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Reuse of the Xylanase Enzyme in the Biobleaching Process of the Sugarcane Bagasse Acetosolv Pulp

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Abstract

In this work, pretreatment-enzymatic series of the bagasse-sugarcane pulp and alkaline extraction of enzyme treated pulp were carried out. In the pretreatment an enzyme dose was utilized and acetosolv pulp suspension of 3% (w/v) with different solvents (distilled water, 0.05 mol/L acetate buffer pH 5.5 and 0.05 mol/L phosphate buffer pH 7.25) stirred at 85 rpm for 2 or 4 h. The enzymes used were pulpzyme and cartazyme, both commercial. The accompaniment of the enzymatic activity was carried out through measurement in initial and finish of each enzymatic pretreatment. The xylanase-treated pulps and xylanase-alkaline-extracted pulps were analyzed regarding kappa number and viscosity. Pulpzyme recovery was better in phosphate buffered medium (84, 46, and 23% for first, second, and third enzymatic treatment, respectively) although in aqueous medium reached only 2% for every treatments. However, the improvement of pulp properties was evidenced only in aqueous medium for pulpzyme. Cartazyme recovery was similar for both solvents (water and acetate buffer), reaching values around 19% for first enzymatic treatment and 9% for second one. Nevertheless, the pulp properties increased only in acetate buffered medium.

Index Entries: Enzymatic pretreatment; xylanase; acetosolv pulping; sugarcane bagasse.

Introduction

Brazil is the largest worldwide producer of sugarcane. At least 10% of the agricultural area, 5.5×10^6 ha of cane, are planted corresponding to an annual production of 357.5×10^6 t of cane *(1)*. The sugarcane bagasse is the principal byproduct of the alcohol industry, used as fuel in mills and its production surplus constitutes an environmentally problem and at the same time would be a renewable font of chemical resources.

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Nowadays the studies of process that utilize agricultural residues for obtaining chemical products are growing. Pulping using organic solvents in aqueous solution known as organosolv is one of these processes. Chlorine utilized in the traditional bleaching sequences reacts with lignin and forms soluble chlorlignins in the basic medium. The low-molecular-weight-compounds (AOX) formed in high amounts own toxic mutagenic and/or carcinogen character *(2,3)*. Environmental laws have put restrictions in using of the chlorine in the bleaching process in paper industry. Then modifications in pulping process and developing alternative ways in bleaching without use of the elementary chlorine were improved, like the bleaching-totally-chlorine-free methods. Hydrogen peroxide is one of the more widely used oxygen-based bleaching chemicals in the pulp and paper industry *(4,5)*.

Enzymes have shown to be a biotechnological alternative in bleaching of pulps, used together with conventional-bleaching-chemical sequences *(6–7)*. The main motive in the use of enzyme is the decrease of the chorine consumed in bleaching, reaching 34% *(2–8)*, decreasing consequently the organochlorine-compound charge produced *(8)*.

The mechanisms of the enzyme action in the pulp are the removal of the redeposited xylan on fibers and the rupture of the lignin–carbohydrate complex. The redeposited xylan on pulp-surface covers the residual lignin becoming it inaccessible to the bleaching reagents. The xylanase hydrolyzes part of this xylan permitting better access of the bleaching reagents to the residual lignin, becoming the removal of this lignin is easier *(7,9,10)*. The lignin–carbohydrate complex theory assumes that there is a union between lignin and polyoses in pulp that restricts the removal of residual lignin. The xylan bond cleavage by xylanase separates the lignin-carbohydrate linkages improving the access of the bleaching reagents and facilitating the lignin removal in the subsequent bleaching chemical sequences *(7–10)*.

The cost of enzyme production has been admitted as the greatest difficulty, impeding economical feasibility of the majority of biomass conversion processes *(11)*. Several strategies have been used to increase the efficiency and reduce the cost of the process. A strategy of much success has been to enhance the enzyme generation productivity for mutation fungus and the culture conduction optimization. The increase of specific activity of the enzymes and enzyme reuse in the process are also strategies to be considered *(12)*. In ethanol production process from lignocellulosic residue, the enzyme recycle lead to reduce the ethanol production cost. Ramos and Saddler *(13)* showed that recovery and recycle of the enzyme β-Glucosidase can reach approx 70% of the original protein added in the first hydrolysis reaction after seven hydrolysis cycles. Greeg et al. *(14)* showed that enzyme recycling using hydrolysis reactors for two cycles decreased the ethanol production cost in 12%. Despite several studies in this area, the reuse of the enzyme xylanase in pulp biobleaching process with intention of decreasing

the cost this enzyme in the paper and cellulose industry was not yet investigated.

Materials and Methods

Acetosolv Pulping

The acetosolv sugarcane bagasse pulping was carried out with acetic acid 93% (v/v), HCl as catalyst, according to Benar *(15)*. The bagasse/solvent ratio was 1 : 14 (w/v). The temperature of pulping was 120°C (temperature of solvent mixture) for 2 or 4 h. The pulp was washed with acetic acid 93% (v/v) and thoroughly washed with water until the neutral pH. The pulp utilized for cartazyme enzyme was the pulp obtained after 2 h, although for pulpzyme enzyme, the pulp was obtained after 4 h because bagasse did not turn in pulp with 2 h pulping.

Enzyme Reuse in the Pulp Pretreatment With Xylanase

Samples of acetosolv pulp with 3% (w/v) consistency were incubated in Erlenmeyer flasks in a shaker at 50°C with 85 rpm stirring for 10 min. Thereon a enzyme quantity, according to Ruzene and Gonçalves *(16)*, 36 IU/g dry pulp for both enzymes, cartazyme and pulpzyme, was incubated in each Erlenmeyer and left to react for 2–4 h. After this time, the pulp was filtered on a Büchner funnel and washed thoroughly with distilled water.

The pulp retained in filter was transferred to a flask and 10 mL of solvent was added. Thereon the pulp was shaked for releasing of adsorbed enzymes. This mix was filtered and the obtained filtrate was blended with the filtrate of the enzymatic pretreatment and utilized in other pulp sample, one other pretreatment was carried out with the same reaction conditions described in the previous paragraph, except the addition of the enzyme. An aliquot of the filtrate was reserved for determination of the enzymatic activity. The procedure of enzyme reuse was repeated once for cartazyme with treatment duration of 4 h and twice for pulpzyme with treatment time of 2 h, decreasing the treatment time for this enzyme. Also the solvent was altered: distilled water and sodium acetate buffer with pH 5.5 and 0.05 mol/L for cartazyme and distilled water and sodium phosphate butter with pH 7.25 and 0.05 mol/L for pulpzyme. A control treatment was carried out with the same reaction conditions described above, except the enzyme addition.

Alkaline Extraction of the Pulp

Samples of acetosolv pulp with 3% (w/v) consistency were treated with sodium hydroxide solution 5% (w/w) for 1 h at 65°C. After, the alkaine-extracted pulp was filtered and thoroughly washed with water until the pH was neutral.

Determination of the Xylanase Enzymatic Activity

The two commercial xylanases, pulpzyme (Novozyme, Bagsvaerd, Denmark) and cartazyme HS (Sandoz, Leed, UK), were used. The cartazyme has pH and temperature optima of 3.0–5.0 and 35–55°C, respectively, with a molecular mass of 21 kDa. The pulpzyme was kindly furnished by Novozyme with batch number CKN00044 and has pH and temperature optima of 6.5–8.0 and 45–60°C, respectively.

The enzymatic activity was determined by measuring the reducing sugar quantity, released hereby Birchwood xylan breakage, according to Bailey et al. *(17)*. A diluted-xylanase solution with buffer was incubated in a substrate solution of Birchwood xylan for 5 min at 50°C. Reducing sugars were dosed by 3,5-dinitrosalicylic acid (DNS) *(18)*. The buffers used in the enzyme dilution were: sodium acetate buffer with pH 5.5 and concentration 0.05 mol/L for cartazyme and sodium phosphate butter with pH 7.25 and concentration 0.05 mol/L for pulpzyme.

Estimation of Kappa Number

Samples of pulp were submitted to the action of 0.1 eq/L $KMnO_4$ at 25°C for 10 min. Adding KI solution in excess stopped the reaction and the $KMnO_4$ consumed was determined from the results of back titrating of the liberated iodine with standard 0.1 eq/L sodium thiosulphate solution. Kappa number was calculated using the volume in mL of 0.1 *N* $KMnO_4$ consumed per gram of pulp *(19)*. Kappa number was determined for xylanase treated pulp and xylanase-alkaline-extracted pulp.

Determination of Viscosity

Viscosity was determined, through an Ostwald viscometer, by steeping of pulp in 12.5 mL of distilled water for 15 min and by dissolving pulp in 12.5 mL of cupriethylenediamine (Cu^{+2} 1 mol/L) for 30 min *(20)*. Viscosity was determined for xylanase treated pulp and xylanase-alkaline-extracted pulp.

Results and Discussion

Acetosolv Pulp Treated With Cartazyme Enzyme

In both solvents, with analysis of viscosity and kappa number ratio of xylanase treatment (X), only in first treatment a improvement in pulp properties was noted, whereas in the other one there was a slight decrease. Therefore with enzymatic treatment followed by alkaline extraction (XE) an improvement was noticed only in the treatment carried out in the buffered medium, because in aqueous medium the values decreased (Fig. 1, Table 1). The cartazyme effect was just enhanced in pulp viscosity analysis, whereas X stage as much as in XE stage, the viscosity suffered a rising. Because the kappa number analysis for XE stage presented similar values,

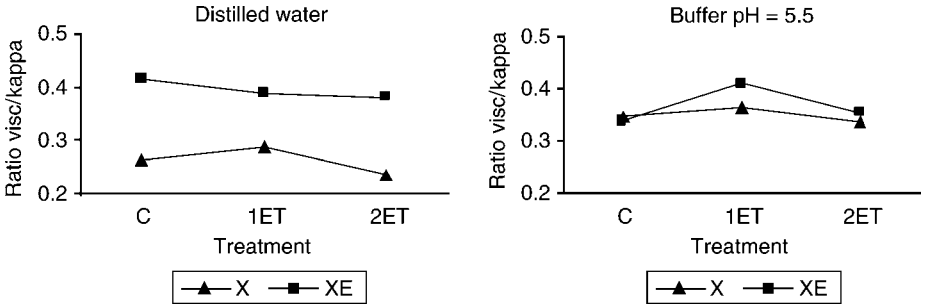

Fig. 1. Graphics of the ratio between viscosity and kappa number of cartazyme treated pulps and cartazyme-alkaline-extracted pulps. ET, enzymatic treatment; C, control treatment (without enzyme); X, xylanase; XE, xylanase and alkaline extraction.

Table 1
Values of Kappa Number and Viscosity of Cartazyme Treated Pulps and Cartazyme-Alkaline-Extracted Pulps

Medium	Sample	Kappa number X	Kappa number XE	Viscosity X (cP)	Viscosity XE (cP)	R X	R XE
Water	Control	28	18	7.3	7.3	0.261	0.415
	1st ET	34 ± 2	20 ± 1	9.8 ± 0.2	7.6 ± 0.6	0.286	0.387
	2nd ET	33 ± 1	18.4 ± 0.5	7.6 ± 0.8	7.0 ± 0.1	0.233	0.381
Buffer pH 5.5	Control	29	16	10.2	5.53	0.347	0.340
	1st ET	31 ± 1	18 ± 1	11.1 ± 2	7.4 ± 0.2	0.363	0.411
	2nd ET	33 ± 5	19.0 ± 0.2	11.2 ± 3	6.7 ± 0.2	0.335	0.352

ET, enzymatic treatment; X, xylanase; XE, xylanase plus alkaline extraction; R, ratio between viscosity and kappa.

that could be explained as bleaching agent was used in excess (NaOH solution 5% w/w), reaching bleaching saturation for this reagent. In such case the enzyme effect could not be checked by this analysis. Besides, in both solvents the enzymatic activity fall were similar, reveling that the use of buffer did not show any advantage regarding distilled water (Fig. 2). The cartazyme presented an enzyme recovery, in both solvents, around 19% and 9% for first and second enzymatic treatment, respectively. This recovery, equal for both solvents, is owing to acidity pulp, turning the aqueous medium slightly acid (similar enzymatic treatment condition in pH 5.5 buffer medium).

Acetosolv Pulp Treated With Pulpzyme Enzyme

In the Pulpzyme enzymatic treatment a significant improving in pulp properties was noted for treatment in aqueous medium, whereas in buffered medium the pulp properties were kept for X stage and reduced for XE stage (Table 2, Fig. 3). Also an increase in pulp viscosity was evidenced in

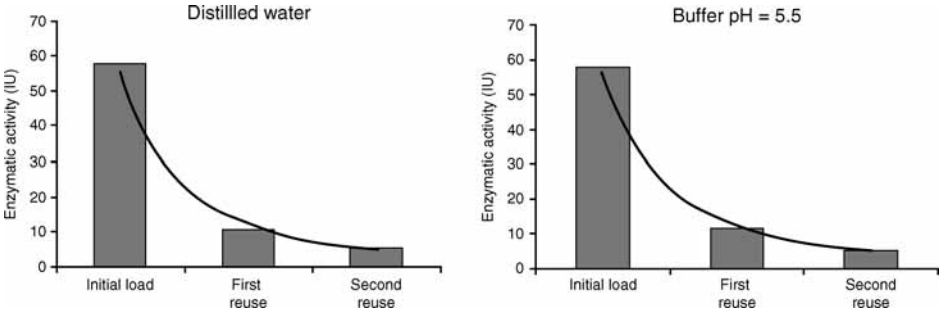

Fig. 2. Graphics of the enzymatic activity fall for the treatments enzymatic of cartazyme enzyme. The first column indicates initial enzymatic load and the others show the enzyme load of first and second reuses.

Table 2
Values of Kappa Number and Viscosity of Pulpzyme Treated Pulps and Pulpzyme-Alkaline-Extracted Pulps

Medium	Sample	Kappa number X	Kappa number XE	Viscosity X (cP)	Viscosity XE (cP)	R X	R XE
Water	Control	25	9.5	13.6	8.7	0.544	0.918
	1st ET	11 ± 2	9 ± 2	13 ± 2	9.4 ± 0.6	1.175	1.106
	2nd ET	22.2 ± 0.5	9.1 ± 0.2	12 ± 2	8.7 ± 0.4	0.559	0.922
	3rd ET	20.9 ± 0.5	9.1 ± 0.6	14 ± 1	9.1 ± 0.6	0.687	1.001
Buffer pH 7.25	Control	37	11	11.2	16.2	0.302	1.496
	1st ET	36.7 ± 0.6	10.7 ± 0.4	10 ± 1	13 ± 1	0.278	1.217
	2nd ET	35.7 ± 0.3	10.6 ± 0.1	12 ± 2	17 ± 3	0.336	1.555
	3rd ET	34.5 ± 0.5	10.2 ± 0.3	12 ± 2	16 ± 1	0.360	1.549

ET, enzymatic treatment; X, xylanase; XE, xylanse plus alkaline extraction; R, ratio between viscosity and kappa.

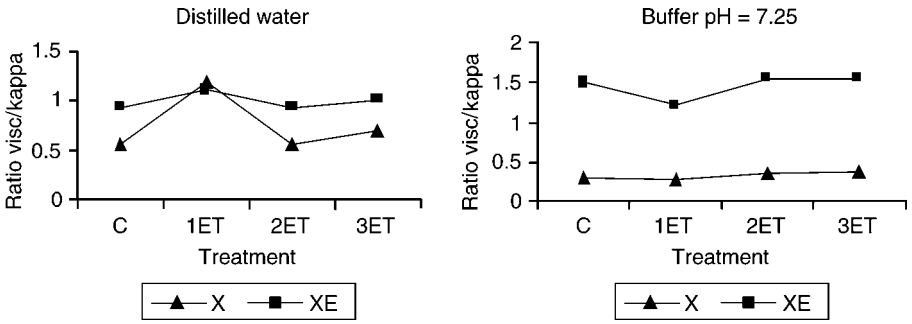

Fig. 3. Graphics of the ratio between viscosity and kappa number of pulpzyme treated pulps and pulpzyme-alkaline-extracted pulps. ET, enzymatic treatment; C, control treatment (without enzyme); X, xylanase; XE, xylanase and alkaline extraction.

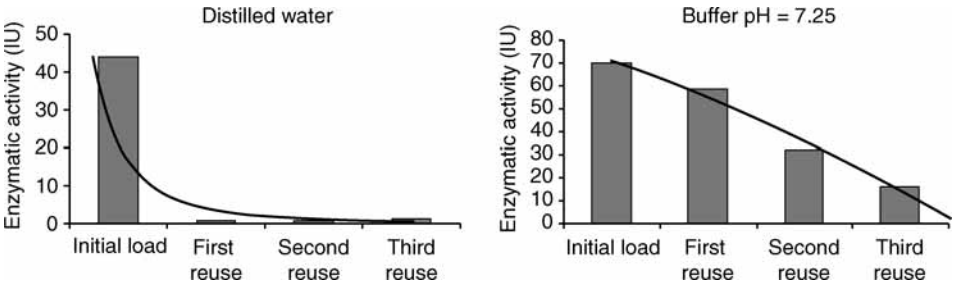

Fig. 4. Graphics of the enzymatic activity fall for the treatments enzymatic of pulpzyme enzyme. The first column indicates initial enzymatic load and the others show the enzyme load of first, second, and third reuses.

both stages and a bleaching saturation (kappa numbers similar) in XE stage. However, the use of buffer showed advantages regarding distilled water in the enzyme recovery. The enzymatic activity fall was very different (Fig. 4), in aqueous medium the enzyme recovery was low (approx 2% for every treatments) whereas in buffered medium the fall was more bland (84%, 46%, and 23% for first, second and third enzymatic treatment respectively).

Conclusions

With these results, it was concluded that pulpzyme enzyme would be more appropriate for use only in one batch in aqueous medium, whereas this enzyme achieves to increase the pulp properties (reduced enough the kappa number and increased the viscosity) and owing to this fact, it has small enzyme recovery in aqueous medium. The pulpzyme enzyme in buffered medium reached values of enzyme recovery higher than in aqueous medium, however the use of this enzyme, in buffered medium, did not revel improving in pulp properties in the studied time (2 h), so suggesting a larger time to evidence an effect of this enzyme. Regarding cartazyme, in buffered medium, this enzyme works better, whereas the increase of viscosity and kappa number ratios were higher than that in aqueous medium regarding the control. The enzyme recovery in both solvents was similar.

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