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RESEARCH ARTICLE OPEN ACCESS

ACID HYDROLYSIS OF CORN COB FOR THE PRODUCTION OF SECOND GENERATION ETHANOL BY SACCHAROMYCES CEREVISIAE ATCC 26602

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ABSTRACT

Corn is one of the most produced crops in Brazil, during its processing it generates rejects such as cobs, stalk, leafs and straw that can be used as biomass for the production of second generation bioethanol (2G). However, for the reutilization of this substrate it is necessary the application of a treatment for the removal of lignin, fragmentation of hemicellulose and cellulose into corresponding sugars to be converted into ethanol by microorganisms. Therefore, the purpose of this research was to perform acid hydrolysis of corn cob and utilizing the hydrolyzate as a culture medium for the production of ethanol 2G by yeast *Saccharomyces cerevisiae* ATCC 26602. Initially it was evaluated the effect of various concentrations of sulfuric acid and various times of exposure to the acid solution to determine the efficiency of the hydrolysis. Next it was carried out the removal of phenolic compounds generated during the hydrolysis (detoxification). Following that, it was analyzed the effect of various agitation speeds in the production of ethanol by the yeast in question. The hydrolysis had more efficiency when performed with 2.5% of sulfuric acid and exposure time of 30 min. The hydrolyzate detoxification resulted in the lowering of phenolic compounds that act as inhibitors of the fermentation. The yeast was capable of utilizing the raw hydrolyzate (RH) and detoxified raw hydrolyzate (DRH) for cellular growth and biosynthesis of ethanol under agitated environment. Maximum ethanol production (8.11 $g.L^{-1}$) was obtained in 100 rpm agitation tests after 36 hours of fermentation. The results imply that detoxified corncob hydrolyzate culture medium could be an efficient alternative for the production of second generation ethanol by the yeast *S. cerevisiae* ATCC 26602. Furthermore, the yeast was able to grow and produce ethanol on the raw hydrolyzate, which removes one stage in the production of 2G ethanol.

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INTRODUCTION

The worldwide concern with the increase of the emissions of gases causing the greenhouse effect, the negative impacts on the global climate and exhaustion of fossil fuels, have motivated the search for alternate methods of renewable energies, therefore making the production of bioethanol interesting and beneficial, since it represents a clean and sustainable source of energy (RUIZ *et al.,* 2016). First generation ethanol is a biofuel produced from microbial fermentation of vegetable raw materials, such as sugar cane, corn and sugar beet (BHARATHIRAJA *et al.,* 2017). However, a large amount of waste is generated to obtain it, becoming a major environmental problem. The production of second generation ethanol (2G) is an alternative to this problem, since, the agro-industrial residues are rich in cellulose and hemicellulose, that can be submitted to chemical or biological hydrolysis, providing the assimilable sugars, for the microorganisms to perform the alcoholic fermentation (OFORI-BOATENG; LEE, 2014). The bioethanol originated

from lignocellulosic materials is a renewable fuel that promises to decrease the problems caused from the use of fossil fuels. Other than the fact that it contributes to the reduction of CO_2 emissions, and ensures a worldwide supply of energy (RUIZ *et al.,* 2016). Amongst all of the various agro-industrial residues, those originated the cultivation of maize, also known as corn, deserves emphasizing because it is one of the most grown cultures worldwide, amounting to more than one billion tons of corn produced in the harvest of 2016/2017. Out of this total, 98.5 million tons were produced in Brazil, making it the third largest grower of corn, only behind the United States and China (USDA, 2018). The stages of processing corn generate large quantities of residues, especially straw and cobs. According to FAO (2018), in 2016 there were 51 867 980 tons of residues originated by the cultivation of maize crops worldwide. The corn cob is produced after threshing for remove the corn kernels (ANUKAM *et al.,* 2017). This residue is mainly composed by cellulose (32.2%), hemicellulose (29%) and lignin (18.8%) (RAJ *et al.,* 2015). The high contents of cellulose and hemicellulose found in this residue make it an interesting source of carbon for fermentable microorganisms, such as *S. cerevisiae*. The process of converting lignocellulosic biomass into assimilable sugars by fermentable microorganisms consists of many steps. In the first step, the material is submitted to a particle size reduction, followed by the hydrolysis of its chemical bonds, by the addition of chemical agents of enzymes that promote the release of fermentable sugars that can finally be assimilated in the stage of fermentation, by microorganisms such as bacteria, yeasts or filamentous fungi (ANWAR *et al.,* 2014). On the alcoholic fermentation, the microorganism most frequently used is the yeast *Saccharomyces cerevisiae* due to its capacity to produce ethanol in high quantities. The strain *S. cerevisiae* ATCC 26602 has shown great capacity to adapt under different conditions of process and substrate produced by acid and enzymatic hydrolysis, even without sterilization or detoxification of the hydrolyzates used as culture medium (MENDES *et al.,* 2016). In view of that, the main objective of this work was to evaluate the effect of various concentrations of sulfuric acid and time of hydrolysis for obtaining fermentable sugars, to analyze the effect of different agitation speeds and fermentation times on a raw hydrolyzate medium and detoxified raw hydrolyzate medium, in the production of second generation ethanol by the yeast *Saccharomyces cerevisiae* ATCC 26602.

MATERIAL AND METHODS

Raw material: The corncobs were acquired from establishments that commercialize corn based products, situated at Municipal Dam of São José do Rio Preto, São Paulo, Brazil (Latitude: 20º49'10.99" S and Longitude: 49º22'45.98" W).

Processing of corncob: The corncobs were cut manually in pieces of approximately 3cm, distributed in trays and set out to dry naturally by sun exposure until reaching a brittle like aspect. Next, the pieces of corncob were crushed and sifted up to 14 mash, conditioned in hermetic bottles and stored under ambient temperature until being needed.

Corncob chemical hydrolysis and detoxification: The hydrolysis of corncob took place in Erlenmeyer bottles of 250ml, at a scale of 10/100 (w/v) of corncob in sulfuric acid (H_2SO_4) with various concentrations (2.5; 5.0; 7.5 and 10%). The bottles were submitted to heating in autoclave at 121 °C/1.1 atm. during 15 and 30 min. Afterwards, the pH of the hydrolyzate material was neutralized (pH 7) with NaOH (50%) and filtered using Whatman nº1 filter for the separation of residual solids, which were discarded. The obtained supernatant was used as substract for the production of ethanol by two methods: raw hydrolyzate (RH), before being submitted into detoxification and detoxified raw hydrolyzate, that had already been through the detoxification stage with activated charcoal, for the removal of inhibitory agents from the microbial fermentation according to the methodology described by Mussatto; Roberto (2004). Before carrying out the fermentation process with the RH and DRH the total sugar values were measured by the phenol-sulfuric method (DUBOIS *et al.,* 1956) and reducing sugars total values by the copper-arsenate method (NELSON, 1944) and (SOMOGYI, 1952). Also, the total phenolic compounds values were analyzed by the method described by Folin-Ciocalteau modified by Chaovanalikit; Wrolstad (2004).

Microorganism, maintenance, inoculum preparation and fermentation medium: The samples of *Saccharomyces cerevisiae* ATCC 26602 yeast were kindly given by the Department of Chemistry Engineering of University of Coimbra - Portugal. The yeast was stored YM Medium composed by glucose (10 $g.L^{-1}$), peptone (5 $g.L^{-1}$), yeast extract (3 $g.L^{-1}$), malt extract (3 $g.L^{-1}$) and agar (20 $g.L^{-1}$) at pH 5.0, after being cultivated for 24h a 30°C. The microorganism strains were stored under freezing conditions (-80ºC), while periodically being reactivated to maintain its viability. The inoculum was prepared by the addition of microorganisms previously cultivated in YM broth in Erlenmeyer bottles of 250ml containing 100ml of YM broth (pH 5.0). The inoculum was standardized by spectrophotometry with an absorbance of 0.6 and wave length of 600nm. The production of ethanol was performed in culture media (pH 7) composed by yeast extract (5 $g.L^{-1}$); KH_2PO_4 (1 $g.L^{-1}$); $MgSO_4.7H_2O$ (1 $g.L^{-1}$), $(NH4)_2SO_4$ (1 $g.L^{-1}$) and a carbon supply (glucose, raw hydrolyzate or raw detoxified hydrolyzate). Effect of culture medium agitation on substrate (carbon source) consumption and ethanol biosynthesis by *S. cerevisiae* Erlenmeyer bottles of 250 ml containing 100 ml of detoxified acid hydrolyzate of corncob and flasks containing 100 ml of raw acid hydrolyzate of corncob, which were incubated on a rotary shaker with agitation of 0.5 and 100 rpm at a constant temperature of 30 ºC. The fermentation of the raw detoxified hydrolyzate occurred during 48h, with samples being taken every 12 h for the purpose of evaluating the ethanol production, cellular biomass values, pH change, sugar values (reducing and total) and residual phenolic compounds. For the raw hydrolyzate the fermentation time was 24h, where, samples were taken every 4h at the phase-lag and at every 2h at the phase-log for the evaluation of cellular growth and ethanol production.

Analytical Methods: The final pH values were determined in the fermentation broth using the potentiometer Digmed pHmeter model DM20. The cellular concentration was determined by turbidimetry in spectrophotometer Biochrom, model Libra S22. The ethanol concentration values were determined by gas chromatography (GC) in the fermented broth free of cells, using a GC Thermo Scientific Model Series Focus TR-WAX column HP-FFAP (25 m x 0.2 mm x 0.3 μm) and flame ionization detector (FID).

The oven temperature was kept at 70 ºC (during the whole isothermal run), running time of 5min, the injector temperature was kept at 230°C; detector temperature at 270 °C and injection of 200 µl of sample vapor. The samples were left at a water bath of 40°C until reaching its point of equilibrium.

RESULTS AND DISCUSSION

effect of sulfuric acid concentration and time of exposure in the efficiency of corncob hydrolysis: Preliminary tests were performed to evaluate the performance of the various concentrations of H_2SO_4 in the hydrolysis of corncob using various heating times to establish the most effective procedure to be used on the biomass treatment. The H_2SO_4 concentration values tested were 2,5; 5,0; 7,5 at heating times of 15 and 30min at a temperature of 121 °C/1.1 atm. for each sample. Based on these treatments, the concentration of total and reducing sugars and phenolic compounds obtained during the hydrolysis were determined and shown in Figures 1, 2 and 3, where their values are quantified for the trials using raw hydrolyzate (RH) and detoxified raw hydrolyzate (DRH). The samples containing corncob hydrolyzate were detoxified by the addition of active carbon, aiming to reduce the content of fermentation inhibitor compounds, created by the addition of H_2SO_4 during the heating process. All experiments were run at a triplicate replica. These analytical determinations were executed to determine and compare the concentration values of which acid could free the highest value of fermentable sugars, that could be used by the yeast in the production of ethanol, during the fermentation process. The highest content of total sugars (38.44 $g.L^{-1}$ a 30.18 $g.L^{-1}$ were obtained when using concentration values of 2.5 to 7.5 % H_2SO_4 and 30min exposure time at 121 °C/1.1 atm. for the samples that had not been submitted to detoxification. On the other hand, when using a concentration of 10% sulfuric acid there was a noticeable reduction on the values of released sugars, shown in Figure 1.

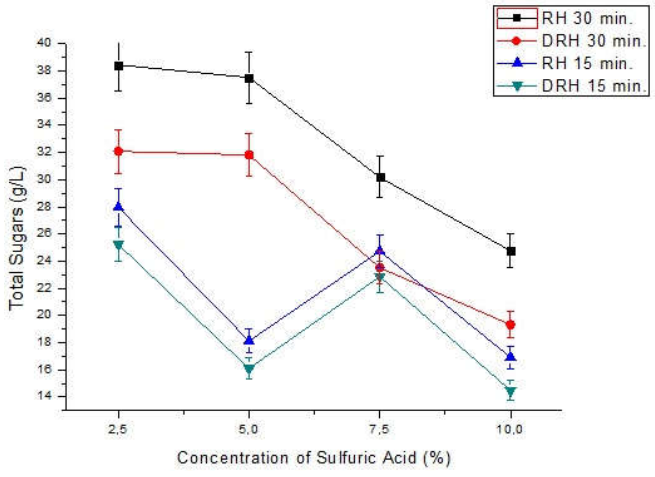

Figure 1. Profile of total sugars ($g.L^{-1}$) found for crude hydrolyzate (RH) and crude detoxified hydrolyzate (DRH), obtained at different sulfuric acid concentrations and heating at 121 ° C / 1.1 atm.

An acid hydrolysis of corncob performed using 5% of H_2SO_4 and heating times of 30, 60 and 120min, El-Zawawy et al. (2011), found inferior results, where only 5.80 $g.L^{-1}$ of total released sugars were obtained after 120min of hydrolysis. For the time of 30min they obtained 4.50 $g.L^{-1}$, while in this present work, while on the same exact conditions (5% de H_2SO_4 e tempo de 30 min.), it was possible to obtain 37.52 $g.L^{-1}$ of total sugars.

The effect of various exposure times showed a possible synergistic effect in correlation with the concentration of sulfuric acid and with the detoxification treatment of the hydrolyzate, as shown in Figure 1. In 30 min of exposure, there was a gradual reduction in sugar release as higher concentrations of H_2SO_4 were used for both RH and DRH. However when using 15min of exposure time on the tests, higher concentrations of sugars were found, when working with sulfuric acid concentrations of 2.5 and 7.5% for RH and DRH, and finally when performing trials with sulfuric acid concentrations of 5 and 10% there was a noticeable decrease of 32 and 34%, respectively (Figure 1). The process of detoxification of the corncob raw hydrolyzate implied a reduction on the total sugars concentration, released after the hydrolysis (Figure 1). In a research on corn straw acid treatment performed by Lu et al. (2007) tested concentrations 2.0; 4.0 e 6.0 % de H_2SO_4 and also varying the temperature between 80, 100 and 120 °C. On their trials, they noticed that the best working conditions were concentration of 2.0% of H_2SO_4 exposure time of 34 min at 120°C. What differs from this present work is that we utilized different concentrations of acid, showing that there is no need to use longer hydrolysis since 15 and 30 min were used at 121 ° C. The reducing sugars concentration levels released during the hydrolysis, on the conditions tested, showed a behavior similar to those of total sugars, as shown in Figure 2. Higher concentrations of sugar were also verified on the trials conducted using 2.5% and 7.5% of H_2SO_4 on both of the heating times mentioned before (15 and 30 min.). After the detoxification, there was also a reduction of the released sugars, as can be seen on Figure 2.

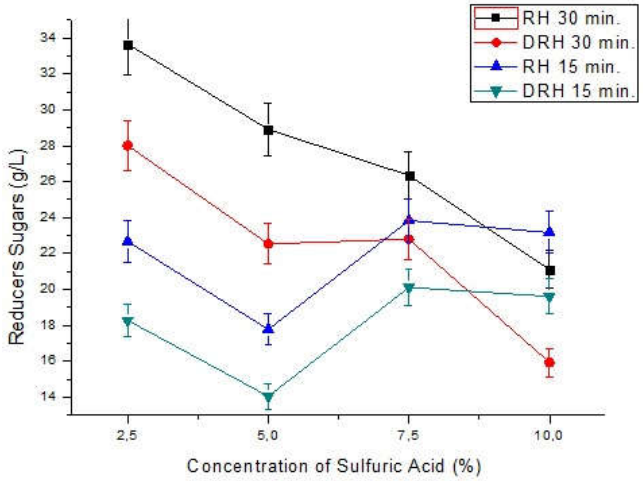

Figure 2. Profile of reducing sugars ($g.L^{-1}$) verified for crude hydrolyzate (RH) and crude detoxified hydrolyzate (DRH), obtained at different sulfuric acid concentrations and heating at 121 ° C / 1.1 atm.

Santos-Rocha et al. (2017) analyzed the total reducing sugars (TRS) content from corncobs and corn straws that had been submitted to hydrothermal pre-treatment with water at 170°C/15 min and 195°C/10 min, while on 200rpm agitation, following that they were hydrolyzed using enzymatic extract Accellerase®1500 (20 $FPU/g_{dry\ biomass}$) for 72 h at 150rpm, was obtained 7.4 $g.L^{-1}$ of TRS for the straws and 12.4 $g.L^{-1}$ of TRS for the corncobs. These results are inferior to the ones obtained in the present work, when comparing the values released sugars. For the acid hydrolysis of corncobs, the maximum release of reducing sugars on trials conducted with 2.5% of H_2SO_4 were found to be 22.67 $g.L^{-1}$ for 15 min and for 30 minutes the results obtained were 33.66 $g.L^{-1}$, both for the raw hydrolyzate without detoxification.

On a different work, by Rocha et al. (2016) they tested an acid pre-treatment for the corncobs and corn straws, analyzing the hydrolysis time, temperature and also the concentrations of H_2SO_4. Following the acid hydrolysis they performed and enzymatic hydrolysis, obtaining higher contents of TRS while using low concentrations of acid. Utilizing 0.5% of H_2SO_4 at 120°C for 15 min., for corn cob 42.6 g.L^{-1} was obtained; for straw 50.9 g.L-^{1} in TRS content. However, it is important to emphasize that the results obtained by these researchers also had enzymatic treatment and that, despite this, there was no increase in the concentration of sugars obtained when compared to the hydrolysis performed in our research. Therefore, the acid hydrolysis could make the fermentative process economically viable, because there would be no need for additional enzymes. The results achieved on our research, in comparison with the ones described, show that it is possible to work with higher temperatures (121°C). This is important because. A higher temperature induces higher cellular disorganization on the lignocellulose structure used as raw material, allowing the fermentable sugars to be more accessible, therefore releasing higher contents of TRS after each one of the pre-treatment stages (DAGNINO *et al.,* 2012), while also using lower concentrations of sulfuric acid.

Effect of detoxification on the removal of phenolic compounds of the raw hydrolyzate corncobs: As previously mentioned, after the process of detoxifying the raw hydrolyzed corn cobs there was a decrease of the total sugars and reducing sugars contents released after the hydrolysis (Figures 1 and 2). This decrease could have occurred during the process detoxification, that aims to remove inhibitor compounds by stages of exposure to activated carbon and centrifugation. The activated charcoal can bond with both the phenolic compounds and the sugars, retaining them, causing the reduction of the final contents. Phenolic compounds and by-products such as furfural and e hydroxymetilfurfural (HMF) are the main inhibitor compounds existing on this kind of lignocellulosic sample submitted to acid hydrolysis and heating at high temperatures. The presence of high concentrations of phenolic compounds in hydrolyzed substrates can result in the inhibition of the microbial fermentative, lessening the efficiency of the fermentative process. The fermentative processes being either biological or physicochemical, are essential approaches taken in order to reduce the concentration of the fermentation inhibitor compounds and for the maximum use of the fermentable sugars by the microbial strain used (ARUMUGAM; ANANDAKUMAR, 2016; DENG; AITA, 2018).

Figure 3 shows the contents of phenolic compounds obtained from the samples of raw hydrolyzed corn cob and detoxified raw hydrolyzed corn cob. The experiments conducted with heating times of 15min., for either the raw hydrolyzate (RH) and the samples submitted to physicochemical detoxification (DRH), it was verified higher contents of phenolic compounds ranging between 0.770 g.L^{-1} and 1.03 g.L^{-1} using 5.0 e 10.0 % of sulfuric acid as the hydrolysis chemical agent, respectively. The lowest content of phenolic compounds was found when using concentrations of 2.5% of sulfuric acid, resulting in 0.393 g L^{-1}. On the other hand, on the experiments conducted with longer hydrolysis times, 30min., resulted on lower contents of phenolic compounds when comparing to those with the duration of 15min., as described in Figure 3. The lowest contents of phenolic compounds were verified at concentrations of 2.5 and 5.0 % of H_2SO_4, resulting in 0.325 e 0.345 g.L^{-1}, demonstrating an heterogeneous behavior between the releasing of phenolic compounds and hydrolyzing concentration agent during the 30min. of processes (Figure 3). Therefore, it is possible to observe that by using higher concentrations of sulfuric acid, higher concentrations of phenolic compounds will be released. The release of phenolic compounds increased during the hydrolysis on each and every one of the sulfuric acid concentrations tested, especially on the samples that had been submitted to detoxification, showing values between 0.005 g L^{-1} and 0.16 g L^{-1} (30 min.) (Figure 3).

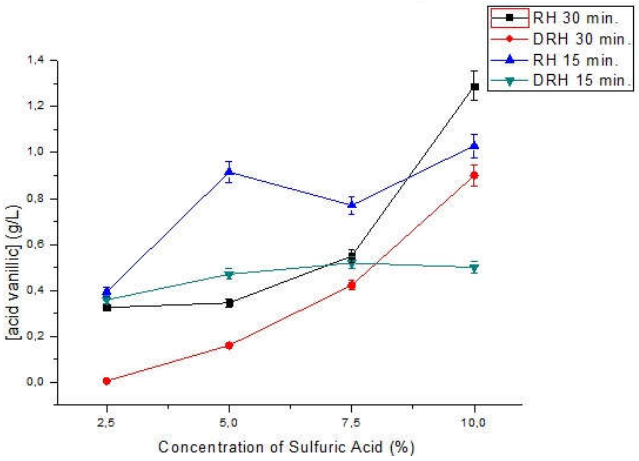

Figure 3. Content of phenolic compounds in corn cob crude (RH) and detoxified hydrolyzate (DRH) obtained in different H_2SO_4 concentrations and autoclaving at 121 ° C / 1.1 atm

Out of all detoxification methods the most commonly applied and economical is by activated charcoal. Various methods of detoxification have been described, Mussatto and Roberto (2004) have tested different brands of activated charcoals on rice straws hydrolyzate and described that the origins and particle size of the charcoal had influence and that the activated charcoals with the smallest granulometry had the best efficiency. Furthermore, Trinca et al. (2017) observed that the duration of the contact between the charcoal and the hydrolyzate medium also influences the detoxifications efficiency. On the present study it was observed that the exposure time of 60 min. of the hydrolyzate to activated charcoal was sufficiently for both times of the hydrolysis treatment, where the concentration of phenolic compounds for the hydrolyzed substrate with 7.5% of H_2SO_4 and 15min. of heating, was found to be 0.77 g de vanillic acid /g and after the detoxification decreased to 0.51 g de vanillic acid /g and for a heating time of 30min. and 7.5% of H_2SO_4 the hydrolyzate substrate with initially 0.549 g of vanillic acid/g decreased to 0.42 g de vanillic acid /g after the detoxification. Therefore, lowering the concentration of phenolic compounds around 65.0% after the detoxification in all of the samples.

In relation to the inhibitory compounds, Michelin et al. (2016) studied the effect of phenolic compounds on the cellulosic and hemicellulosic activities of the sugar cane bagasse previously treated with hot water under pressure at 180 or 200°C during 30min. These scientists stated that the pre-treatment released phenolic compounds that inhibit and/or deactivate the cellulase and hemicellulase enzymes. Besides that, a couple of other samples also had undergone pre-treatment with acetone and it was observed that the phenolic compounds released by them strongly deactivated both the β-glycosidase e a β-xylosidase enzymes.

However, the temperature conditions applied in this work (121 °C) was less drastic than the ones used by these authors. High temperatures cause degradation of the sugars, leading to the creation of acids, furfural, HMF and other compounds that can be harmful to the microbial growth. On his work, Trinca et al. (2017), assessed the influence of the presence of phenolic compounds formed on the hydrolyzate of soybean hulls, that were used as substrate for ethanol production. On this research it was observed that the when the samples were heated there was a higher release of phenolic compounds and that the release of these compounds would increase as higher concentrations of H_2SO_4 were applied, just as mentioned on this present work. At higher concentrations of H_2SO_4 (3.5%, 4.0% and 4.5%) it was observed up to 0.6 mg/g of phenolic compounds, whereas at lower concentrations (2.0%) only 0.2 mg/g were found. When heating was applied, there was higher release of phenolic compounds when using concentrations of 5.0% H_2SO_4 during 15 min. of heating (1.97 g/g). However, Trinca et al. (2017) stated that heating is necessary in order to increase the discharge of fermentable sugars while also lowering the effective time of the hydrolysis, even if it generates phenolic compounds (TRINCA *et al.,* 2017). Based on the data obtained, the best conditions for the hydrolysis are 2.5% sulfuric acid and heating time of 30min. therefore, these parameters were chosen to be used in this present research, for the hydrolysis of corn cobs for the production of fermentable sugars to be used on the fermentative process of ethanol production by the yeast *Saccharomyces cerevisiae* ATCC 26602.

Effect of corncob chemical hydrolysis and detoxification release of fermentable sugars: In the light of the data obtained on this work, the conditions chosen were of 2.5% sulfuric acid concentration and heating time of 30min. as the most effective parameters for the acid hydrolysis of corn cob. Having selected these conditions, a new hydrolysis of the corn cobs was performed, and again the contents of total and reduced sugars and phenolic compounds were determined (Figure 1). Shortly after the hydrolysis of the corn cobs, the raw hydrolyzate presented 31.14 g L^{-1} of total sugars, being in most part 31.14 g L^{-1} of reducing sugars (Table 1). This raw hydrolyzate was concentrated by evaporation, to evaluate the influence of various substrate concentrations in the biosynthesis of ethanol by the yeast in question. After 48 hour of evaporation it was obtained a corn cob hydrolyzate with 61.54 g.L^{-1} of total sugars, of which 88.33% are reducing sugars. After the detoxification, the concentrated hydrolyzate now had 59.91 g.L^{-1}, meaning a loss of 2.64% of total sugars during the detoxification process. Out of all the total sugars that were determined, after the detoxification, 85.42% were identified as reducing sugars. The increase of reducing sugars content after the concentration of the substrate is an important stage, for there to be an ample availability of assimilable sugars, such as glucose and xylose by the fermenting microorganisms.

Table 1. Contents of total and reducing sugars and phenolic compounds on the hidrolyzates of corn cob, after acid hydrolysis (H_2SO_4, 2.5%/30 min.), concentration and detoxification

•	• RH	• CH	• CDH
• TS (g L^{-1})	• 31.14 ± 1.74	• 61.54 ± 2.21	• 59.91 ± 3.09
• RS (g L^{-1})	• 25.02 ± 0.92	• 54.36 ± 0.31	• 51.18 ± 2.06
• PC (g L^{-1})	• 0.80 ± 0.07	• 0.84 ± 0.04	• 0.29 ± 0.01

TS: Total sugar; RS: reducing sugars; PC: Phenolic compounds; RH: Raw hydrolyzate; CH: Concentred hydrolyzate; CDH: Concentred and detoxicated hydrolyzate.

On Table 1 it is also possible to observe that after the detoxification there was an expressive reduction (63.75%) of phenolic compounds, demonstration the effectiveness of the activated charcoal. 3.4 effect of the agitation of detoxified raw hydrolyzate of corn cob used as culture medium for the production of ethanol by *S. cerevisiae.* In order to determine what kind of effect the agitation caused on the culture mediuns that contained the concentrated detoxified hydrolyzate (CDH), with an initial concentration of 51.18 g.L^{-1} of reducing sugars, for the production of ethanol by the yeast *S. cerevisiae*, tests were conducted at 50 and 100rpm during 48h of fermentation at 30°C. The sugar consumption was monitored every 12h during the whole 48h of the fermentative process and the results are displayed at Figure 4.

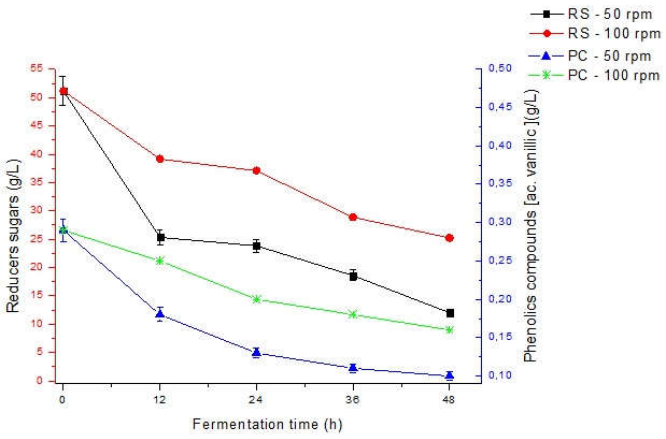

Figure 4. Consumption profile of reducing sugars (g.L^{-1}) and contents of phenolic compounds (g vanillic acid/g) present in the hydrolyzate medium, during 48h of fermentation at 30°C and agitation of 50 and 100rpm

On this Figure 4, it is possible to observe that a gradual reduction of the reducing sugars has occurred within the rise of the fermentation time, indicating that the microorganism was able to consume the available sugars in the hydrolyzate substrate, especially for the tests conducted at agitation of 50 rpm (Figure 4). At 100 rpm, there was a lesser consumption of reducing sugars, most likely because of the higher incorporation of oxygen into the medium, rising the respiratory rates and consequently, causing a deviation of the yeast natural´s biosynthetic route. Gonçalves et al. (2016) described in his work the high fermentative capacity which *Saccharomyces cerevisiae* PE-2 has in hydrolyzates that have various sugars present in its medium, when compared to other laboratorial and industrials strains of microorganisms. In relation to the contents of residual phenolic compounds present in the hydrolyzate after the detoxification, there were no shown signs of inhibitory effect to the yeast, and it was observed that a significant reduction occurred of their contents, suffering a reduction of 65.51 % in 48 hours of processing, as shown in Figure 4. This decrease of contents could have been caused either due to the formation of the enzyme alcohol dehydrogenase by the yeast *S. cerevisiae*, this enzyme is responsible for the reduction of the furfural created or by yeast´s capacity to absorb organic acids. In a selection done by Wimalasena et al. (2014), of 90 strains of *Saccharomyces* spp the yeasts showed levels of tolerance to acetic acid, formic acid, furfural, HMF and vanillin. The *S. cerevisiae yeast* has the capacity of converting some of the inhibitory phenolic compounds into other compounds, such as converting furfural into furfurilic alcohol, this way, the reduction rate of the

furfural rises together along with the development of the yeast. In Wang´s et al. (2013) study the *S. cerevisiae* showed signs of tolerance at the presence of higher concentrations of inhibitory agents and rapidly degraded the furfural and HMF resulting in the increase of ethanol production and consumption rates of glucose. The fermentative process took place during a period of 48 h at 30 °C, under different conditions of agitation (Figure 5). Figure 5 represents the production of ethanol and growth rate of the yeast *S. cerevisiae* ATCC 26602 in the culture medium containing the detoxified corn cob hydrolyzate at a concentration of 51.18 g L^{-1}.

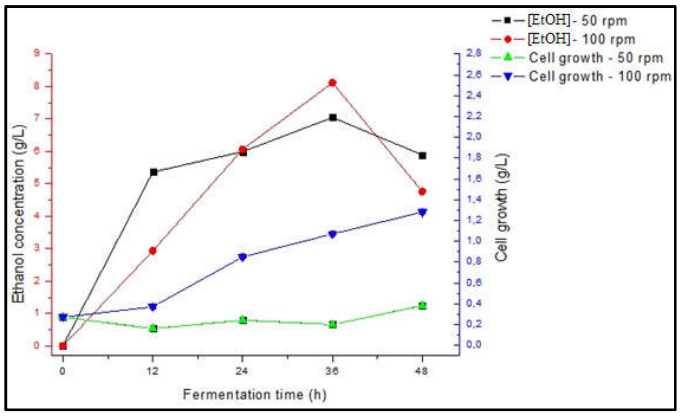

Figure 5. Production of ethanol and cellular growth rate during 48 h of fermentation at 30°C in medium containing corn cob hydrolyzate as a source of carbon and agitation of 50 and 100rpm

The results show the yeast was capable of utilizing the corn cob hydrolyzate as carbon source, for both its growth and for the production of ethanol as soon as the first 12 h of cultivation. It's possible to observe a rising growth in the ethanol contents until 36 h of process and after that a minor reduction at the end of the fermentation, at 48 h. The maximum production of ethanol was observed at 36h of fermentation for both of the agitation conditions, obtaining values 8.11 g.L^{-1} for 100 rpm and 7.04 g.L^{-1} for 50 rpm (Figure 5). In regards of the cellular growth, the yeast was able to develop under both agitation conditions proposed, especially for the tests conducted at 100 rpm, where the maximum cellular concentration was 1.28 g L^{-1}. The cultivation of *S. cerevisiae* in a medium with high contents of glucose, the yeast initially uses the glucose to for its own growth and when the source of glucose is exhausted, the yeast starts to consume the by-product of its own metabolism for its survival, such as ethanol. This change from consuming glucose to ethanol is known as diauxic shift and explains the drop of ethanol contents in the culture medium (LAVOVÁ *et al.,* 2014). This can be observed in Figure 5. In his work Rocha et al. (2016) evaluated the production of ethanol from acid treatment applied in corn cobs and corn stove in fermentation at 30°C, and 200 rpm during 24 hours. The highest point of fermentative efficiency was achieved using hydrolyzate with 0.5% H_2SO_4 at 120°C during 15min., obtaining 2.2 g.L^{-1} of ethanol for the corn cobs and 9.3 g.L^{-1} of ethanol for the corn straw. Santos-Rocha et al. (2017) also studied the production of ethanol from corn cobs and corn straws by submitting them to a hydrothermal treatment with water in a fermentation of 24 h/30°C/200 rpm, obtaining 3.3 g.L^{-1} of ethanol for the corn straws treated at 170°C during 15min. and 5.5 g.L^{-1} of ethanol for the corn cobs treated at 195°C/10min. The efficiency of the fermentation found by these authors for the corn cobs was better than the one found in this present work, being 8.11 g.L^{-1} and 36 h of fermentation. Figure 5 shows the growth of the yeast increased gradually during 48h of fermentation (around 1.0 g.L^{-1}). The highest growth observed was of 1.28 g.L^{-1} with agitation of 100 rpm. Also on Figure 5, it is possible to observe that the best ethanol production by the yeast occurred at 36 h of fermentation, for both culture mediums with agitations of 50 rpm (8.11 g.L^{-1} of ethanol) and 100rpm (7.04 g.L^{-1} of ethanol). After 36h, on the final stage of fermentation, it is possible to observe a decrease in the ethanol contents and a rise on the cellular development, what can be explained by the shortage of nutrients in medium by the formation of secondary products that can cause cellular stress, by the quantity of toxic compounds present, even if in lower quantities and even if by the yeasts behavior itself (diauxie). Lavová et al. (2014) stated that for two strains of *S. cerevisiae* the diauxic shift occurred after around 8 to 12 hours of incubation. When initiating the fermentation, the medium containing the detoxified raw hydrolyzate of corn cob presented a pH of 7.26 (surrounding neutrality) which maintained practically constant throughout the first 24 h of the fermentation process, shortly after, declining reaching 5.98 (Table 2).

Table 2. pH behavior in the fermentation medium during ethanol production by *S. cerevisiae* in culture medium containing corn cob hydrolyzate incubated at 30 °C for 48 h in the detoxified raw hydrolyzate of corn cob (DRH)

Time of fermentation (h)	pH (50 rpm)	pH (100 rpm)
0	7.26 ± 0.08	7.26 ± 0.08
12	6.74 ± 0.14	6.90 ± 0.12
24	7.12 ± 0.06	6.70 ± 0.08
36	6.40 ± 0.17	6.27 ± 0.15
48	6.06 ± 0.12	5.98 ± 0.13

This behavior occurs because during the absorption and use of the fermentable sugars by the yeast, it produces CO_2, acetic acid and organic acids, consequently, the pH of the culture medium lowers, which indicates an efficient development of the fermentation (FU; PEIRIS, 2008). 3.5 Effect of agitating the raw hydrolyzate of corn cob used as culture medium for the production of ethanol by *S. cerevisiae* Acid hydrolisys was performed with the previoulsy chosen parameters, 2.5% of sulfuric acid and 30 min of heating time, and the evaluating the total and reducing sugars content and phenolic compounds content (Table 3). After the hidrolysys the value of total sugar content was of 32.60 g.L^{-1}, being composed of 88.00% of reducing sugars. For the standardization of the substrate concentration, it was concentrated by evaporation until reaching 40 g.L^{-1} of reducing sugars and then, converted by the yeast for the production of ethanol. After the evaporation, a hydrolyzate of corn cob with 54.08 g.L^{-1} of total sugars was achieved, out of which 76.20% are reducing sugars, resulting in a concentration of 40 g.L^{-1} (Table 2).

Table 3 – Contents of total and reducing sugar and phenolic compounds in corn cob hydrolyzate, after acid hydrolysis (H_2SO_4, 2.5%/30 min.)

	RH	CH
TS (g L^{-1})	32.60 ± 2.36	54.08 ± 2.76
RS (g L^{-1})	28.69 ± 1.36	41.20 ± 1.15
PC (g L^{-1})	0.97 ± 0.03	3.03 ±0.03

TS: Total sugar; RS: reducing sugars; PC: Phenolic compounds; RH: Raw hydrolyzate; CH: Concentered hydrolyzate.

The concentration of phenolic compounds was affected during the evaporation of the substrate, increasing from 0/97 g

vanillic acid /g to 3.03 g vanillic acid /g. These compounds could interfere in the yeasts growth, as well as in the production of ethanol. The fermentation was conducted during 24 h at 30°C and submitted to an agitation of 0, 50 and 100 rpm to evaluate the effect it would have in the production of ethanol in the culture medium of corn cob raw hydrolyzate. The initial concentration of reducing sugars was found to be 41.20 $g.L^{-1}$. The cellular growth and ethanol production are shown in Figure 6.

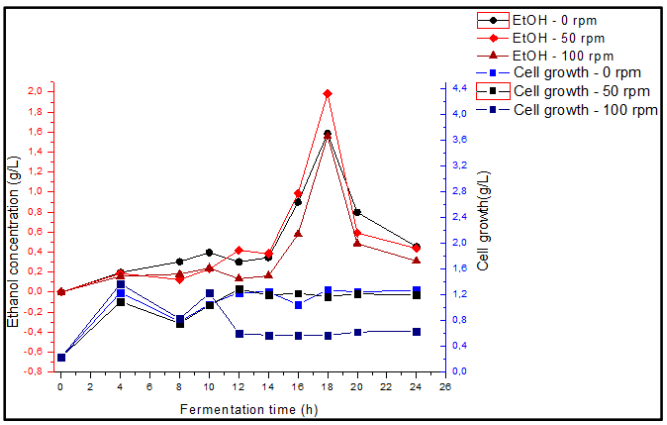

Figure 6. Production of ethanol and cellular growth during 24 h of fermentation at 30°C in medium containing corn cob raw hydrolyzate as a source of carbon and agitation of 0, 50 and 100rpm

In Figure 6, it is possible to observe that the yeast was capable of consuming the corn cob raw hydrolyzate showing cellular growth in the first 4 h of fermentation, with and without agitation. Besides that, it was also observed that there was ethanol production between 16 and 20 h of fermentation. The highest production rates were verified at 18 h of fermentation for all of the conditions tested. Out of all of these, the agitation speed that provided the highest concentration of ethanol was of 50 rpm, 1.98 $g.L^{-1}$, followed by 0 rpm, 1.58 $g.L^{-1}$ and 100 rpm, 1.55 $g.L^{-1}$. In regards of the cellular growth, it maintained an average of 1.0 $g.L^{-1}$ and its highest peak was at 1.23 $g.L^{-1}$ in 18h of fermentation. Sarawan et al. (2019) assessed the optimization of the use activated charcoal, on the detoxification of acid hydrolyzate of sorghum leafs, applying a fermentation of 30 °C at 120 rpm during 24 h for the raw hydrolyzate and detoxified hydrolyzate of sorghum leafs. The authors confirmed that the detoxification improved both the cellular growth and efficiency of ethanol production. Likewise, for the raw substrate of corn cob, which showed cellular growth and production of ethanol even with the detoxification of the hydrolyzate. However, due to the detoxification there was a higher growth and ethanol production by the yeast *S. cerevisiae* ATCC 26602, in the detoxified corn cob hydrolyzate.

Conclusion

The acid hydrolysis of corn cobs was efficient since it provided a high content of fermentable sugars. The conditions of hydrolysis that presented the most efficiency at the release of sugars were of 2.5% of sulfuric acid during 30min. of heating time in autoclave. The detoxification was efficient for the removal/reduction of phenolic compounds produced during the hydrolysis, decreasing around 65% the content of these compounds present in the hydrolyzate. The yeast *Saccharomyces cerevisiae* ATCC 26602 was capable of utilizing the various concentrations of hydrolyzate for cellular growth and ethanol synthesis under the agitated conditions studied. The maximum ethanol production was observed in detoxified raw hydrolyzate after 36 h of process under agitation of 100 rpm (8.11 $g.L^{-1}$). The corncob hydrolyzate has shown to be an alternative substrate for the production of ethanol by the yeast *S. cerevisiae* ATCC 26602. Furthermore, the yeast was able to use this same hydrolyzate, with it having undergone the process of detoxification, to develop itself and produce ethanol, which shortens a stage of second generation (2G) ethanol production.

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