0 with 50% sodium hydroxide (m/m). Each hydrolysate was centrifuged at 3600 rpm for 20 minutes to separate the remaining husks. After centrifugation, it was filtered through No. 1 Whatman filter paper to completely remove the cassava husk (remaining cake). During the preparation of the fermentation media, the hydrolysates were concentrated in a water bath at 60ºC until they reached the desired concentration, when necessary. In each hydrolysate, total sugars were determined (Dubois et al., 1956); reducing sugars (Nelson, 1944; Somogyi, 1952) and phenolic compounds (Chaovanalikit, Wrolstad, 2004).

2.1.2 Detoxification

Powdered activated carbon was used in this procedure in the proportion of 1 g for every 40 g of hydrolysate. The mixture was shaken in a refrigerated orbital incubator (Shaker) for 1 hour at 200 rpm and 30ºC, centrifuged at 2000g for 30 minutes and then filtered through No. 1 Whatman filter paper. The analysis of reducing sugars, phenolic compounds and total sugars was then performed, before and after each detox step. Ultimately, the supernatant obtained was stored at a temperature below 0ºC, where the crude and detoxified hydrolysates were used for subsequent use in the fermentation medium.

2.2 Micro-organism and Maintenance Medium

The microorganism used for the fermentation of the hydrolysate was the yeast *Saccharomyces cerevisiae* ATCC 26602. The yeast was provided by the Department of Chemical Engineering at the University of Coimbra - Portugal. The yeast storage medium was composed of (g/L): malt extract, 3; yeast extract, 3; meat peptone, 5; glucose, 10; and agar, 20. Stored at a temperature of 4 ºC.

2.2.1 Pre-inoculum preparation

The *S. cerevisiae* strain was inoculated into test tubes containing 10 mL of maintenance medium, as described in item 2.1, and incubated in an oven at 30°C for 24 h. 5 mL of sterile sodium alginate was added to each tube and, with the aid of a platinum loop, the microbial mass was removed from the tube. Then, they were poured into 100 mL of previously sterilized sodium alginate.

2.2.2 Cell immobilization - Cell entrapment in alginate gel

S. cerevisiae ATCC 26602 cells under pre-established conditions (item 4.2.2) were added to a 2.0% sodium alginate solution (Dynamica), previously prepared and sterilized, under agitation for 15 minutes. Afterwards, it was dripped (using a 10 mL pipette) into a 3.0% calcium chloride solution (Sigma) under agitation, obtaining calcium alginate spheres approximately 2.5 mm in diameter. and 0.06 g; kept for 30 minutes under agitation, after this period, 2 grams of these spheres were added to each of the fermentation media.

2.3 Fermentation Media

2.3.1 Synthetic medium

The synthetic medium was carried out in a basal medium (pH 7) composed of yeast extract (5 $g.L^{-1}$); KH_2PO_4 (1 $g.L^{-1}$); $MgSO_4.7H_2O$ (1 $g.L^{-1}$), $(NH_4)_2SO_4$ (1 $g.L^{-1}$). For assays using yeast, glucose solution was used. The sugar solution was sterilized separately at 121°C for 15 min, and after cooling to room temperature, it was aseptically mixed at the time of preparation with the synthetic medium, previously prepared in distilled water and sterilized in order for the medium to obtain a sugar concentration of 50 g/L.

2.3.2 Medium containing hydrolyzed cassava peel residues

The detoxified and non-detoxified hydrolysates were standardized based on the concentration of reducing sugars. The hydrolyzed broths were concentrated to 50 g/L of reducing sugars and then they were sterilized separately at 121°C for 15 min., cooled to room temperature and then added to the synthetic medium, previously prepared and sterilized.

2.4 Fermentation Conditions

The experiments were carried out to observe the production of bioethanol by the yeast *S. cerevisiae* ATCC 26602. The fermentations took place in 125 mL Erlenmeyers containing 50 mL of the culture media and approximately 2 g of sodium alginate beads containing the immobilized yeast.

2.4.1 Evaluation of variables that can influence the yeast *Saccharomyces cerevisiae*

The determination of the working conditions of the yeast was carried out using synthetic medium added with glucose (50 g/L), during a 12-hour fermentation, taking samples every two hours.

The best fermentation time was determined from a static fermentation (without agitation), for which the samples were

incubated in a B.O.D oven at 30 °C, after each fermentation time, the pH was determined. The rest of the medium was centrifuged at 3600 rpm for 15 min to separate the cells, an aliquot was also captured for ethanol analysis. The experiment was performed in duplicate.

2.4.2 Evaluation of fermentations using hydrolysates

Fermentations using detoxified and non-detoxified hydrolysates were carried out under conditions similar to those described previously.

For ethanol production, a concentration of reducing sugars in the hydrolysate of 50 g/L was used, the fermentation was carried out without agitation, incubating the hydrolysates in a B.O.D incubator at 30 ºC for 12 hours, taking samples after 4, 6, 8, 10 and 12 hours of fermentation, the pH was determined at each time, as well as a sample was taken and centrifuged at 3600 rpm for 15 ml for cell separation and aliquots were collected for ethanol analysis. The experiment was performed in duplicate.

2.5 Analitycal Methods

The determination of the amount of glucose present in the hydrolysates was performed only for samples that did not undergo heat treatment, by means of an enzymatic reaction of β-glucosidase with the use of staining reagents in the reading of absorbances in a spectrophotometer (510 nm).

The final pH was determined directly in the fermented broth using the pH meter Digmed model DM20.

Cell concentration was determined by turbidimetry using a Biochrom spectrophotometer, model Libra S22.

Ethanol was determined by gas chromatography in cell-free fermented broth, using a Thermo Scientific Model Focus chromatograph with flame ionization detector (FID) and HP-FFAP column (25 m x 0.2 mm x 0.3 µm); oven temperature at 70 ºC (maintaining this temperature throughout the isothermal run); 5 min run time; injector temperature of 230 °C; detector temperature of 270 °C; injection of 200 µl of sample steam. The samples were left in a water bath at a temperature of 40 °C (until reaching equilibrium).

3. Results and Discussion

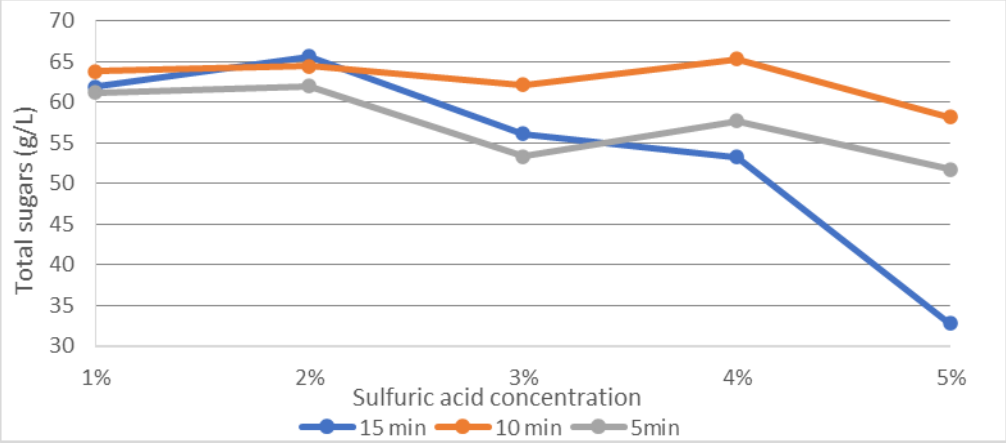
3.1 Standardization of the Acid Hydrolysis

Various acid hydrolysis was performed to determine the best reaction time of sulfuric acid in contact with the biomass. For this, at the end of each test, the levels of total sugars, reducing agents and phenolic compounds present in the hydrolyzed medium were evaluated.

The best hydrolysis conditions were chosen considering the concentration of H_2SO_4 and the process time that allowed a greater release of sugars under heating conditions in an autoclave at 121 ºC and 1 kgf/cm2.

The results from the evaluation of total sugars carried out in the hydrolyzed medium are shown in Figure 1.

Figure 1 – Values of the concentrations of total sugars (TS) released during the acid hydrolysis of cassava residue from different concentrations of H_2SO_4 and heating times.

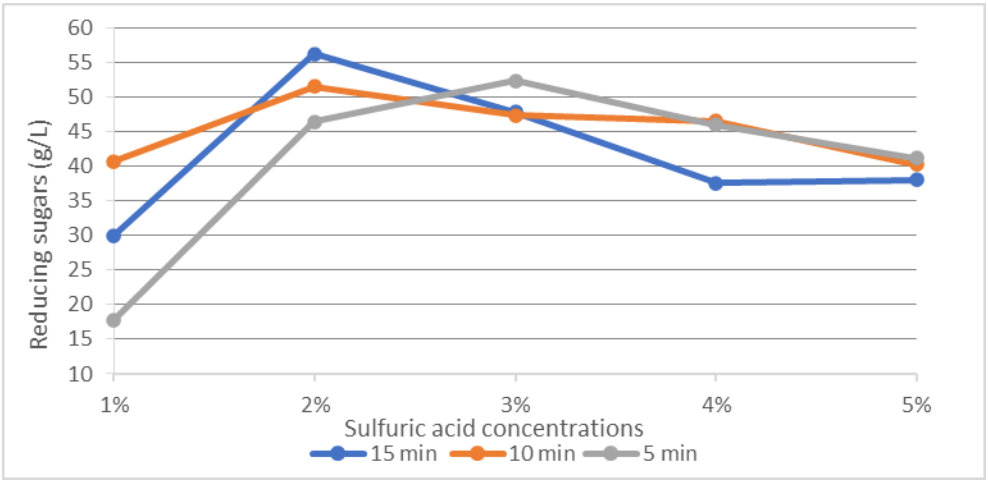


Source: Authors.

Figure 1 shows the amount of total sugars (TS) released in each concentration of H_2SO_4 used (1.0 to 5.0%), with the heating times in autoclave for 5, 10 and 15 min., where it is possible to observe that the highest release of TS was with the use of 2.0% of H_2SO_4 and 15 minutes of heating, where 65.61 g/L of total sugars were released. This helped to choose the concentration of H_2SO_4 and the heating time of the hydrolysis. In 10 min. and 4% concentration, the same result was obtained, with an increase of 5 min. being more advantageous of heating than the increase of acid in hydrolysis, as the use of a higher concentration of acid can be harmful, increasing the release of inhibitory compounds.

Figure 2 shows the results obtained from the analysis of reducing sugars in hydrolyzed biomass with and without detoxification. In Figure 2 it is possible to visualize that the hydrolysis condition that presented the best result was with the use of a concentration of 2.0% of H_2SO_4, where a higher concentration of released reducing sugars was found, equivalent to 56.26 g/L with heating in an autoclave for 15 minutes.

Figure 2 – Values of the concentrations of reducing sugars (RS) released during the acid hydrolysis of cassava residue from different concentrations of H_2SO_4 and heating times.



Source: Authors.

Hashem and Darwish (2010) used potato residues as biomass for the production of ethanol from *S. cerevisiae*, and their results showed that using 1.0% H_2SO_4 at 100 °C for 1 h was sufficient to hydrolyze all the starch contained. in the residue. However, they preferred to standardize the acid hydrolysis at 2.0%, thus achieving the release of reducing sugars close to 18 g/L, values lower than those obtained in the present work, 56.26 g/L, in addition to having a longer reaction time having consequently higher energy expenditure.

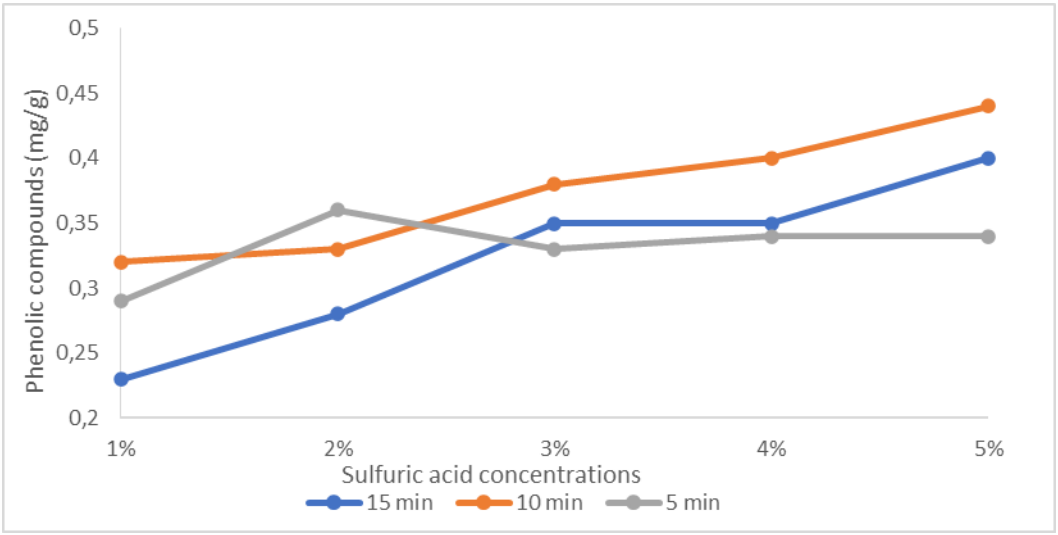
The use of low concentrations of H_2SO_4 reduces the risk of corrosion of equipment and glassware, releases a smaller amount of toxic compounds and reduces the cost of the process by using less reagent. (Tomas-Pejó et al., 2011).

Figure 3 shows the results of the analysis of phenolic compounds after acid hydrolysis with heating in an autoclave for 5, 10 and 15 min. of duration. In Figure 3, it can be seen that the 10 min. releases the greatest amount of phenolic compounds at the used concentrations of H_2SO_4, when compared with the use of 5 and 15 min. of heating, with the exception of the concentration of 2.0% for the time of 5 min. where there was a higher concentration than for 10 and 15 min. heating (Figure 3).

The analysis of phenolic compounds (Figure 3) showed that the highest release of the compounds occurred when using a concentration of 5.0% of H_2SO_4 during 10 minutes of heating (0.44 mg/g cassava residue). From these results, it was possible to verify a direct relationship with the concentration of H_2SO_4 and the time of the reaction in autoclave, because in general, the longer the time and the higher the concentration of H_2SO_4 used, the greater the indices of phenolic compounds formed. This shows that the most advantageous is to use a lower concentration of acid to avoid the formation of inhibitory compounds.

Magalhães (2011) found that using high acid concentrations associated with a prolonged heating time can lead to the transformation and accumulation of toxic compounds. On the other hand, they allow a greater degradation of the sugars available in the substrate, and are potential inhibitors of fermentation of certain yeasts and bacteria.

Figure 3 – Amount of phenolic compounds released from the hydrolysis of cassava residues using different heating times and sulfuric acid concentrations.



Source: Authors.

Loureiro et al. (2020), using a 5% concentration of H_2SO_4 and a longer heating time, 2 hours at 120 °C; obtaining 44.68 g/L of RS. With a lower concentration, in our research, 56.26 g/L of RS were obtained, however, with heating for only 10 min. in autoclave at 121 °C. This shorter heating time allows for a faster and cheaper process and furthermore prevents the formation of PhC.

The cassava residue presents in its composition low concentrations of lignin, where after its degradation, there were low levels of phenolic compounds released. Due to the low levels of phenolic compounds found in the analyses, it is expected that they do not influence fermentation, causing a possible inhibition of *S. cerevisiae* ATCC 26602. Inhibitors cause an increase in environmental stress for the fermentative microorganism, while the concentration of Ethanol increases, since excess environmental stresses can result in cell death if the cell's ability to cope with stress is exceeded (Palmqvist, Hahn-Hägerdal, 2000).

The aggressiveness of the hydrolysis process with dilute acid on the concentration of fermentable sugars and generation of sugar degradation products as well as the fermentation capacity of wood hydrolysate was studied by Larsson et al. (1999 b), for this he varied the parameters of time (1-30 min.), temperature (150-240°C), and H_2SO_4 concentration of 0.5 - 4.4% (m/m) of dry matter. It was observed that as there was the formation of furfural and HMF, there was consequently a decrease in the concentration of fermentable sugars, as these compounds accumulated, there was a decrease in the fermentative performance. The solution found by the authors to have high yields of fermentable sugars as well as high fermentation power was to define the optimal parameters of 0.5% H_2SO_4, 225°C, 5 minutes of reaction and 0.5% H_2SO_4, 210°C, 10 minutes, respectively.

Possibly the decrease in total sugars released in the cassava residue during acid treatments and heating times are the reasons cited by the authors above. It can be seen in Figure 1, the higher the acid concentration (greater than 3% of sulfuric acid) and treatment times, the greater the degradation of sugars, resulting in their decline, probably due to the formation of secondary compounds such as furfural and HMF, but such compounds were not quantified in the present work.

3.2 Hydrolysate Detoxification

The purpose of this procedure is to promote the reduction of inhibitory compounds generated during the acid hydrolysis step or during the hydrolysate concentration step (Figure 3). To verify the effectiveness of the process, analysis of the phenolic compounds was carried out in the crude hydrolysate (before the detoxification process and after the concentration of the hydrolysate). After this process, the amount of reducing and total sugars was also analyzed. Table 1 shows the concentration of phenolic compounds (PhC), total (TS) and reducing sugars (RS) in the crude hydrolysate (CH) and in the detoxified hydrolysate (after being concentrated up to 50 g/L of reducing sugars) (DH), subjected to fermentation.

Table 1 – Phenolic compounds, total sugars and reducing sugars before and after the detoxification process (after concentration of the medium).

	Phenolic Compounds (mg/g de cassava residue)	**Total Sugar (g/L)**	**Reducing Sugar (g/L)**
Crude Hydrolysate	0,544 ± 0,04	51,327 ± 4,68	68,830 ± 2,13
Detoxified Hydrolysate	0,191 ± 0,00	35,072 ± 3,65	60,287 ± 1,71

Source: Authors.

In Table 1, it is possible to observe that the detoxification was efficient, as it resulted in a reduction of approximately three times in the levels of phenolic compounds present in the detoxified hydrolyzed medium that was used for fermentation.

Therefore, from the results found in the analysis of total sugars and reducing sugars obtained from the acid hydrolysis of cassava residues, and also from the evaluation of the concentration of phenolic compounds released in the hydrolyzed medium, it was possible to identify that the concentration and heating time that showed the best results were 2.0% for 15 min. heating in the hydrolysis (65.31 g/L of total sugars, 56.26 g/L of reducing sugars and 0.28 g/L of phenolic compounds). These

parameters were chosen as the best for carrying out the fermentation.

3.3 Fermentations

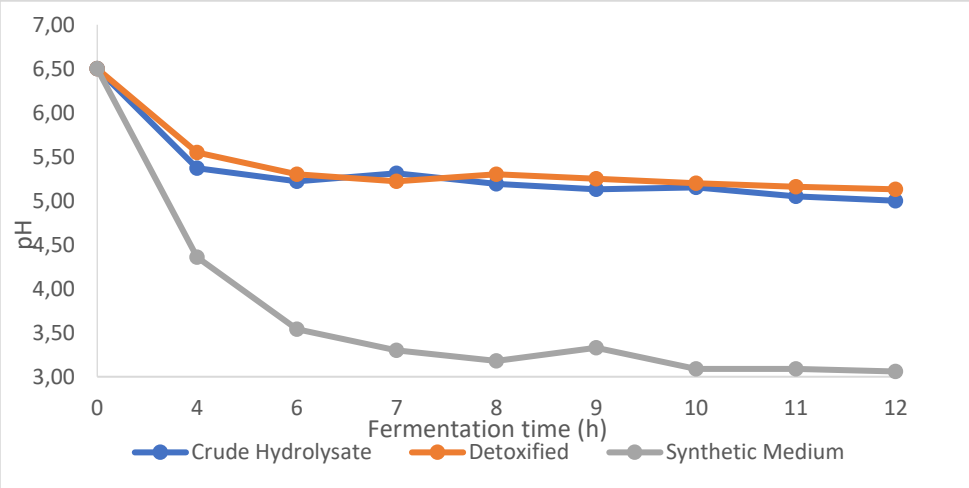
The experiments were carried out with the three-culture media: synthetic medium; half with the detoxified hydrolysate and half with the crude hydrolysate (without the detoxification process), in order to evaluate the behavior of the yeast immobilized on sodium alginate spheres.

The following conditions were used to carry out the fermentation: temperature of 30 ºC, pH of 6.5, incubation period of 12 hours, without agitation, with a concentration of reducing sugars of 50 g/L. The value of the initial inoculum for each fermentation was 2 g of alginate beads containing the immobilized yeast.

In Figure 4 it is possible to observe that there is a reduction in pH as the incubation time increases in both samples. It is also possible to observe that, for the standard synthetic medium, added with glucose, there is a marked reduction during the first 6 hours of fermentation, where after this period there were no more significant changes in pH.

The pH of the fermentation medium decreased due to the increase in biomass, since, during its development, glucose absorption occurs and the release of acids and other by-products of its metabolism, consequently, decreases the pH of the culture medium (Silva et al., 2020).

Figure 4 – pH variation in hydrolysates without detoxification, detoxified and synthetic medium used as substrates in culture media for 12-hour fermentation by *S. cerevisiae* yeast immobilized on sodium alginate spheres.



Source: Authors.

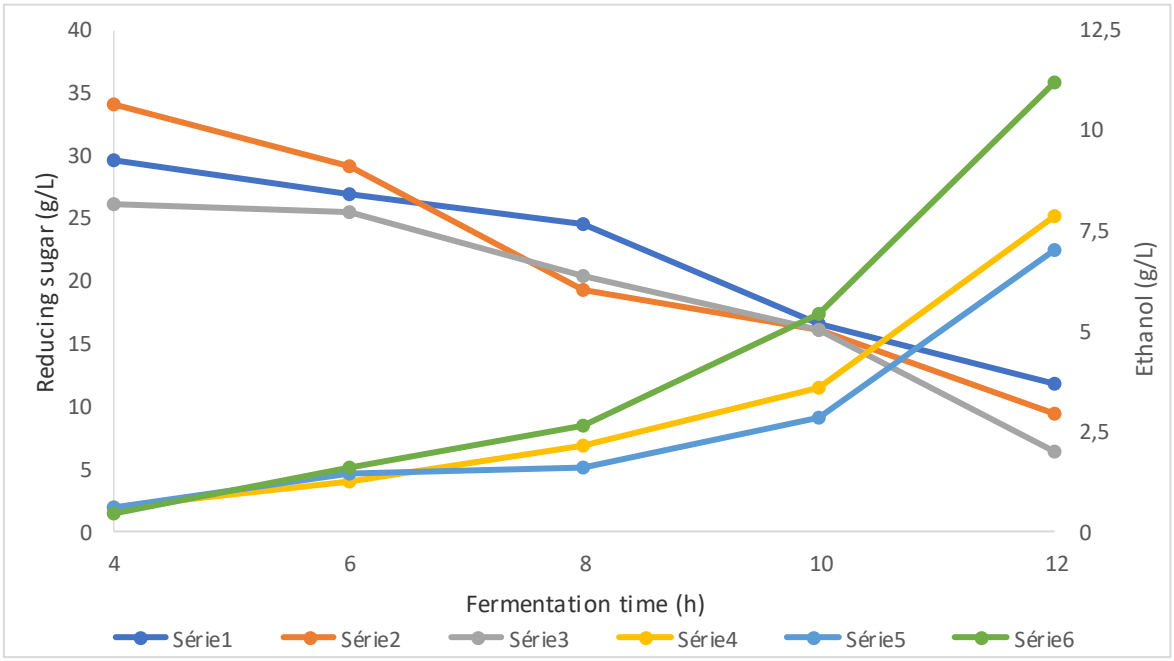
Furthermore, pH can cause fermentation inhibition, leading to a change in cellular metabolism, acid dissociation results in the accumulation of protons that generate intracellular acidification that inhibits yeast growth, forms by-products and free radicals. Immobilization promotes advantages in this sense, as it protects cells from adverse conditions in the environment, such as pH, inhibitory compounds, etc. (Park; Chang, 2000).

Figure 5 shows the ethanol production in relation to the fermentation time and the substrate added to the culture medium used (SM: Synthetic Medium; DH: Detoxified Hydrolysate; CH: Crude Hydrolysate without Detoxification). Also, it is possible to verify the consumption of sugar by the yeast, which decreased over the 12 hours of fermentation, showing the production of metabolites.

The maximum production of ethanol occurred in the time of 12 h of incubation, with the use of detoxified medium this content was 7.85 g/L of ethanol, with the crude hydrolysate it was 7.07 g/L of ethanol with a productivity of 0.59 g/L and

theoretical yield of 76.52%. The use of the synthetic medium presented a production of 12 g/L of ethanol.

Figure 5 – Profile of ethanol production and consumption of fermentable sugars by *S. cerevisiae* ATCC 26602 in the medium containing Detoxified Hydrolysate (DH), Crude Hydrolysate (CH) and Synthetic Medium (SM) in an initial concentration of Reducing Sugar (RS) of 50 g/L, 30 °C without agitation.



Source: Authors.

It is observed that the variation in ethanol concentration between detoxified and non-detoxified media is small. The largest generator of phenolic compounds in plant cell matrices is lignin, cassava residues, however, have a low amount of such compound and therefore this residue releases a low amount of phenolic compounds in the acid hydrolysis step. This factor, associated with the protection against adverse conditions offered by alginate, may have been important factors in the low difference between the production of ethanol from hydrolyzed, crude and detoxified media. In addition to these factors, the literature shows that low concentrations of acids in the medium provide a stimulating effect on ethanol production by *S. cerevisiae* (Pampulha; Loureiro-Dias, 1989).

This indicates that the microbial inhibition generated by the phenolic compounds was practically null in the metabolism of the yeast *S. cerevisiae* ATCC 26602 (Figure 5).

Behera et al. (2010) in their studies compared ethanol production in *S. cerevisiae* in marula flower hydrolysates, the conditions used were pH 6.5, temperature of 30°C for 96 hours of fermentation. With a productivity of 0.25 g/L.h, lower than that obtained in the present work for 12 hours of fermentation, this value is close to the average productivity of the samples in a period of 8 hours of fermentation. One of the possible reasons for this difference is the amount of substrate used in this work, which was quadrupled in the work cited, the other is the optimization of the process caused by the immobilization of the yeast in sodium alginate spheres.

4. Conclusion

The sulfuric acid concentration that showed the greatest efficiency in the release of fermentable sugars by the acid hydrolysis of cassava residues was 2% (v/v) with a heating time of 15 minutes (121 ºC), releasing 56.26 g/L of reducing

sugars; 65.27 g/L of total sugars and a low concentration of phenolic compounds (0.28 mg/g cassava residue).

In the alcoholic fermentation with initial concentration of reducing sugar 50 g/L, 12 hours of fermentation at 30ºC, flasks kept without agitation, initial pH of 6.5, 7.02 g/L of ethanol were obtained with a productivity of 0.59 g/L and theoretical yield of 76.52% with the crude hydrolyzed medium (without previous detoxification); 7.85 g/L of ethanol with a productivity of 0.65 g/L.h and theoretical yield of 81.30% from the detoxified medium.

The yeast showed resistance to inhibitory or interfering compounds from hydrolysis, with protection against adversities offered by immobilization in alginate spheres. Therefore, detoxification is not a necessary step for the bioethanol production process with *S. cerevisiae* ATCC 26602, under the conditions of the present experiment.

In view of the results obtained in this project, it is concluded that the use of cassava husk residue for bioethanol production using *S. cerevisiae* ATCC 26602 immobilized in sodium alginate spheres is viable, with this arises the need for appropriate technologies for the use of easily accessible and low-cost raw materials for the production of fuel ethanol, a viable outlet for energy production in a sustainable, renewable and cheap way.

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