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# Selective hydrolysis of amorphous cellulosic fines for improvement in drainage of recycled pulp based on ratios of cellulase components

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ABSTRACT

Recycled pulp contains large amount of fines having high surface area and amorphous cellulose. These fines adsorb water to large extent and dewatering rate is lower compared to virgin pulp. Selective hydrolysis of these excess ultra fines through cellulase enzymes can be utilized for enhancing the drainage rate. The concept of monocomponent cellulase treatment of recycled pulp for improvement in drainage and the understanding whether effectiveness depends on a specific type of cellulase component (endoglucanase/cellobiohydrolase) is described. The improvement of ~15–23% in pulp drainability was achieved along with better paper properties.



## Introduction

Recycled fibers have become increasingly important as raw material in the pulp and paper industry. In spite of several advantages offered by paper recycling such as savings of wood for making pulp through substitution of virgin pulp, several problems are also associated with the recycling of waste paper such as deinking of different types of post consumer papers, drainability of recycled pulp, stickies contamination and lower strength. One of the main problems with recycled fiber is that due to high relative surface area of fines, dewatering rate is lower compared to virgin pulp. These fines and fibrils which cause lower rate of drainage in secondary fiber are reported to decisively consist of amorphous cellulose [1,2]. Thus; the productivity of the paper making process is considerably decreased compared to virgin pulp. Dewatering of the pulp strongly affects the energy efficiency of paper machine and thus the cost efficiency of papermaking. Enhanced water removal in former, press, and dryer sections enables lower energy consumption or increased production capacity. Alternately, due to improved drainage, a shorter drying section would decrease investments costs.

Conventionally, dewatering is increased on a paper machine by using drainage aids in the former section or more intense wet pressing in the press section. Use of drainage aids can worsen formation, and high wet press levels decrease the bulk of the end-product [3]. Therefore, novel pulp modification and dewatering innovations are required with new high capacity paper machines. The adverse effect of secondary fines on drainage could be more effectively overcome by treating them in such a way as to reduce their effective surface area [4]. Enzyme usage for selective and controlled hydrolysis of fines will be more cost effective solution for drainage improvement compared to screening of fines from cost efficiency perspective.

Dewatering of different paper and board grades has been reported to be improved due to enzymatic treatments [5]. Cellulase enzymes are reported to modify the interfacial properties of recycled fibers, increasing the water affinity, which in turn change the technical properties of pulp and paper, such as drainability and strength [6].

The term cellulase actually refers to a complex of three hydrolytic components, recognized on the basis of substrate specificity or mode of action: It is generally accepted that cellulose degradation by cellulases is initiated by endoglucanase randomly cleaving internal glucosidic bonds along the length of the cellulose

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chain in the amorphous regions, decreasing the DP of the substrate. Then the newly created chain ends become available sites for cellobiohydrolase action. Cellobiohydrolases are processive enzymes that initiate their action from the ends of the cellulose chains, producing primarily cellobiose that is then released to solution. They attack the crystalline parts of the substrate and decrease the DP of the substrate, albeit slowly. The β-D-glucosidase or cellobiase converts cellooligosaccharides and cellobiose to glucose.

Jackson et al. [7] suggested that enzymes can either flocculate or hydrolyze fines and remove fibrils from the surface of large fines. The enzyme aided flocculation occurs when a low enzyme dosage is used. In this case, fines and small fiber particles aggregate with each other or with the larger fibers, decreasing the amount of small particles in pulp and consequently improving pulp drainage. On the other hand, at higher enzyme concentration, flocculation becomes less significant, and hydrolysis of fines begins to predominate.

It has been also shown that although enzymatic treatment of fibers decreased the amount of amorphous and gel-like polysaccharide layer on the surface, it did not affect the amount of fines [8]. On the other hand, enzymes may behave in a manner similar to retention aids and polymers facilitating the flocculation of the small fiber particles [9].

Several commercial cellulase enzymes are available which claim to improve the drainage of secondary fibers. But using mixtures of cellulases can be disadvantageous for certain pulp properties. When applying cellulase enzyme mixtures, identification of the key component, responsible for the required effect on pulp and paper properties, is required. Studies with pure cellulose have shown that the rate of hydrolysis of amorphous cellulose is five to thirty times higher than that of crystalline cellulose [10].

Therefore, further knowledge on the complex "fiber enzyme" interactions and more data on the effect of enzymatic treatments on the pulp and paper properties is required to develop a rational design of enzymatic fiber upgradation. The following research investigates the impact of three different cellulase enzyme products on the structure, drainage, viscosity and paper strength for recycled fiber based pulp furnish. The present studies have shown that it is possible to achieve significant freeness increase by judicious selection of the enzyme component, its dosage and the duration of treatment, while still not causing unacceptable levels of degradation to the fibers. Scanning electron micrographs of enzyme treated fibers were also examined to help better understand the mechanisms behind enzymatic fiber modification. The crystallinity index and apparent crystalline size for untreated and enzyme treated pulps were analyzed by X-ray diffraction.

## Experimental

### *Cellulosic pulp*

Wood-free bleached recycled pulp constituting sorted office pack, coated book stock and old record was procured from a recycled fiber based paper mill in Northern India. The cationic charge demand and zeta potential of the bleached recycled pulp was 7.05 μequiv./L and −21.9 mV, respectively. About 200 mL pulp slurry (0.33% consistency) was filtered through 200 mm screen and 10 mL of the filtrate was taken as the sample. The charge was measured on Mutek PCD 03 pH Particle Charge Detector and the sample was titrated with cationic polymer to neutralize the charge. About 500 mL pulp slurry (0.33% consistency) was taken and mixed thoroughly before measurement of zeta potential. The zeta potential of the pulp slurry was measured using Mutek SZP 06.

### *Cellulase enzyme products*

The three different commercial enzymes preparations used for this study were procured from Novozymes (Denmark) and Dyadic (USA). While the three enzymes contain both major classes of cellulases, they differ significantly in their relative activity of endoglucanases, exoglucanases (cellobiohydrolases) and activity shown relative to degradation of filter paper (denoted as FPase activity). The enzyme preparations were characterized by their activity against standard substrates purchased from Sigma Aldrich, USA. The activities are presented in international units (IU) per milliliter of enzyme preparation. Fibercare D (denoted as Endoglucanase, EG) and Fibercare R (denoted as cellulase mix A) from Novozymes, Denmark were engineered blend of mainly endoglucanases with side activities of cellobiohydrolase and mixed blend of both endoglucanase and cellobiohydrolase enzymes components in sufficient quantity, respectively. Fibrezyme LBR (denoted as cellulase mix B) from Dyadic, USA was a natural blend of enzymes having more cellobiohydrolase activity.

### *Characterization of enzyme products*

The endoglucanase activity of enzyme preparations was determined using sodium carboxymethyl cellulose (CMC) as a substrate [11]. 0.5 mL of suitably diluted enzyme solution was incubated together with 0.5 mL of 2% CMC solution in sodium phosphate buffer (0.05 M, pH 7.0) for 30 min at 45 °C. The enzyme activity was expressed in IU/mL.

The exocellulase (cellobiohydrolase, CBH) was determined with p-nitrophenyl-β-D-glucoside (pNPG) (Sigma Aldrich, USA) as a substrate [13]. Aliquot of 10 mM substrate stock solution (0.05 mL) was mixed with 0.85 mL of 0.1 M acetate buffer, pH 5.0, and preheated at 40 °C for 5 min. The enzymatic reaction was started by the addition of 0.1 mL of suitably diluted enzyme and preheated at 40 °C for 5 min. After exactly 10 min of incubation of the mixture at 40 °C, the reaction was stopped by the addition of 0.5 mL of 1 M sodium carbonate solution. Then the absorbance at 400 nm was measured on a spectrophotometer against a substrate blank that was prepared and incubated in the same way as the sample with enzyme, except the acetate buffer (0.1 mL) was added to the blank instead of enzyme. The quantity of p-nitrophenol released was determined using its extinction coefficient (18,300 $M^{-1}$ $cm^{-1}$), and then the enzyme activity was calculated.

The FPase activity was determined by incubating 0.5 mL of suitably diluted enzyme with 50 mg of Whatman No. 1 filter paper of 1 cm × 6 cm size [11]. After 60 min of incubation at 50 °C, the reducing sugars were measured in the supernatant by dinitrosalicylic acid procedure [12], with glucose as a standard. The activity was expressed in FPU/mL.

### *Enzyme treatments*

All enzyme treatments were done at 4% consistency, 45 °C and pH ∼ 7. Pulp suspension was warmed up to desired temperature and the pH was adjusted by addition of aluminum sulfate, which is used as retention aid and flocculating agent in pulp and paper industry. The enzyme based products were added at varying dosages ranging from 0.01 to 0.025%. Reaction mixtures were continuously stirred using an IKA laboratory stirrer at 500 rpm and incubated with the pulp slurry for 30 minutes prior to filtering the slurry through a Buchner funnel using a Whatman no.1 filter paper. The filtrate was collected and amount of reducing sugar (as glucose equivalents) released from pulp was determined spectrophotometrically at 540 nm according to the DNS method [12]. Then the enzyme reaction was stopped by keeping the pulp at 90 °C temperature for 10 min. The pulp was then washed thoroughly.