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

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Abstract: Pretreatment and enzymatic hydrolysis are two important steps within the process of converting lignocellulosic biomass into glucose. Sugarcane bagasse (BCA) ground into particles smaller than 40 mesh was pre-treated with peracetic acid (APA) at concentrations of 5, 10 and 15% (v/v), in a time of 6 to 24h and temperature of 50 and 90 ºC in a solid:liquid ratio of 1:10. The pretreated BCA was subjected to hydrolysis with commercial enzymes in a solid:liquid ratio of 1:30, with 100 µL of cellulase mixtures and 100µL of Aspergilus niger laccase (807 U/g), in citrate-phosphate buffer (50 mM; pH 4.8) for 72h at 50 ºC under agitation at 150 rpm. The results of pre-treatment with APA showed an efficiency in the delignification of BCA of 71.1% and the glycan fraction increased from 44.78% to 53.06% in the solid residue of BCA recovered after pre-treatment carried out with APA 15 %, time of 24 hours at 50 ºC. Within a mass balance analysis, a solids recovery of 66.9% was observed for the pre-treatment stage with APA. Therefore, for every 100 g of pre-treated BCA, within the process, residue with 53.06g of glycan and 6.5g of lignin was recovered and for every 100g of BCA recovered after pre-treatment and enzymatically treated, it was possible obtain 56.88g of glycan hydrolyzed into glucose. Within these results, it is possible to conclude that acid pretreatment with APA and hydrolysis with commercial enzymes proved to be very efficient in converting glycan into glucose.

Keywords: Glucose; Cellulases; Lignocellulosic biomass.

INTRODUCTION

The availability of low-cost and available forestry and agricultural residues can be used to produce ethanol and other chemicals with virtually no additional land requirements or impacts on food and timber crops (MAEDA, et al., 2013). Sugarcane is industrially exploited in the production of sugar and ethanol. This exploitation generates a large surplus of sugarcane bagasse, which within the sugar and alcohol industries is used to produce energy through burning to generate steam in large boilers. In the last two decades, the possibility of obtaining sugars from the polysaccharides that form the cell wall of sugarcane bagasse biomass has also been studied to increase ethanol production without causing an increase in the area under sugarcane cultivation. -of sugar. Studies reveal that around 50% of sugarcane bagasse with additional straw can be used to obtain monomeric sugars through pre-treatment and enzymatic hydrolysis within conventional industrial facilities (MATEI, et al., 2020; SARTORI, et al., 2019).

Sugarcane bagasse is mainly composed of glycan (35-45%), xylan (20-30%) and lignin (15-25%). For effective conversion of lignocellulosic biomass, pre-treatment strategies have been explored in recent decades of research, using dilute acids, alkalis, ionic liquid, organosolv process and others. Peracetic acid (APA) has been used in the pulping and bleaching industries. APA has a relatively weaker O-O binding energy (159 kJ.mol-1) than hydrogen peroxide H2O2 (213 kJ.mol-1) and can intensely generate radicals. The homolytic cleavage of the O-O bond can generate acetyloxyl (H3CC=OO·) and hydroxyl (·OH) radicals that are very reactive and promote the delignification of lignocellulosic biomass (ZHUANG, et al., 2022). Enzymatic treatment, with commercial cellulases or produced in situ, subsequent

to the delignified biomass in the acid pre-treatment 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 peracetic acid to evaluate the delignification of lignocellulosic biomass at different temperatures and residence times in a thermostated water bath. 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 PERACETIC ACID

In this experimental process, 5g of dry mass of BCA was placed in 250 mL conical flasks adding 50 mL of peracetic acid according to variations in concentration (5%, 10% and 15% v/v), time (6 to 24h) at temperatures of 50ºC and 90ºC for each pre-treatment process in a water bath and steady state. 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 sugarcane biomass pretreated with peracetic 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 rotary shaker thermostated at 150 rpm. Mass of biomass pretreated with peracetic 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, 54 ºC for 10 min. The supernatant of each sample was analyzed for the amount of glucose released during the enzymatic hydrolysis time.

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 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 with800 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 the amount of 1 µM 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 pre-treated with peracetic acid and post-enzymatic 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 to a glucose calibration curve (g/L) within the same assay conditions as described in Miller, 1959. 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 in 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

Samples of fresh sugarcane bagasse and pre-treated with peracetic 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. 3 mL of H2SO4 at a concentration of 72% were added to each test tube, 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 H2SO4 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 peracetic acid was performed by GOD/POD glucose assay using equation 1.

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

In which:

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 sugarcane bagasse residue after pre-treatment was calculated using equation 2.

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

In which:

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

M_{BCA} – BCA putty 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 GLUCOSE SOLUBILIZATION FROM APA-PRETREATED BCA

The BCA pre-treatment process was carried out with diluted peracetic acid in concentrations ranging from 5% to 15% (v/v), temperatures of 50ºC and 90ºC, within a residence time varying from 6 to 24h in steady state. in a thermostated water bath (Table 1). The ratio between the mass of BCA and the volume of peracetic 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 55.9% to 80.4% after drying in an oven until constant weight, with the greatest solid recovery being caused by a lower concentration of peracetic acid with a lower hydrolysis of cellulose into reducing sugars. The recovered filtrate showed, by spectrophotometric analysis, a concentration of reducing sugars ranging from 1.32 to 4.48 g/L (Table 1). The relationship between the results of reducing sugars and the pre-treatment time was not clear since it presented a higher concentration of glucose in a shorter time (6h), at the same temperature and concentration of diluted acid, despite the solid residue recovered for treatment of enzymatic hydrolysis did not show a significant difference between the two treatment times, which was between 80.4% and 79.3%, respectively (Table 1). Analyzing the higher yield in solid recovery and lower glucose solubilization, the process parameters indicate greater treatment efficiency at a concentration of 5% peracetic acid, at a lower temperature, 50 ºC and a time of 24 hours.

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. 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).

The results of reducing sugars, which represent the total amount of hexoses, pentoses and oligosaccharides present in the filtrate after pre-treatment with peracetic acid, 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). Therefore, the lowest concentration of glucose (1.32 g/L) can be observed in the treatment with peracetic acid at a concentration of 5%, 50 ºC for 24h, presenting a significant difference between the other experiments. 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 CA subjected 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 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 pre-treatment with peracetic acid ranging from 5% to 15% (v/v) with residence time ranging from 6 to 24h at temperatures of 50ºC and 90ºC is presented (Table 2).

The variation in the lignin fraction by analysis with in natura BCA showed significant loss, but the glycan variation showed gains and losses in the post-pretreatment composition. Within the results obtained in all pre-treatments, lignin varied from 3.66% to 22.11%, representing a component loss of 83.73% to 1.70%. Regarding the increase in the proportion of glycan, the gain in cellulose mass in the recovered solid waste was from 2.27% to 15.84% (Table 2). Statistically, the pre-treatment carried out with 5% (v/v) peracetic acid, at a temperature of 50 ºC for 18h, provided greater preservation of the glycan fraction, despite the solubilization of lignin not being very satisfactory at 57.5%. when compared with other treatments. 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).

Regardless of the statistics, the greatest glycan retention (+15.84%) in the solid residue occurred when the pre-treatment was carried out with a diluted solution of 5% peracetic acid at 50 ºC for 18h, with lignin solubilization around 57.5%, treatment with 15% peracetic acid at 50 ºC for 24h showed an increase in glycan (+8.28%), but with higher lignin solubilization at 71.10%, which was also selected for treatment in next step of enzymatic hydrolysis (Table 2). The high solubilization of lignin during treatment may be linked to strong nucleophilic radicals arising from the decomposition of APA and which promote the decomposition and dissolution of lignin by reactions with nucleophilic sites, including the aromatic rings and the aliphatic side chain of lignin (ZHUANG, et al., 2022).

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 ºC for up to 72 hours. The 100 µL volume of enzymes provided effective activity of the enzymes in U/g of pre-treated residue in cellulases (endo-glucanase – 18.8 U/g, ß-glucosidase – 28 U/g, xylanase – 14.6 U/g) and 100 µL of laccase from A. niger (807 U/g). The ratio between solid and citrate buffer containing enzyme volumes was established at 1:30 (m/v), resulting in a ratio between cellulases and β-glucosidase at 1:1.3 without considering exo-glucanases that may be present (Figure 2). BCA without acid pretreatment was used as a control.

The enzymatic hydrolysis treatment using BCA pretreated with 5% APA (v/v) for 18h at 50 ºC did not show a significant difference when compared with BCA without pretreatment. Both experiments achieved a level of glycan hydrolysis of approximately 15% in 72h. This implies that BCA grinding conditions below 425µm already present an ideal characteristic for the hydrolysis treatment of in natura biomass, as the mechanical grinding process increases the surface area of the matter and reduces the crystallinity of the cellulose, providing greater efficiency in the hydrolysis

Exhibition	Conc. % (v/v)	Temperature (ºC)	Time (h)	Residue (%)	Reducing sugars (g/L)
1	5	50	6	79,3	4,48 ± 0,44
2			18	73,1	2,21 ± 0,37
3			24	80,4	1,32 ± 0,30
4	10	50	18	60,5	2,45 ± 0,63
5			24	69,6	3,15 ± 0,86
6	15	50	18	55,9	2,63 ± 0,26
7			24	66,9	3,44 ± 0,88
8	15	90	6	59,4	2,74 ± 0,49

Table 1- Percentage of residual solid and glucose concentration in the filtrate (g/L) of BCA after pre-treatment with peracetic acid (5% to 15% v/v) and residence time (6 to 24h). The experiments were carried out at 50 ºC and 90 ºC in a water bath without shaking.

Exhibition	C v/v	T (h)	Glycan (%)	P <0,05	Lignin (%)	*Análise difference (%)	
						Lignin	Glycan
BCA			44,78 ± 3,14	BC	22,49 ± 1,36		
1	5	6	53,26 ± 13,67	B	22,11 ± 4,36	- 0,38	+ 8,48
2		18	60,62 ± 4,54	C	9,56 ± 2,50	-12,93	+15,84
3		24	52,20 ± 1,14	BC	8,57 ± 1,06	-13,92	+ 7,42
4	10	18	43,62 ± 9,52	BC	5,22 ± 1,81	-17,27	- 1,16
5		24	47,05 ±10,29	BC	13,03 ± 3,90	-9,46	+ 2,27
6	15	18	21,76 ± 2,81	A	3,66 ± 2,50	-18,83	- 23,02
7		24	53,06 ± 3,73	BC	6,50 ± 2,41	-15,99	+ 8,28
8·	15	6	56,15 ± 12,01	BC	9,43 ± 3,50	-13,06	+11,37

Table 2 - Chemical composition of BCA in natura and after pre-treatment with peracetic acid (5% to 15% v/v) and residence time (6 to 24h). The experiments were carried out at 50 ºC and 90 ºC in a water bath without shaking.

• Experiment 8 carried out at a temperature of 90 ºC, the other experiments carried out at a temperature of 50 ºC.

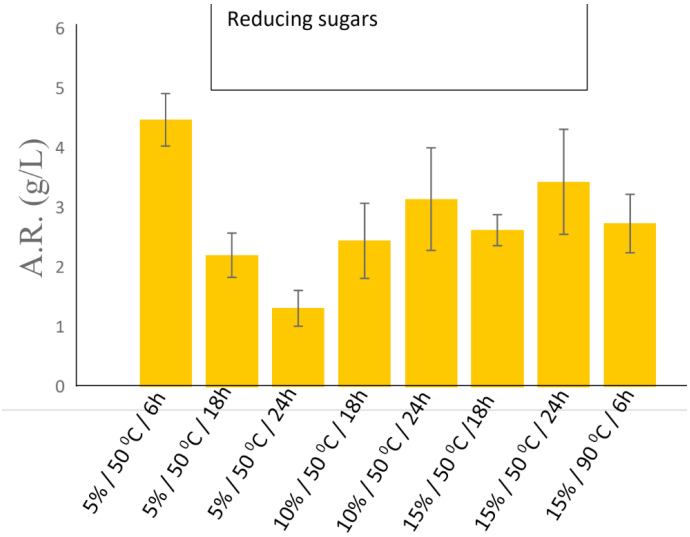

Figure 1 - Concentration of reducing sugars (g/L) present in the BCA filtrate pre-treated with peracetic acid (5% to 15% v/v) and residence time (6 to 24h). The experiments were carried out at 50 ºC and 90 ºC in a water bath without shaking.

treatment by allowing greater absorption of enzymes for converting cellulose into glucose (DEVI, et al. 2021; PENDSE; DESHMUKH; PANDE, 2023).

More promising results were obtained with BCA pre-treated with 15% APA for 24h at 50 ºC, promoting within 24 hours of enzymatic treatment high levels of glycan (37%) solubilized in glucose monomers. Until the end of 72h, the hydrolysis of cellulose into glucose increased almost linearly until 56.88% of solubilized glycan was obtained (Figure 2).

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.

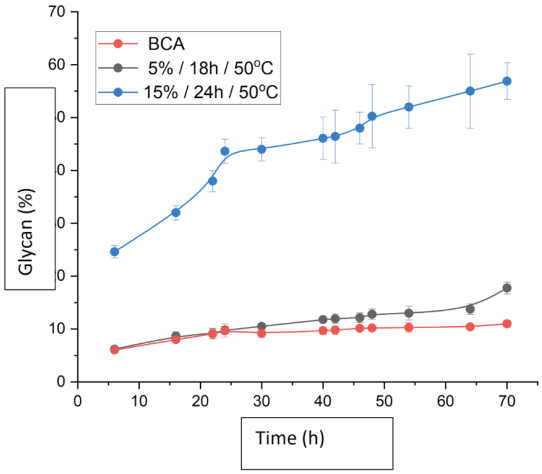

Figure 2 - Enzymatic hydrolysis of BCA pretreated with peracetic acid (5% to 15% v/v) and residence time (6 to 24h). The hydrolysis experiments were carried out with 100 µL of cellulases and 100 µL of laccase in a solid:liquid ratio of 1:30 (w/v) in citrate-phosphate buffer (50 mM; pH 4.8) at 50 ºC for 72h under stirring at 200 rpm.

ZHUANG *et al.* (2022) evaluated the enzymatic hydrolysis of BCA in natura and pretreated 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 combination: APA/$FeCl_3$ obtained hydrolyzed glucose at a level of 313.0 mg/g (ZHUANG, et al., 2022).

MASS BALANCE

The mass balance of the BCA conversion process into glucose by pretreatment with diluted peracetic acid at 15% (v/v) for 24h at 50 ºC within a solid:liquid ratio of 1:10, followed by enzymatic hydrolysis at 50 rpm and 50 ºC in citrate-phosphate buffer (50 mM, pH=5.0) with cellulase mixture (endoglucanase – 18.8 U/g, β-glucosidase – 28 U/g and xylanase –

14.6 U/g) and laccase (807 U/g) at a solid:liquid ratio of 1:30 was performed.

For every 100g of BCA, 44.78g is glycan and 22.4g is lignin. Pre-treatment of 100g of BCA with 15% (v/v) peracetic acid for 24h at 50 ºC in a 1:10 ratio between solid and liquid provided the recovery of 66.9g of solid residue with 71.1% lignin solubilized, with an increase in glycan to 53.06g. 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 56.88g of buffer-soluble glycan. This relatively higher value after pre-treatment may be related to the evaporation of water at 50 ºC for 72h.

FINAL CONSIDERATIONS

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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