Improvement of Kraft Bagasse Pulp Bleachability by Treatment with Fungi and their Enzymes

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ABSTRACT

Aspergillus carneus and Trichoderma harzianum fungi or their enzymes such as glucose oxidase and xylanase were used for improvement of kraft bagasse pulp qualities. Trichoderma harzianum NRC-10 produced the maximum xylanase 234 U/ml/min when 6% pulp consistency was used against 73.1 U/ml/min xylanase production for Aspergillus carneus NRC-6. The reduction in hemicellulose level for 4 percent NaOH treated pulp increased by about 48% due to fungi processing. Incubation of pulp for 18 h with glucose oxidase or xylanase reduced the hemicellulose level by 23-37%. Treatment of pulp with mixed fungi culture improves the pulp bleachability while treatment with single fungi improves the strength properties of produced paper sheets. Crude glucose oxidase or xylanase treatments of pulp slightly improve its bleachability while coupling enzymes increase the pulp brightness by 6.2%.

INTRODUCTION

The world suffers presently from the pollution specially the water pollution with several contributing sources. Pulp bleaching is one source where chlorinated phenolic compounds produced during conventional pulp bleaching are toxic and resistant to biodegradation (1). Many fungi play important role in the biobleaching of whole wood or kraft pulp, specially the white or brown rot fungi (2). Bacillus pumilus cultures (3), Aspergillus species as well as other fungi such as Aureabasidium pullulans (4), Saccharomonospora viridis (5), and Trichoderma harzianum, reduce xylan content in unbleached kraft pulp (6). Lignin is chemically linked to the hemicellulose with a frequency of about 1 in every 35-phenyl propane units (7). Therefore, removal of a small portion of hemicellulose might be sufficient to open up the polymer and facilitate the solvent removal of residual lignin, leading to a decrease in energy demand during beating. It also reduces the active chlorine consumption during bleaching (8) by about 25%. Lingin is industrially degraded with peroxide, generated further from glucose oxidase (9). Also, gluconic acid, a product of glucose oxidase activity, is an efficient shelter, which increases brightness of pulp (10). Several studies recorded that mixtures of hemicellulases with different conduct can be more effective than a single enzyme activity (10).

In this paper, improvements in kraft pulp bleaching brought about cultures of Aspergillus carneus NRC-6, Trichoderma harzianum NRC-10 alone or mixed and also by coupling their enzymes xylanase and glucose oxidase are presented.

EXPERIMENTAL

Materials and Methods

Chemicals

Xylan was obtained from oat spelt and glactomannan was obtained from Locust bean gum (Sigma). Carboxymethyl cellulose (CMC) type 6LT was acquirer from Hercules, Inc., Wilmington, Delaware, USA. Crude malt extract, 45% w/v, was purchased from Yeast Company, Zigazag, Egypt, kraft bagasse pulp with 4.3% lignin, 22.8% pentosan, 69% α-cellulose and 0.6% ash was obtained from Egyptian Sugar Company, Edfo Mill.

Fungi

Aspergillus carneus NRC-6 and Trichoderma

harzianum NRC-10 were isolated from Egyptian sugar cane bagasse maintained on potato-dextrose agar slants and identified according to standard method (11).

Xylanase Assay

Xylanase activity was determined according to the following procedure; 0.5 ml of 0.01% xylan in 0.1 mol citrate buffer (pH 5.0) was incubated for 10 min with 0.4 ml distilled water and 0.1 ml of appropriately diluted enzyme (total volume 1 ml). The reaction was stopped and the reducing sugars were determined according to Miller's DNS method (12). The xylanase unit is defined as the amount of enzyme releasing 1 μ mol of xylose equivalent/ml/min.

Glucose Oxidase: Gluconic acid, a product of glucose oxidase action, was determined in distilled water according to Coop method (13). One unit of glucose oxidase catalyzes conversion of 1 μ mol D-glucose to gluconic acid/ml/min. The enzyme protein in the fermented broth was determined by the method of Lowry et al (14).

Bleaching

Different pulps, before and after enzymatic treatments, were bleached by the DED method. Bleaching was carried out at 70°C for 1h at a liquor ratio of 10:1 for every step. Chlorine dioxide was 4%, based on pulp, in D1 and 3 in DII. Extraction in between (E) was carried out with 3 percent NaOH. Paper sheet formation and paper properties were conducted according to standard methods. Pentosan content was determined according to Tappi standard methods, Tappi T 223 om-84 and permanganate number according to Tappi T 214 wd-76.

Glucose oxidase was produced by A. carneus NRC-6 in the gluconic acid production medium (15) and extracted as a crude enzyme (16). Different amounts of bagasse pulp meal were used in 100-ml o fl percent crude malt extract in a 250-ml conical flask. Each flask was inoculated with standard inoculum slant of A. carneus NRC-6 or T. harzianum NRC-10 and then incubated for 12 days at 28°C at 200 rpm.

The kraft bagasse pulp meal was soaked overnight at 28°C with different concentrations of NaOH (2, 4, 8, 12 or 16% w/w), washed with distilled water till it became neutral. The equivalent of optimum amount of kraft pulp (6 g/flask) was substituted with wet weight of pretreated pulp with alkali and the medium constituents as previously used. After autoclaving, each flask was inoculated with A. carneus NRC-6 and/or T. harzianum NRC-10 and incubated at 28°C at 200 rpm for 12 days.

Different amounts of the fermented broth of T. harzianum NRC-10, from 0.05 to 0.25 ml were applied and the equivalent crude protein was determined. The enzyme production was carried out under conditions of 0.5 ml of 0.01% substrate in 0.1 mol citrate buffers and the total volume was made up to 1 ml by distilled water and incubated at 25°C for 10 min and the activity was determined as aforementioned method.

Pulp treated with crude xylanase, produced by T. harzianum NRC-10, and glucose oxidase, produced b A. carneus NRC-6, either alone or in combination with each other under optimum conditions and buffer, after 18 h of incubation at optimum enzymes concentrations.

Optimum units of xylanase as well as glucose oxidase were added to 10 g of the crude kraft

Table 1. Effect of different consistency of kraft bagasse pulp treated with A. carneus NRC-6 and T. harzianum NRC-10 on the xylanase production and hemicellulose

	A. Carneus NRC-6			T. harzianum NRC-10		
Pulp weight g/flask	Xylanase activity U/ml/min	Hemicellulose, %	RRH %	Xylanase activity U/ml/min	Hemicellulose, %	RRH %
2	34.3	14.1	38.15	88.0	14.9	34.64
4	58.2	13.6	40.35	166	13.6	40.35
6	73.1	12.9	43.42	234	9.63	57.76
8	40.1	16:8	26.31	52.6	16.2	28.94

Incubated at 28°C at 200 rpm for 12 d; initial concentration of hemicellulose = 22.8 percent

RRH : Relative removal of hemicellulose.

bagasse pulp and 4% NaOH treated pulp under optimum conditions of the reaction mixture using 100 ml buffer at 25°C for different incubation periods, at 6 h intervals.

The tested fungi in the medium containing 1% crude malt extract carried out the bleaching of 4% alkali pretreated kraft pulp. Either the fungal cultures applied singly for 12 d or mixed after 4 d fermentation with the other fungus culture and the incubation was extended to 12 d at 28°C and 200 rpm in 250 ml conical flasks

The bleaching of 4% alkali pretreated kraft pulp was carried out by the optimum units of xylanase and glucose oxidase or mixture of them under optimum incubation period at room temperature using 10 g pulp in 100 ml of the proper buffer in 500 ml conical flasks. Then the bleaching carried out and the properties of the pulp tested.

RESULTS AND DISCUSSION

The data presented in Table 1 show that the optimum consistency of kraft bagasse pulp was 6% (w/v) to produce maximum xylanase activity and reduce hemicellulose levels by both fungi. A carneus NRC-6 produced about 73.1 U/ml/min xylanase in the fermented broth and reduced the hemiceullulose contents to 12.9%. While T. harzianum NRC-10 recorded xylanase activity 234 U/ml/min and reduced the hemicellulose level to 9.63% (< 50% of original concentration, 22.%), whereas purified xylanase from T. harzianum reduced the xylan content of unbleached kraft pulp 25% (6). Moreover, at consistencies more than 6% bagasse, the medium became like a solid state and it was difficult carry out the reaction and the processing. These results in Table 1 confirm that 6% pulp

Table 2. Effect of A. carneus NRC-6 and T. harzianum NRC-10 on hemicellulose content and xylanase activity after alkali treatment of pulp

NaOH	H percent	A. carneus NRC-6			T. Harzianum NRC-10		
percent (w/w)	after NaOH treatment	H %	RRH %	Xylanase U/ml	H %	RRH %	xylanase U/ml
0	22.8	14.3	37.3	23.0	14.5	36.4	86.4
2	20.5	11.0	46.3	23.5	12.3	40.0	80.4
4	19.1	9.7	49.2	9.5	10.1	47.1	68.1
8	17.2	9.0	47.7	8.1	10.3	40.1	55.5
12	16.9	9.7	42.6	7.0	10.2	39.6	57.2
16	15.4	8.9	42.2	4.9	9.6	37.7	43.6

Incubated at 28°C at 200 rpm for 12 d; H: Hemicellulose content, RRH: Relative Removal of Hemicellulose

Table 3. Activity at different concentrations of crude hemicellulases produced By T. harzianum NRC-10 at different substrate and different buffer

Crude hemicellulase volume, ml	Protein equivalent, mg	Xylanase unit/ml	Mannanase unit/ml	CMCase unit/ml
0.05	9.0	15.1	38.2	0.0050
0.10	18.0	11.3	22.3	0.0037
0.15	27.0	6.44	11.2	0.0037
0.20	36.0	4.56	7.79	0.0011
0.25	45.0	2.40	5.33	0.0005
Citrate buffer	9.0	15.1	38.2	0.0050
Acetate buffer	9.0	11.1	21.2	0.0040

Incubated at 25°C for 10 min, the enzyme reaction carried out in citrate buffer.

Table 4. Effect of incubation time on hemicellulose % of crude and alkali treated kraft pulp by optimum crude xylanase and glucose oxidase

Incubation time, h	Xylanase		Glucose oxidase	
	crude kraft pulp	4 % NaOH treated pulp	Crude kraft pulp	4 % NaOH treated pulp
	H%	H%	H%	H%
0	21.3	19.1	21.3	19.1
6	19.1	18.2	19.6	18.1
12	16.5	15.9	16.9	16.2
18	14.4	11.9	15.5	13.2
24	14.4	11.8	15.4	13.1
10 g in 100 ml buffer at room temperature, H: Hemicellulose content				

consistency offer the maximum reduction of 43.4 and 57.8% of hemicellulose level by A. carneus NRC-6 and T. harzianum NRC-10, respectively compared to the control pulp. Despite the lower xylanase production by A. carneus, the hemicellulose level of pulp treated with it was lower than that with T. harzianum. This is due to the action of the other enzymes that produced with it in the medium specially glucose oxidase. The results obtained indicate that the crude glucose oxidase activity was 3.4 U/ml/mg crude protein.

The results presented in Table 2 indicate that the hemicellulose level was gradually reduced with increasing alkali concentration and the use of fungi increased relative hemicellulose removal. The relative removal of hemicellulose (RRH% loss in hemicellulose due to enzymatic action), by A. carneus NRC-6 increased with increasing alkali concentration, upto 4% NaOH, where RRH was 49.2%, then it decreased with higher alkali concentrations. The maximum production of xylanase (23.5 U/ml) was obtained for the pulp treated with 2% NaOH that decreased with increasing alkali concentration. The limited production of xylanase by A. carneus NRC-6 which reduced the hemicellulose level during kraft pulp fermentation is attributed to glucose oxidase system of A. carneus NRC-6 and its role in pulp bleaching. Also, T. harzianum NRC-10 reduced the hemicellulose with RRH 47.1% when the pulp was treated with 4% NaOH, while the maximum xylanase activity was recorded only for the control (without alkali treatment, and soaked in water only) and decreased with increasing alkali concentrations. The treatment of pulp with diluted NaOH caused a swelling of the bulk lignin and xylan (open pores in structure) and chemical bonds such as ester linkages of lignin are broken. Also, acetyl groups of xylan are removed. So, such treatment greatly facilitates the enzymatic attack (17), while lower enzyme concentrations were used.

The results presented in Table 3 show that, the xylanase activity in crude hemicellulases of T. harzianum NRC-10 was 15.1, mannanase 38.2 and CMCase about 0.005 U/ml. This result indicated that the cellulase determined as CMCase (5u/liter) was an insignificant product in the crude liquor. Besides, the citrate buffer was better than the acetate buffer for the xylanase activity, The citrate buffer reduced lignin content (kappa number) by 5% (18).

The result in Table 4 show a gradual decrease in hemicellulose level with increasing incubation time. The proper incubation time of the tested enzymes was 18 h where the hemicellulose content decreased by xylanase to 14.4% for crude kraft pulp and 11.9% alkali pretreated pulp. While

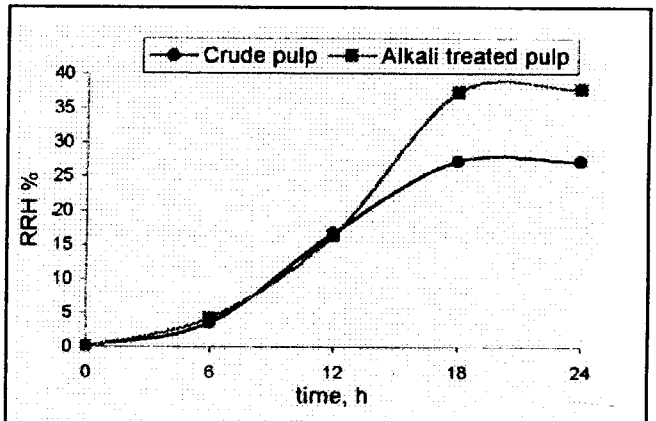

Fig. 1 Effect of incubation time on relative removal of hemicellulose (RRH) for pulp treated with xylanase

Table 5. Effect of A. carneus NRC-6 and T. Harzianum NRC-10 growth on pulp and paper properties

Treated sample	Hemicellulose percent		Permanganate number		Brightness percent		Breaking length, m		Tear factor	
	B*	B**	B*	B**	B*	B**	B*	B*	B**	B**
4 % NaOH treated pulp	20.5	19.8	9.4	1.69	41.4	75.0	2479	2764	87.6	90.8
A. carneus	14.4	12.2	8.4	1.69	40.4	73.7	3874	3942	83.9	83.0
T. harzianum	17.9	15.6	9.16	1.69	39.6	73.7	4286	4718	92.8	88.8
A. carneus + T. harzianum	16.0	11.9	9.78	1.87	41.7	75.9	2432	3247	58.3	66.8
+ A. carneus	17.5	16.6	9.16	1.87	42.4	75.3	2771	3274	72.3	69.6

Incubated at 200 rpm for 12 d at 28°C, B* Samples before bleaching, B** Samples after bleaching

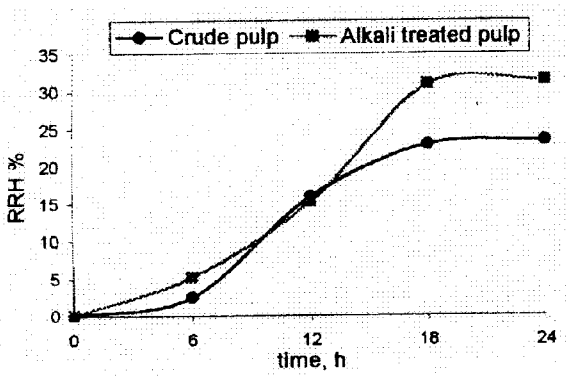

Fig. 2 Effect of incubation time on relative removal of hemicellulose (RRH) for pulp treated with glucose oxidase

glucose oxidase reduced the hemicellulose content of 15.5% and 13.2% for crude and 4% alkali pretreated pulp, respectively. Longer incubation duration showed little significant improvement. Fig. 1 shows the effect of incubation time on percentage of the relative removal of hemicellulose for pulp treated with xylanase. It could be seen that the maximum removal of hemicellulose for crude pulp and alkali treated pulp was achieved after 18 h incubation. It is evident that relative reduction in hemicellulose is much noticeable for alkali treated pulp. Comparable results for pulp treated with glucose oxidase could be noticed from Fig. 2. It could also be noticed that xylanase reduced more hemicellulose than glucose oxidase.

The data in Table 5 summarize the fermented pulp properties before and after the chemical bleaching. The hemicellulose level was reduced to 14.4 and 16.0% before bleaching; in samples cultured with A. carneus NRC-6 alone or in conjunction with T. harzianum NRC-10 respectively, and after bleaching the corresponding figures were 12.2 and 11.9%. Fermentation with A. carneus NRC-6 lowered the permanganate number to 8.4 while culturing with T. harzianum NRC-10 lowered it to 9.16 compared to that for the control. The greater permanganate number due to mixed culture fermentation before chemical bleaching could be related to the presence of hexenuronic acid that contained a double bond, which increases consumption of permanganate thereby increasing its value (19). The brightness of samples fermented with mixed cultures were increased to 41.7 and 42.2 before bleaching and to 75.9 and 75.2 after bleaching for A. carneus NRC-6 mixed with T. harzianum NRC-10 and T. harzianum NRC-10 mixed with A. carneus NRC-6, respectively, which is more than that for controls and those treated with single fungus alone.

The breaking length increased to different extent by different fungi treatment, specially with cultures of T. harzianum NRC-10 and A. carneus NRC-6 where values of 4718 and 3942 m were recorded, respectively, after chemical bleaching followed by mixed culture treatment when compared with control. The tear factor of the pulp decreased upon fungi treatment, especially with the mixed culture. Finally mixed fungi treatment yielded more bleachable pulp for greater brightness after bleaching. Strength properties of different pulp samples treated with fungi increased while the hemicellulose content decreased by about 35%.

Table 6 shows that the hemicellulose decreased slightly when treated with crude enzymes for bleached pulp compared to control samples in the citrate buffer. It is known that during xylanase prebleaching there is significant decrease in xylan

Table 6. Effect of crude enzymes on pulp and paper properties

Property	Hemicellulose %		Permanganate number		Brightness %		Breaking length, m		Tear factor	
	B*	B**	B*	B**	B*	B**	B*	B*	B**	B**
4 % NaOH treated pulp	20.5	19.8	9.4	1.69	41.4	75.0	2479	2764	87.6	90.8
Control citrate buffer	17.4	13.1	9.0	1.33	44.2	76.0	2783	2933	86.1	92.8
Crude xylanase pH5	13.4	12.9	9.7	1.6	43.9	76.9	2517	20.91	46.6	40.1
Glucose oxidase pH7	14.2	13.3	9.1	1.6	42.7	76.2	2668	2561	82.2	90.4
Crude xylanase pH5 + glucose oxidase pH7	13.3	12.1	9.0	1.69	42.0	81.2	2588	2009	16.1	23.1

10 g in 100 ml buffer at room temperature for 18 h, B* Samples before bleaching, B** Samples after bleaching.

DP while only small amount of xylan is removed (20).

Permanganate number, indication of lignin content, showed that citrate buffer moderately reduced the lignin content (18). Enzymatic treatment had little effect on permanganate number before bleaching. It is known that the hemicellulose treatment is an indirect bleaching method. In kraft pulping, xylane tends to readsorb on the cellulose micro fibrils surface as a result of decreased alkali concentration at the end of the cook (21). So removal of xylan by xylanase, from outer surfaces of fibres improves the extractability of lignin by exposing lignin surfaces (22). Also, increased solubilization of xylan-lignin complexes from kraft pulp has been observed by xylanase treatment (23).

Brightness values, the important property to indicate pulp bleachability, showed that all enzymatic treatments of pulp before bleaching increased the brightness. Xylanase and glucose oxidase recorded 0.9 and 0.2 more brightness units for bleached pulp than the buffered controls. Xylanase coupled with glucose oxidase have the brightness 81.2%, more than that of controls and other treatments due to the synergetic effect of xylan removal and peroxide bleaching. While, the citrate buffer increased the brightness before chemical bleaching to 44.2%, more than the other enzyme treatments.

Breaking length and tear factor, indications of paper sheet strength properties, showed that a slight decrease in them were observed for pulps treated with glucose oxidase while pulps treated with xylanase or mixed enzymes showed inferior properties when compared with controls or buffered controls. The deterioration in strength properties for these samples may be due to the contamination of xylanase with cellulose enzyme. Cellulose causes a degradation of cellulose fibre, and reduces the degree of polymerization and viscosity of the pulp and hence resulted in lower strength properties of the paper sheets prepared from such pulps (24). Therefore, to avoid deterioration of paper sheet strength properties, cellulase free xylanase must only be used, using a mutant of T. harzianum (6).

CONCLUSION

Greater xylanase activity and lower hemicellulose content were detected, for both A. carneus and T. harzianum, when 6 g of kraft bagasse pulp was treated in 100 ml medium contain 1% malt extract. Hemicellulose was reduced to 9.7 percent by A. carneus NRC-6 and to 10.1% by T. harzianum NRC-10 for kraft pulp pretreated with 4% NaOH. The fungi also improve the breaking length to carneus and T. harzianum, respectively, and the tear factor was improved only by T. harzianum the pulp brightness. The optimum of incubation period time for the maximum units production of crude xylanase and crude glucose oxidase was 18 h which reduced hemicellulose content of alkali pretreated kraft pulp to 11.9 and 13.2%, respectively.

The coupling enzymes, xylanase and ISO brightness by 6.2% but the tear factor was reduced. Citrate buffer before and after bleaching improved the strength properties obviously.

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