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PRE-TREATMENT WITH HYDROCHLORIC ACID AND ENZYMATIC HYDROLYSIS OF SUGAR CANE BAGASS TO OBTAIN GLUCOSE

Luana Pereira Vasconcelos de Abreu
Graduation Student in Bioprocess Engineering and Biotechnology, Pibic/UFT, Universidade Federal do Tocantins

Ezequiel Marcelino da Silva
PhD in Industrial Biotechnology, Bioprocess Engineering and Biotechnology Course, Universidade Federal do Tocantins

Abstract: Pretreatment and enzymatic hydrolysis are two important steps within the process of converting lignocellulosic biomass into glucose. In this work, sugarcane bagasse (BCA) ground into particles smaller than 40 mesh (< 425 μm) was pre-treated with hydrochloric acid (HCl) in different concentrations (0.75, 1.0 and 1.25 mol/L), in time varying from 30 to 90 min by autoclaving (121 ºC / 1 atm) in solid ratio :net 1:10. The pretreated BCA was subjected to hydrolysis with commercial enzymes at a solid:liquid ratio of 1:30, with100 μL of cellulase mixtures and 100 μL of Aspergilus niger laccase, in citrate-phosphate buffer (50 mM; pH 4.8) for 72h at 50 ºC under agitation at 150 rpm. Pre-treatment with HCl showed an efficiency in delignification of BCA of 59.2% and the glycan fraction increased to 64.48% in the solid residue of BCA recovered after pre-treatment carried out with HCl 1.25 mol/L, time 30 min. Within these operational parameters, enzymatic hydrolysis continued for 72h. Within a mass balance analysis, a solids recovery of 42.8% was observed for the pre-treatment stage with HCl. Therefore, for every 100 g of pre-treated BCA, within the process, residue with 64.48 g of glycan and 9.23 g of lignin was recovered and for every 100 g of BCA recovered after pre-treatment and enzymatically treated with cellulases and laccase it was possible to obtain 12.0g of glycan hydrolyzed into glucose. It is concluded that pre-treatment with dilute acid and hydrolysis with commercial enzymes showed efficiency in converting BCA glycan into glucose.
Keywords: laccase; Cellulases; Lignocellulosic biomass.

INTRODUCTION

The initiative to use energy from renewable sources has been promoted to reduce the exploration and use of non-renewable sources such as coal, natural gas and crude oil, which have caused environmental problems related to ozone depletion, water pollution and the generation of solid waste (SOLARTE-TORO, et al., 2019; AMOAH, et al., 2019). For these reasons, the use of renewable resources from agro-industries has stood out within several areas of technological innovation by academic researchers and the processing industry itself. Renewable resources, such as lignocellulosic biomass, have been gaining prominence in the production of energy and high-value-added inputs (MATEI, et al., 2020). Within this focus, the conversion of these biomasses into second-generation bioethanol has been developing and being applied industrially (FANELLI, et al., 2020; SOLARTE-TORO, et al., 2019). This industrial field is increasingly connected to developing more efficient physical-chemical and biochemical processes in the production of biofuels, aiming at lower consumption of energy, water, chemical inputs and generation of waste from pre-treatments and subsequent treatments that may cause damage to the environment. environment (PLACIOS-BERECHE, et al., 2022). The use of renewable resources also generates waste, often requiring treatment before disposal and which can, in some way, be reused to produce new products with high added value.

Sugarcane bagasse is composed on average of 40-45% cellulose, 30-35% hemicellulose and 20-30% lignin (PENDSE; DESHMUKH; PANDE, 2023). For effective conversion of lignocellulosic biomass, pre-treatment strategies using diluted acids, alkalis, ionic liquids, organosolv process and others have been explored in the last decades of research. Due to the simplicity, low cost of reagents and effectiveness of the pre-treatment process with dilute acid, it is considered the technology closest to commercialization. The process is carried out using solid to liquid ratios of 1:10 (m/v) and which allows the almost complete hydrolysis of the hemicellulosic

fraction, as well as the partial solubilization of the lignin corresponding to sugar yields close to 90% that are achieved by enzymatic hydrolysis post pre-treatment (SOLARTE-TORO, et al., 2019). Sulfuric (H2SO4) and hydrochloric (HCl) acids are considered strong and with high dissociation of ions in water, releasing H+ cations and SO42- and Cl- anions, respectively. The potentially oxidizing SO42- and Cl- anions, even when diluted, are very reactive with organic compounds present in lignin, causing their dissolution through the oxidation reaction of the lignocellulosic biomass molecules. Diluted H2SO4 is the most used in the pre-treatment of lignocellulosic biomass to remove lignin and part of the hemicellulose that promote an access barrier to cellulose (MOTAUNG; ANANDJIWALA, 2015). However, other dilute acids are also sources of research into their beneficial effects on lignin removal. Therefore, pre-treatment with HCl can be considered, in diluted concentration, effective in removing lignin. Enzymatic treatment, with commercial cellulases or those produced in situ, subsequent to delignified biomass in pre-treatment with dilute acid promotes the conversion of the cellulose fraction into glucose for subsequent fermentation to second generation bioethanol.

In this work, BCA was subjected to pre-treatment with diluted hydrochloric acid in different concentrations to evaluate the delignification of lignocellulosic biomass at different residence times in an autoclave. The solid recovered after pre-treatment was subjected to enzymatic hydrolysis with a mixture of cellulases and A. niger laccase in a buffered medium under agitation to obtain glucose by depolymerization of the remaining glycan.

MATERIALS AND METHODS

Sugarcane bagasse (BCA) was obtained by donations from traders at fairs and commercial spaces. The bagasse went through an outdoor drying process until its moisture content was reduced as much as possible. The bagasse was dried in an oven at 60 ºC for the time necessary to extract the remaining moisture so it could be ground. The sugarcane bagasse was crushed into smaller fractions with the help of a machete. These pieces were subjected to grinding in a knife mill, obtaining particle sizes smaller than 40 mesh. (425 μm).

PRETREATMENT OF BCA WITH HYDROCHLORIC ACID

In this experimental process, 5g of dry mass of BCA was placed in 250 mL conical flasks adding 50 mL of hydrochloric acid according to variations in concentration (0.75, 1.0 and 1.25 M) and time (30. 60 and 90 min) for each pretreatment process and conducted by autoclaving at 1 atm / 121 °C separately. Once the process was complete, all material was filtered through a Whatman No. 1 vacuum paper filter. The filtrate was collected and stored in test tubes with lids for subsequent analysis of glucose and reducing sugars. The solid material retained in the filter underwent washing processes with twice the volume of distilled water (100 mL) and dried in an oven at 80ºC for 24 hours, and was then stored in "zip-lock" plastic bags for subsequent characterization of the pre-treated biomass. and enzymatic hydrolysis process.

ENZYMATIC HYDROLYSIS

Enzymatic hydrolysis of BCA biomass pretreated with hydrochloric acid was conducted with a mixture of cellulases such as endo-glucanase (CMCase), ß-glucosidase and xylanases in citrate-phosphate buffer (pH 4.8; 50 mM), temperature of 50ºC for up to 72h in a thermostated rotary shaker at 150 rpm.

Mass of biomass pretreated with hydrochloric acid (1g) was placed in 250 mL conical flasks with 30 mL of citrate-phosphate buffer with 100 µL of cellulases (endo-glucanase – 18.8 U/g, ß-glucosidase – 28 U/g, xylanase – 14.6 U/g) and 100 µL of laccase from Aspergilus niger (807 U/g). A 1 mL sample was collected at different hydrolysis times, boiled for 5 min in boiling water and centrifuged at 10,000 rpm, 4 ºC for 10 min. The supernatant of each sample was analyzed for the amount of glucose released during the enzymatic hydrolysis time.

PRE-TREATMENT CHARACTERIZATION ANALYZES

ENZYME ACTIVITIES

The enzymatic activity of cellulase (endoglucanase) was defined in units of reducing sugars released during the hydrolysis of CMC (Carboxymethylcellulose) (Sigma–St. Louis, USA) at 0.44% in 50 mM citrate-phosphate buffer, pH 4.8 at 50 °C (TANAKA, et al., 1986). The assay was performed with 900 µL of 0.44% CMC prepared in citrate-phosphate buffer with 100 µL of enzyme for 5 min. 1000 µL of DNS reagent was added and boiled for 5 min in boiling water and cooled in an ice bath. The enzymatic activity of xylanase (endo- and exo-xylanase) was also defined in units of reducing sugars released during the hydrolysis of beech xylan (Sigma–St. Louis, USA) at 1.0% in citrate-phosphate buffer (BAILEY; BIELY; POUTANEN, 1992). The assay was performed with 800 µL of substrate and 200 µL of enzyme for 5 min. 1000 µL of DNS reagent was added and boiled for 5 min in boiling water and cooled on ice. The glucose and xylose results were obtained in comparison to the glucose and xylose calibration curves at different concentrations (µmol) using the same methodology as the DNS reagent (MILLER, 1959).

The enzymatic activity of ß-glucosidase was defined in p-nitrophenol units released from p-nitrophenyl- ß-D-glucopyranoside (Sigma–St. Louis, USA) at 0.1% in 50 mM citrate-phosphate buffer, pH 4, 8 to 50 ºC (TAN; MAYERS; SADDLER, 1987). The assay was performed by mixing 900 µL of substrate with 100 µL of enzyme and incubated for 5 min and adding 1000 µL of saturated sodium tetraborate solution. The quantification of p-nitrophenol was obtained in comparison to a curve of p-nitrophenol at different concentrations (µmol) adding saturated sodium tetraborate and the color intensity was read on a spectrophotometer at 410 nm. One unit of enzyme activity is considered as the amount of 1 µmol of glucose, xylose and p-nitrophenol released per min per mL of enzyme extract (U/mL).

Laccase activity was determined by oxidation of 1.0 mM ABTS (2,2'-azinobis-3-benzothiazoline-sulfonic acid) (Sigma – St. Louis, USA) in 50 mM citrate-phosphate buffer, pH 4, 8 to 50 ºC (NIKU-PAAVOLA, et al., 1988). The test was carried out with 800 µL of citrate-phosphate buffer, 100 µL of ABTS and the oxidation reaction started with 100 µL of enzyme. Oxidation was monitored by kinetics in a spectrophotometer for 1.5 min at 420 nm. One unit of laccase enzymatic activity is considered to be the amount of 1µM of ABTS ($\varepsilon_{420\ nm}$ = 65 mM^{-1} cm^{-1}) per min per mL of enzyme extract (U/mL).

QUANTIFICATION OF FREE REDUCING SUGARS AND GLUCOSE

All filtrates from samples pretreated with hydrochloric acid and enzymatic post-hydrolysis were quantified for the concentration of reducing sugars and free glucose, with reducing sugars representing the total sum of sugar oligomers that may be present in the samples plus free glucose. Reducing sugar was determined by the DNS method (3.5 dinitrosalicylic acid) and

quantified by comparison with a glucose calibration curve (g/L) under the same test conditions as described in MILLER (1959). The quantification of free glucose was determined by the supernatant reaction method with the glucose diagnostic KIT (GOD/POD) within the methodology presented by the manufacturer. The method consists of the reaction of the enzyme glucose oxidase responsible for the oxidation of glucose to gluconic acid and hydrogen peroxide. Through an oxidative coupling reaction catalyzed by peroxidase (POD). The oxidation of POD by H2O2 leads the enzyme to an oxidation state that oxidizes 4-aminoantipyrine and phenol, forming a red complex (quinoneimine), whose absorbance, measured at 510 nm, is directly proportional to the glucose concentration in the sample, within the same test conditions. A glucose calibration curve at different concentrations (g/L) was assembled under the same assay conditions.

CHEMICAL CHARACTERIZATION OF BIOMASS

The fresh BCA samples and pre-treated with hydrochloric acid were characterized by the methods described by FERRAZ (2000). To characterize the chemical composition, the tests were carried out in triplicate using 300 mg (dry mass) of sample. Before weighing the samples, the moisture content of the samples was determined and then weighed in test tubes. In each test tube, 3 mL of H_2SO_4 at a concentration of 72%, followed by incubation in a water bath at 30° C for 1 hour. After this period, the contents of each tube were transferred to 250 mL Erlenmeyer flasks adding 79 mL of distilled water to obtain a final concentration of 4% m/m of H_2SO_4 and the Erlenmeyer flasks were autoclaved at 121°C for 1 hour.

After hydrolysis, the material was cooled to room temperature and subsequently filtered through a No. 3 sintered porous ceramic filter to determine insoluble lignin by gravimetry, and soluble lignin by reading on a spectrophotometer at 205 nm. (ε_{205}=105 L/g) in 10-fold dilution. The volumes of the filtered solutions were made up to 200 ml with distilled water in a volumetric flask.

Previous estimation of glycan content in BCA and BCA pretreated with hydrochloric acid was performed by GOD/POD glucose assay using equation 1.

$$\text{Glycan}(\%) = \frac{\text{C glucose(g/L) x 100 x 0,9}}{\text{C sample(g/L)de BCA}} \qquad (1)$$

Where:

C glucose (g/L) – equivalent to the concentration of glucose in the filtered liquid.

C sample (g/L) - equivalent to the BCA mass (0.3 g in 82 mL) for chemical characterization of the pre-treatment and (1g in 30 mL) in the enzymatic hydrolysis process.

The percentage of BCA residue post pretreatment is calculated using equation 2.

$$\text{Residue}\ (\%) = \frac{\text{M(g) of BCA pre T x 100}}{\text{M } BCA(\text{g})\ in\ natura} \qquad (2)$$

Where:

M (g) – mass of BCA retained in filtration after pretreatment.

M_{BCA} – BCA - before pre treatment

STATISTICAL ANALYSIS

To evaluate pre-treatments and enzymatic hydrolysis treatments, a completely randomized design was used. The results obtained in practice were subjected to multiple variance analysis "Fisher's LSD" to verify the smallest significant difference between the means of the treatments carried out in triplicate ($P < 0.05$). Data analysis was studied using the STATGRAPHIC-18.0 statistical program.

RESULTS AND DISCUSSION

Sugarcane bagasse, after being air-dried and ground in knife mills, showed ultrafine particle sizes smaller than 40 mesh (< 425μm) in 95%. Reduction of particle size in micrometers assists in greater pre-treatment and enzymatic hydrolysis efficiency. The mechanical grinding process increases the surface area of the matter and reduces the crystallinity of the cellulose, providing greater efficiency in the removal of lignin with pre-treatment with dilute acid and greater absorption of enzymes in the hydrolysis treatment to release glucose (DEVI, et al., 2021; PENDSE; DESHMUKH; PANDE, 2023).

SOLIDS RECOVERY AND SUGAR SOLUBILIZATION FROM PRETREATED BCA

The BCA pre-treatment process was carried out with diluted hydrochloric acid in concentrations ranging from 0.75 to 1.25 mol/L, within a residence time ranging from 30 to 90min by autoclaving (121 ºC / 1atm), (Table 1). The ratio between the mass of BCA and the volume of hydrochloric acid was set at 1:10 (m/v) in all experiments.

Filtration of BCA post pre-treatment under different conditions resulted in solid residue varying from 42.4% to 50.2% after drying in an oven until constant weight, with the greatest solid recovery being caused by a lower concentration of HCl (0. 75 mol/L) with cellulose hydrolysis into soluble glucose at 4.10 g/L. The recovered filtrate presented, by spectrophotometric analysis, a glucose concentration ranging from 2.59 to 4.76 g/L, with no significant difference at the 95% level between treatments (Table 1 and Figure 1). The concentration of soluble reducing sugars, which represents the content of polyoses formed by total hexose and pentose sugars present in BCA, after pre-treatments ranged from 6.54 to 11.38 g/L (Table 1).

Analyzing the highest yield in solid recovery (50.2%), it does not indicate that the concentrations of glucose (4.10 g/L) and reducing sugars (10.32 g/L) soluble after pre-treatment were the lowest, as the lowest solubilizations of glucose (2.59mg/L) and reducing sugars (6.54 g/L) were found at factors of 1.25 mol/L-90 min and 1.25 mol/L-30 min, respectively.

Exhibition	Conc. (M)	Time (min)	Residue(%)	Glucose (g/L)	Reducing sugars (g/L)
1	0,75	30	49,6	3,28 ± 0,74	9,09 ± 1,40
2		60	50,2	4,10 ± 1,24	10,32 ± 0,14
3		90	49,0	4,76 ± 0,76	11,38 ± 1,02
4	1,0	30	44,8	3,42 ± 0,87	10,27 ± 0,12
5		60	47,2	4,20 ± 0,50	8,88 ± 0,08
6		90	49,0	3,81 ± 0,34	8,86 ± 2,43
7	1,25	30	42,8	3,38 ± 0,50	6,54 ± 1,02
8		60	42,4	3,04 ± 0,37	8,11 ± 2,05
9		90	43,2	2,59 ± 2,13	8,29 ± 1,19

Table 1 – Percentage of residual solid and glucose concentration (g/L) in the BCA filtrate after pretreatment with hydrochloric acid (0.75 to 1.25M) and residence time (30 to 90 min). The experiments were carried out at 121 ºC and 1 atm by autoclaving.

The consumption of organic matter during the process of eliminating lignin, hemicellulose and even a little cellulose, which normally occurs due to solubilization of these components in an acidic medium, is expected and this reduces the recovery of solids for the enzymatic hydrolysis treatment of cellulose. What is expected is greater retention of the cellulose fraction after pre-treatment to increase the glucose yield. In fact, the more cellulose and hemicellulose remaining in the pretreatment residue, the greater the ethanol yield per mass of BCA.

The results for glucose and reducing sugars represent the total amount of hexoses, pentoses and oligosaccharides present in the filtrate after pre-treatment with HCl and

imply the evaluation of the best process in which a greater quantity of polysaccharides can be retained in the residue recovered in the pre-treatment. (Figure 1). In fact, recovery of 80.3% of solids, with solubilization of 0.5% of glycan, was obtained in the pre-treatment of Alamo with a solution of hydrogen peroxide (PH) and acetic acid (AA) in a ratio of 8: 2 (v/v) in the presence of 100 mM H2SO4 at 60 ºC for 2h (LIN, et al., 2023). Pre-treatment of BCA at 15% (m/v) solids load with diluted acid/alkali and sequentially provided greater solids recovery by 83% with excellent elimination of lignin and hemicellulose present in the biomass (HEMANSI; SAINI, 2023). RISANTO et al. (2023) observed low glucose libration (2.87 g/L) in the pre-treatment of BCA with 1% maleic acid (m/m) with 6 g of solid load carried out at 180 ºC for 45 min compared to treatment with H2SO4 1% with greater glucose solubilization at 4.09 g/L. They observed that the use of maleic acid promotes a minimum conversion of glucose into furfural and 5-hydroxymethylfurfural when compared to H2SO4 and this leads to less inhibition during the subsequent enzymatic hydrolysis treatment of the solid residue obtained.

LIN et al. (2023) performed pre-treatment with PH: AA in the ratio 8:2 (v/v) followed by treatment with 2% AA at 170 ºC for 30 min and observed the presence of 3.2% of solubilized glycan and 51.6 % of solid recovered in this treatment. Pretreatment of BCA and rice husk before subjecting to enzymatic hydrolysis with crude enzymatic extract, rich in laccase in the removal of lignin in the presence of HBT, DMP and HBA resulted in glucose release at 3.86 mg/g and 3.20 mg/g, respectively, indicating greater exposure of cellulose and hemicellulose fibers for subsequent enzymatic hydrolysis treatment (MATEI, et al., 2020).

CHEMICAL COMPOSITION OF GLYCAN AND LIGNIN AFTER PRETREATMENT

The chemical composition of the glycan and lignin components in the solids recovered after pretreatment with HCl ranging from 0.75 to 1.25 mol/L (2.74% to 4.56% m/v) with residence time ranging from 30 at 90 min in an autoclave at 121 ºC and 1 atm pressure is shown (Table 2).

The variation in the lignin fraction by analysis with fresh BCA showed significant loss, ranging from -6.08% to -15.45%. The variation in the glycan fraction showed a significant gain, ranging from +2.14% to +19.69% (Table 2). These results lead to the evaluation of the lignin and glycan composition in the solid residue recovered after pre-treatment of 7.04% and 67.65%, respectively for the treatment carried out for 90 min at a concentration of 1.25 mol/L.

Statistically, the pre-treatments carried out with 1.25 mol/L HCl for 30 and 90 min were those that provided greater preservation of the glycan fraction at 64.48% and 67.65%, respectively, even though the solubilization of the Lignin was not the largest, leaving a small fraction of 9.23% and 7.04%, respectively. Pre-treatment of BCA with APA removed 40.6% of lignin and 58.2% of xylan and pre-treatment with APA (2%) in the presence of FeCl3 (0.1 mol/L), 70-130 ºC under agitation at a solid: liquid ratio of 1:10 removed xylan by 43.0%. The decrease in solids recovery was mainly attributed to the fractionation of hemicellulose and lignin, preserving cellulose by 98.6% and 93.4% by FeCl3 and APA, respectively (ZHUANG, et al., 2022).

In analysis of the pre-treatment process time and considering the three established concentrations (0.75, 1.0 and 1.25 mol/L), 30 min is the shortest time and this can generate savings in this pre-treatment stage. treatment (Table 2). Therefore, it was decided to develop

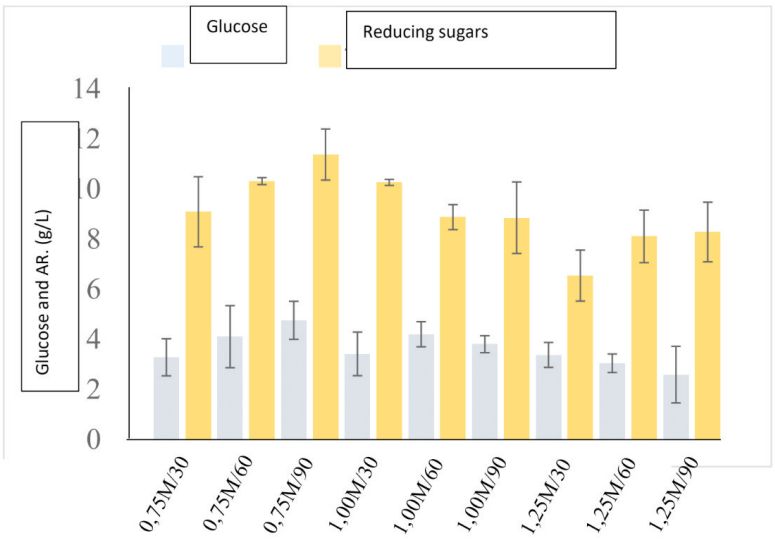

Figure 1 - Concentration of glucose (g/L) and reducing sugars (g/L) present in the BCA filtrate pre-treated with hydrochloric acid (0.75 to 1.25M) and residence time (30 to 90 min). The experiments were carried out at 121 ºC and 1 atm by autoclaving.

Exhibition.	Conc. (M)	Time (min)	Glycan (%)	P < 0,05	Lignin (%)	*Analysis (difference %)	
						Lignin	Glycan
BCA			44,78 ± 3,14	A	22,49 ± 1,36		
1	0,75	30	56,76 ± 6,67	ABC	9,63 ±1,11	-12,86	+11,97
2		60	54,43 ± 7,68	ABC	16,42 ± 2,44	- 6,08	+ 9,64
3		90	52,94 ±3,98	ABC	13,90 ± 2,62	- 8,59	+ 8,15
4	1,0	30	49,61 ± 3,73	ABC	9,41 ± 1,02	-13,08	+ 4,82
5		60	43,55 ± 0,98	ABC	9,23 ± 6,35	-13,26	- 1,23
6		90	46,92 ± 0,80	AB	8,83 ± 3,46	-13,66	+ 2,14
7	1,25	30	64,48 ± 6,32	C	9,23 ±3,46	-13,26	+19,69
8		60	58,68 ± 2,42	BC	11,07 ± 0,29	-11,42	+13,90
9		90	67,65 ± 1,84	C	7,04 ± 0,51	-15,45	+16,23

Table 2 - Chemical composition of BCA in natura and after pre-treatment with hydrochloric acid (0.75 to 1.25M) and residence time (30 to 90 min). The experiments were carried out at 121 ºC and 1 atm by autoclaving.

* Positive and negative values refer to loss or gain of lignin or glycan.

the enzymatic hydrolysis process of the three solid wastes that were carried out in a shorter execution time (30 min) for different concentrations of HCl (Table 2).

The variation in the chemical composition of bamboo shoot peels analyzed by TANG et al. (2023) during pretreatment with diluted H2SO4 ranging from 0 to 2 g/L showed 99% removal of the xylan fraction when the acid concentration was 1 g/L, while the recovery of solids and glycan decreases rapidly for slow from 58% to 35% and 73% to 46%, respectively. These results presented were related to dilute acid pretreatment in which xylan is easily hydrolyzed into oligomers and monosaccharides, while partially dissolved lignin condenses and precipitates on the surface of the substrates, increasing its content in composition. As for glycan, the rapid decline occurred due to the increase in acid concentration, probably due to the effect of H^+ in its swelling that leads to the depolymerization of glycan and xylan at the same time (TANG et al., 2023).

ENZYMATIC HYDROLYSIS OF PRETREATED WASTE

The enzymatic hydrolysis step followed post-pretreatment of BCA using commercial enzymes, cellulase mixture (Sigma ®) and A. niger laccase (Sigma ®) with a volume of 100 µL of both in citrate-phosphate buffer (50 mM; pH 4.8) at 50 °C for up to 72 hours. The volume of 100 µL of enzymes provided an effective activity of the enzymes in U/g of pre-treated residue in cellulases (endo-glucanase - 18,8 U/g, ß- glycosidase – 28 U/g, xylanase – 14.6 U/g) and 100 µL d of Lacase de *A. niger* (807 U/g). The ratio between solid and citrate buffer containing enzyme volumes was established at 1:30 (m/v) finalizing a relationship between cellulases and β-glycosidase in 1:1.3 without considering exo-glucanases that may be present (Figure 2). BCA without acid pretreatment was used as a control.

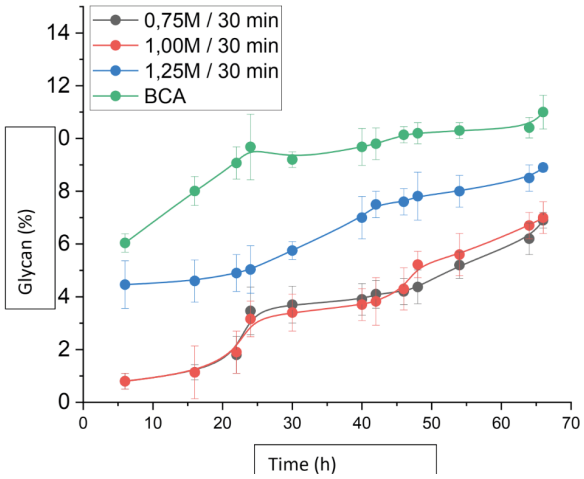

Figure 2 - Enzymatic hydrolysis of BCA pretreated with hydrochloric acid (0.75 to 1.25M) and residence time (30 to 90 min). The experiments were carried out at 121 °C and 1 atm by autoclaving. The hydrolysis experiments were carried out with 100 µL of cellulases and 100 µL of laccase in a solid: liquid ratio of 1:30 (w/v) in citrate-phosphate buffer (50 mM; pH 4.8) at 50 °C for 72h under stirring at 200 rpm.

The enzymatic hydrolysis treatment using BCA pretreated with 1.25 mol/L HCl for 30 min at 50 °C showed a significant difference when compared with other concentrations of the acid, but it was relatively smaller than the control using BCA without pretreatment. The two experiments at lower acid concentrations did not show different results between them during the entire enzymatic hydrolysis time. The enzymatic hydrolysis of the control, BCA without acid pre-treatment, implies that BCA grinding conditions below 425 µm already present an ideal characteristic for the hydrolysis treatment of in natura biomass, as the mechanical grinding process increases the surface area of matter and reduces the crystallinity of cellulose, providing greater efficiency in the hydrolysis treatment by allowing greater absorption of enzymes for

converting cellulose into glucose without the presence of inhibitors (DEVI, et al., 2021; PENDSE; DESHMUKH; PANDE, 2023).

The fact that the hydrolysis behavior of BCA treated at different acid concentrations was not superior to BCA without pretreatment may have been caused by the presence of inhibitors such as furfural, 5-hydroxymethylfurfural and other compounds derived from lignin by reactions caused by acid at high temperature and pressure imposed in the pre-treatment (Figure 2). From another perspective, despite the formation of inhibitory compounds, the process of washing the residual solid material was not sufficient to solubilize inhibitors that remained impregnated in the final cellulosic matrix.

More promising results were obtained with BCA pretreated with 1.25 mol/L HCl for 30 min at 50 ºC, promoting within 68 h of enzymatic treatment the level of glycan by almost 12% solubilized in glucose monomers (Figure 2). RISANTO et al. (2023) analyzed the effect of enzymes on the hydrolysis of BCA pretreated with maleic acid at a concentration of 0.5 to 1.0% (w/v). During hydrolysis, a small amount of xylan was hydrolyzed to xylose by xylanase and the increase in the concentration of maleic acid in the pre-treatment caused greater saccharification into glucose from 4.13 to 4.33 g/L in BCA in the presence of Cellic Cetec2 for 72h at 50 ºC, pH 5.0 at 120 rpm. The use of acid-base catalyst; catalyst prepared with bamboo shoot bark (CBB) treated with ultrasonified NaOH at 60 ºC for 4h treated with SnO2 and H2SO4 – UST-Sn-CBB; post pretreatment with 0.1 g/L H2SO4 increased the level of hydrolysis with Cellic Cetec2 from 68% to 97% in efficiency. The authors even attributed a relevant result to the maximum solubilization of hemicellulose in pre-treatment with dilute acid (TANG, et al., 2023).

Hydrolysis treatment of BCA and CA (rice husk) with Cellic Cetec, after crude pretreatment, rich in laccase in the removal of lignin in the presence of HBT, DMP, HBA, PH and Tween 80 in sodium acetate buffer (50 mM; pH 5.0) at 50 ºC, presented high concentrations of free glucose in cellulose hydrolysis resulting in 203 mg/g and 140 mg/g, respectively (MATEI, et al., 2020). Enzymatic hydrolysis carried out with cellulignin suspension (25 g/L), recovered from acid/base pretreatment, in citrate buffer (50 mM; pH 5.0) in the presence of a mixture of Multifect ® cellulase and enzymatic extracts of P. funiculosum and T. harzianum adjusted to 12.5 FPU/g in equal proportions achieved free glucose concentration at 18.9 g/L and 18.42 g/L within 18h with P. funiculosum and T. harzianum, respectively (MAEDA, et al., 2011). Furthermore, the authors observed low levels of cellobiose concentration during hydrolysis, a fact that is related to the ratio between FPase and β-glucosidase of the fungal enzymatic extracts of 1:4.5 and 1:3.7, respectively. ZHUANG et al. (2022) evaluated the enzymatic hydrolysis of BCA in natura and pre-treated with APA (2%) combined with FeCl3 (0.1 mol/L) for 72h using a solid: liquid ratio of 2:100 in sodium acetate buffer (50 mM; pH 4.8) containing 20 U of Cellic Cetec2/g of solid, under stirring at 150 rpm at 50 ºC. The untreated BCA | presented a glucose release of 69.75 mg/g, while the one treated with APA alone released 190.25 mg/g and the APA/FeCl3 combination obtained hydrolyzed glucose at a level of 313.0 mg/g (ZHUANG, et al. al., 2022).

For every 100g of BCA, 44.78g is glycan and 22.4g is lignin. Pre-treatment of 100g of BCA with 1.25 M HCl (mol/L) for 30 min at a 1:10 ratio between solid and liquid provided the recovery of 42.8g of solid residue with less lignin. Of the 22.4 g mass of lignin, 13.26 g were solubilized in the pre-treatment, increasing the glycan content to 64.48 g. In the

next step, solid residue recovered by filtration was subjected to enzymatic hydrolysis using a mixture of hydrolytic and oxidative enzymes for 72h, establishing a ratio between solid and citrate-phosphate buffer (50 mM; pH 4.8) of 1:30 (m/ v). For every 100g of solid waste pretreated and subjected to enzymatic hydrolysis, within these physical-chemical parameters, it provides the recovery of 12.0g of buffer-soluble glycan. This relatively lower value after pre-treatment may be related to the presence of enzyme inhibitors that hydrolyze the cellulosic matrix with lignin still present in the post-pre-treatment residue.

CONCLUSIONS

Pre-treatment of BCA with low concentration peracetic acid proved to be efficient in removing lignin with low loss of glucose during the delignification process. The dissolution of lignin during pre-treatment caused an increase in the percentage of glycan in the solid recovered by filtration. Enzymatic hydrolysis carried out with a mixture of cellulases in the presence of A. niger laccase was also efficient in depolymerizing the remaining cellulose after pre-treatment, achieving a high yield of glucose.

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