IMPACT OF CELLULASE ENZYME TREATMENT ON STRENGTH, MORPHOLOGY AND CRYSTALLINITY OF DEINKED PULP

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In recent years, enzyme applications in the pulp and paper industry have become remarkable. In this research, *Trichoderma* cellulase was used on deinked pulp and its effects on fiber morphology, crystallinity and strength of pulp were studied. Three parameters were considered: dosage of enzyme, time and temperature of enzymatic treatment. The results showed that the enzymatic treatment enhanced the mechanical properties of the treated pulps. Also, water retention value and drainage were improved by the cellulase treatment. The effect of the enzymatic treatment on the drainage of pulp showed that pulp drainability was improved. The results of handsheet and pulp evaluation indicated that 0.3% cellulase treatment for 60 minutes of at 30 °C were the optimum treating conditions. In treated pulps, structural changes, such as fibrillation, were observed by SEM. The measurement of X-ray diffraction indicated that the crystallinity index of treated pulp was lower than that of the control pulp.

Keywords: enzyme, cellulose, deinked pulp, drainage degree, WRV, strength, morphology, crystalinity

INTRODUCTION

Enzymes can be regarded as catalysts for biochemical reactions. This implies that a relatively small amount of an enzyme may be enough to catalyze a biochemical reaction. On the other hand, if enzymes are not present, the reaction either might not occur or might proceed more slowly. Enzymes are unique, which means each enzyme has its specific function, aiding in a specific biochemical reaction.[1] Such potential has motivated new research and development paths on the utilization and application of various enzymes in different aspects, including bioremediation of effluents, pulping, bleaching and fiber modification and enhancement.

Among enzymes, cellulose has attracted interest since the 1950s. Cellulase has been used increasingly in pulp and paper industry during recent years. Likewise, the consumption of recycled papers has been increasing in the last decades. Generally, the properties of recycled pulp are adversely affected by their history of processing and use. Thus, there are limitations in recycling of paper. Since the 1970s, enzymes have been used for modification of fiber properties.[2] The main goals of paper recycling are deinking and removing pollutant particles, without any loss in strength and brightness properties of paper.[3] However, as has been shown in many studies, the recycling of cellulosic fibers, especially in the case of wood-free pulps, is usually associated with a loss of physical strength of pulp.[4]

Enzymes can be used to alter the internal structure of cellulose fibers and aid in the separation of fibers prior to washing and flotation operations, as well as enhancing fiber strength. The cellulases can be classified based on their mode of action: endoglucanases, cellobiohydrolases and β-d-glucosidases. Endoglucanases randomly attack the amorphous regions of the cellulose substrate, yielding longer chain oligomers. Cellobiohydrolases are exoenzymes, which degrade the crystalline cellulose, releasing cellobiose. Both types of enzymes hydrolyse β-1,4-glycosidic bonds. The β-d-glucosidase also called cellobiase converts cellooligosaccharides and cellobiose to glucose.[5,6] An appropriate use of endoglucanases can increase the drainage of recycled pulp to a degree that is comparable to the effects of polymeric additives. Adding enzymes prior to refining can increase pulp freeness and the speed of the paper machine.[7] In addition, the application of cellulases and hemicellulases improves drainability and yet, it is an environmentally friendly method.[8] Cellulase removes fine particles from pulp[1,8,9,10] and helps to increase the freeness of the pulp.[2,11]

The treatment of recycled paper with cellulase can also increase handsheet strength properties, which can be due to improved fiber flexibility and morphology.[6]

The objective of the present study is to investigate the effect of cellulase on pulp drainage, water retention value (WRV), and strength properties of enzymatically treated deinked pulp.

EXPERIMENTAL

Materials

The deinked pulp used in this study was supplied by the Latif Papermaking Co. (Hashtgerd, Iran) and consisted of 50% mixed office waste (MOW) and 50% printing house white cuttings. The applied enzyme was cellulase (endo-1,4-β cellulase) from *Trichoderma virid* purchased from Merck Chemical Co. (Dormstadt, Germany). The optimal processing conditions for this enzyme were indicated by the producer as the following: temperature 30-50 °C, pH 3.5-5, reaction time 0.5-2 h. Cellulase activities were measured as 1 U/mg using carboxymethylcellulose (CMC), and the released sugars were measured by the dinitrosalicylic acid method (DNS), using glucose as standard monosacharide. The pH of the suspension was maintained at 4.8-5, by adding acetate buffer (1 M).

Methods

Enzymatic treatment

Three different sets of treatment conditions were applied: two cellulase dosages (0.1 and 0.3% based on dry weight of pulp equivalent to 22 U and 66 U, respectively), two treatment temperatures (30, 50 °C) and three treatment times (30, 60, and 120 minutes). A pulp consistency of 3% and a pH of 5 were maintained as constant factors for all experiments. A conventional laboratory water bath was used for adjusting the temperature. The pulp suspensions containing enzyme were mixed before charging to the reaction beaker and then during the treatment period, the suspension was stirred at 10 min intervals. The enzyme reaction was stopped by boiling the suspension. Untreated deinked pulps were used as control samples.

Determination of pulp and paper properties

Laboratory handsheets were prepared according to SCAN C 26:67 procedures. Pulp and paper properties were evaluated following relevant standard test methods: drainage degree, SCAN C 19:65; WRV;[3,10] tensile index, ISO 12192; burst index, ISO 2758; folding endurance, ISO 5626, and tear index, Tappi T414 om-04.

Morphological characteristics of the treated deinked fibers were evaluated by scanning electron microscope. The crystallinity of cellulose was determined with X-ray diffraction (XRD) method, as defined by Segal *et al.*,[7] using equation 1:

$$CrI = ((I_{002} - I_{amorph}) / I_{002}) \times 100 \quad (1)$$

CrI is degree of crystallinity, I_{002} is the maximum diffraction intensity of the (002) plane and I_{amorph} is the diffraction intensity at 2θ =18°.

RESULTS AND DISCUSSION

The properties of enzyme treated and control pulps are summarized in Table 1. The enzymatic treatments altered the drainage degree of the pulp leading to improved drainability. This phenomenon is attributed to the ability of the enzyme to either flocculate fines or remove fibrils from the surface of fibers and larger fines.[8] Verma *et al.*[17] and Mayeli *et al.*[12] stated that fine particles and fibrils intensity was reduced by enzymatic treatment and the drainability of pulp increased accordingly. An enzyme dosage of 0.3% and a treatment temperature of 50 °C proved more effective in improving drainability. It was observed that more fine particles and fibrils were removed at 50 °C and 0.3% enzyme dosage (Fig. 1).

Water retention value (WRV) as a measure of structural integrity of fibers showed that treated pulps retain more water (higher WRV) compared to control pulp.[2,10,11] This may be due to the hydrolysis of lower molecular weight carbohydrates and even to partial deterioration of crystalline structure of cellulose initiating the formation of more hydrogen bonds between cellulose and water molecules.[2] Verma *et al.* [17] stated that the increased solubilisation of amorphous cellulose mediated by cellulase treatments improved water retention values and, as a consequence, drainability was improved. The maximum WRV was reached applying 0.3% enzyme dosage, 60 min and 30 °C.

Gama *et al.*[11] and Jeffries *et al.*[5] reported that cellulase modified fiber surface characteristics in favor of better bond formation and increased fiber-fiber contact, thereby improving the strength properties of paper. Also, Jokinen *et al.*[18] and Nomura[13] reported a similar finding on combined application of cellulase and cellobiase.

Table 1
Effect of enzyme treatment on deinked pulp and paper properties

Trial No.	Temp (°C)	Time (min)	Enzyme dosage		Drainage degree (°SR)	WRV (%)	Burst index ($KPam^2/g$)	Tear index (mNm^2/g)	Tensile index (Nm/g)	Folding endurance
			IU	%						
Control	18	0	0	0	14.5^{d}	20.83^{a*}	1.48^{ab}	9.71^{b}	27.57^{a}	3.3^{ab}
T1	30	30	22U	0.1	14^{cd}	22.43^{f}	1.69^{d}	11.32^{j}	29.09^{e}	4.7^{c}
T2	30	60	22U	0.1	13.5^{bc}	22.60^{i}	1.53^{bc}	10.49^{de}	29.63^{f}	4.7^{c}
T3	30	120	22U	0.1	13^{abc}	21.68^{c}	1.45^{a*}	10.57^{ef}	28.14^{b}	4^{bc}
T4	30	30	66U	0.3	13^{abc}	22.72^{j}	1.57^{c}	10.39^{d}	29.90	4.7^{c}
T5	30	60	66U	0.3	12.5^{ab}	23.36^{k}	1.71^{d}	11.08^{i}	30.53^{h}	5.7^{d}
T6	30	120	66U	0.3	12^{a*}	22.11^{d}	1.48^{ab}	9.49^{a*}	29.15^{e}	4^{bc}
T7	50	30	22U	0.1	13.5^{bc}	22.28^{e}	1.53^{bc}	9.84^{b}	28.74^{d}	3.7^{ab}
T8	50	60	22U	0.1	13^{abc}	22.47^{g}	1.55^{bc}	10.74^{g}	29.18^{e}	4.7^{c}
T9	50	120	22U	0.1	12.5^{ab}	22.21^{d}	1.59^{c}	10.15^{c}	27.65^{a*}	3^{a*}
T10	50	30	66U	0.3	12.5^{ab}	22.50^{g}	1.56^{bc}	10.73^{g}	29.25^{e}	4^{bc}
T11	50	60	66U	0.3	12.5^{ab}	22.57^{h}	1.54^{bc}	10.67^{fg}	29.75^{fg}	4^{bc}
T12	50	120	66U	0.3	12.5^{ab}	21.32^{b}	1.53^{bc}	10.91^{h}	28.52^{c}	3^{a*}

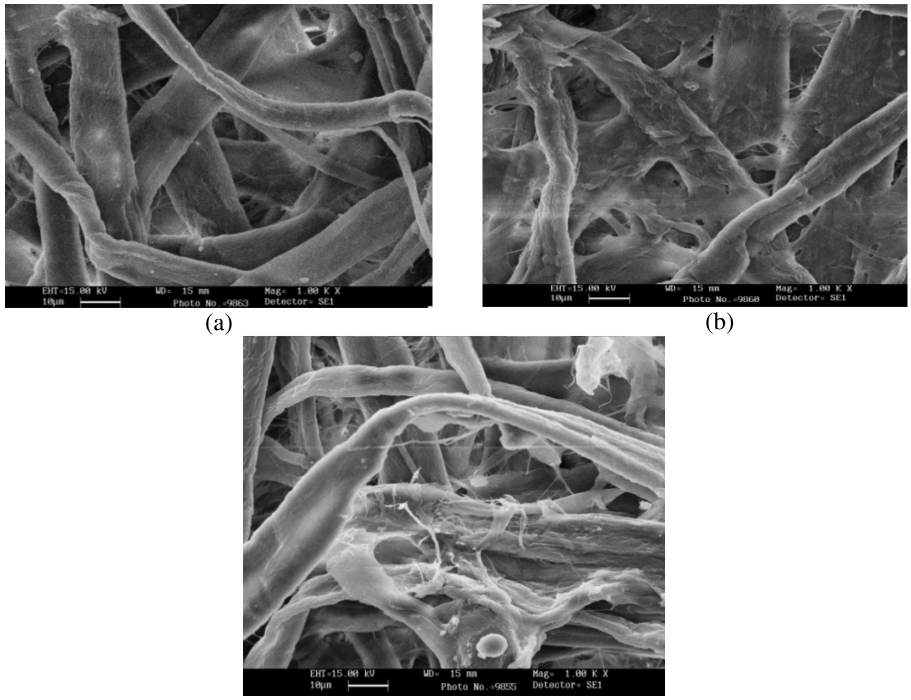
* The lowercase letters indicate Duncan ranking of the averages

Both tensile and burst indices were improved as a result of cellulase treatment. It is believed that a tighter fibrous network with closer fiber-to-fiber contact is formed.[7,9] As our results indicate, a higher tensile index was reached at 30 °C, 60 min and 0.3% enzyme dosage. No significant difference was observed when applying different cellulase dosages and temperatures, while treatment time was significantly effective on burst index. The maximum burst index was observed after 60 minute treatment time was used.

The tear index of cellulase treated pulps was higher than that of the control sample, which was attributed to the improvement in fiber flexibility and to the removal of microfibrils.[6,9] Cellulase dosage and treatment temperatures did not have any significant effect on tear index, but the impact of treatment time on tear index was statistically significant. It was observed that as the treatment time increased to 120 minutes, the fiber structure weakened and its inherent strength reduced, while the integrity of fibers and tear index marginally deteriorated.

Generally, the enzymatic treatment of pulp improved fiber flexibility and folding endurance.[6] Cellulase treatment at 30 °C resulted in more fiber-to-fiber bonds compared to control pulps. There was no statistically significant difference in the effectiveness of cellulase dosages on folding endurance, but 60 min treatment time can be considered as optimum.

Cellulase treatment generated two types of changes in pulp fibers; internal structure modification and surface roughening. To evaluate and characterize the surface of the fibers, the morphology of cellulase treated and control fibers was examined using scanning electron microscopy (SEM). The SEM micrographs of the handsheets showed that fiber surface was effectively changed and, as a consequence of the treatment, fiber-to-fiber bonding was obviously improved (Fig. 1 b, c). Such fiber surface roughening plays a significant role in increasing the strength of the recycled pulps.[9,11] The maximum strengths, surface roughness and flexibility of fibers were observed at 0.3% enzyme dosage, 30 °C and 60 min treatment conditions (Table 1 and Fig. 1).



(c)

Figure 1: SEM images (1000X) of untreated (a) and treated pulps: (enzyme: 0.3%, temperature: 30 °C, time: 60 min) (b), (enzyme: 0.3%, temperature: 50 °C, time: 60 min) (c), (1000X)

Table 2
Crystallinity index of treated and control pulps

Trial No.	Temp (°C)	Time (min)	Enzyme dosage IU	Enzyme dosage %	Crystallinity index (%)
control	18	0	0	0	37.61
T2	30	60	22U	0.1	36.27
T5	30	60	66U	0.3	32.39
T9	50	120	22U	0.1	29.33
T11	50	60	66U	0.3	30.05

Four treatment conditions (Table 2) were selected as representative of all treatments for the evaluation of fiber crystallinity as an index of fiber microstructure alteration. The crystallinity index of treated fibers was lower than that of the control, indicating that cellulase deteriorated the crystalline structure of the fibers.[2] The reduction in crystallinity was the expression of the slight decrease in crystalline to amorphous ratio of the fibers caused by the cellulase treatment. The increased ratio of amorphous regions, measured by the water absorption of fibers, was inversely related to high WRV (Table 1).

CONCLUSION

The impact of the enzymatic treatment on the pulp and paper properties of deinked pulp was evaluated. The effectiveness of *Trichoderma* cellulase was examined, and the following results were obtained:

1. The cellulase treatment reduced the pulp drainage degree. The lowest drainage degree was reached when applying 50 °C and 0.3% (66 U) enzyme dosage.
2. Concerning the strength of the handsheets, the optimum enzymatic treatment was 0.3% (66 U) enzyme dosage, 30 °C and 60 min,

producing pulp with the following properties: tensile index, 30.53 Nm/g; tear index, 11.08 mNm^2/g; burst index, 1.71 $KPam^2/g$; folding endurance, 5.7, WRV 23.36%; and drainage degree, 12.5 °SR.
3. SEM micrographs were showed that the pulps treated with 0.3% (66 U) enzyme dosage at 30 °C and 60 min exhibited a more modified fiber surface and increased fiber-to-fiber bonding.
4. The X-ray diffraction (XRD) measurement showed that the crystallinity index of the treated fibers was lower than that of the control fibers.

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