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Enzymatic Deinking: Effectiveness and Mechanisms

T. Welt and R.J. Dinus

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ENZYMATIC DEINKING: EFFECTIVENESS AND MECHANISMS

Thomas Welt and Ronald J. Dinus
Institute of Paper Science and Technology

SUMMARY

Enzymatic deinking represents a new approach for processing waste paper into quality products. The objectives of the present research were to clarify the role of factors affecting effectiveness of cellulase treatment, and to identify the mechanisms underlying ink removal. A commercial cellulase preparation was used to deink a highly crosslinked soybean oil-based ink from a newsheet consisting of 17% semibleached kraft pulp (SBKP) and 83% thermomechanical pulp (TMP). Ink detached via enzymatic treatment, water controls, and conventional chemical deinking was removed from pulp suspensions in a standard laboratory flotation cell. Effects of various process parameters were investigated in fractional factorial trials. Effectiveness was evaluated in terms of handsheet brightness, residual ink counts, and ink areas before and after flotation.

Several process parameters proved significant, but the most important were enzyme concentration and disintegration time. Cellulase treatment increased brightness and reduced ink counts and areas to values significantly lower than those of water controls. Deinking results, however, did not match those obtained via chemical deinking.

Apparently enzymatic treatment fostered removal of large ink particles by hastening and increasing the separation of fibrous materials covered by large areas of ink. Trials with unprinted paper confirmed that cellulase treatment accelerated separation of fibrous materials. Scanning electron micrographs, prepared after treatment of pure SBKP and TMP papers, showed that SBKP fibers were most affected by cellulase treatment. Some localized degradation was observed, but external fibrils were not removed with any great frequency. Effects of cellulase treatment on TMP fibers were lesser, and restricted primarily to occasional shortening and/or removal of fibrils. Similar effects were noted in photomicrographs of fibers from newsheet.

Based on these findings, the primary role of cellulases in deinking appears to involve separation of ink-fiber agglomerates, and simple dislodging or breaking of ink particles as fibrous materials separate in response to mechanical action during disintegration. Understanding the role of cellulases in deinking, nonetheless, requires clear appreciation of their effects on the different types of fibers present in waste paper. Although not investigated during the present research, fines of both pulp types are also involved by virtue of their significant presence in ink-fiber agglomerates. Given their high specific surface area, fines are subject to preferential attack by cellulases. The role of fines in enzymatically enhanced separation of ink-fiber agglomerates is the subject of ongoing research.

1. Introduction

The use of waste paper as raw material for papermaking has increased dramatically over the last decade, and is expected to continue increasing rapidly through the turn of the century. Profitable conversion of this resource into quality products demands an effective and efficient means for removing contaminants; inks being one serious problem. According to Jaakko Pöyry, worldwide deinking capacity will rise to 31 million tons by 2001, with particularly large expansions for newsprint, printing, and tissue grades (1). Enzymatic deinking may help meet such goals. Useful enzymes, e.g., cellulases, are now available in larger quantities and at lower cost than in the past (2). Results from laboratory, pilot plant, and mill trials appear promising, with enzymatic treatment yielding residual ink areas on a par with or better than those produced by chemical treatment (3).

1.1 Enzymes and Deinking

Enzymes can be used to attack either ink or fibers. Lipases and esterases are used to degrade vegetable oil-based inks. Pectinases, hemicellulases, cellulases, and lignolytic enzymes are believed to alter fiber surfaces or bonds in the vicinity of ink particles, thereby facilitating ink removal by washing or flotation.

Research on enzymatic deinking largely has focused on cellulases. These enzymes are components of large systems in lignocellulolytic fungi and bacteria that hydrolyze cellulose to water-soluble sugars. The nature and action of these and related enzymes have been summarized by Wood and Garcia-Campayo (4), by Eriksson (5), and by Eriksson et al. (6). Hydrolysis of crystalline cellulose requires a three-part system comprised of endo-1,4-β-glucanases, exo-1,4-β-glucanases, and 1,4,-β-glucosidases. Endoglucanases hydrolyze amorphous cellulose and soluble derivatives by randomly splitting internal β-1,4-glucosidic linkages along cellulose molecules. Products include glucose, cellobiose, and other oligomers. Exoglucanases hydrolyze cellulose molecules from the nonreducing end and release glucose or cellobiose units. Glucosidases degrade cellobiose and other oligomers into glucose monomers (6, 7).

1.2 Enzymatic Deinking - Possible Mechanisms

According to a recent review (3), cellulase activity releases ink particles into suspension from fibers and/or fines and reduces ink areas by one or a combination of mechanisms:

1. Enzyme attack fosters disaggregation of ink-fiber complexes during pulping (8), thereby reducing the number and size of residual ink spots.
2. Enzymes attack at sites where ink is bonded to fibers, thereby freeing ink particles from individual fibers (9).

This paper summarizes recent research on the use of a commercial cellulase preparation to remove a highly crosslinked soybean oil-based ink from a typical USA newsheet. This combination of ink, paper, and enzyme facilitated the investigation of underlying mechanisms as well as process effectiveness. The present paper concerns ink removal as affected by

enzymatically enhanced fiber separation. Future publications will deal with ink removal from individual fibers and the role of fines.

2. Materials and Methods

2.1 Enzymes

The cellulase preparation used in this research, GC Cellulase (GCC) was donated by Genencor International, Finland. GCC is a mixture of several cellulases derived from a selected strain of *Trichoderma reesei*. The preparation also contained some hemicellulases. Filter paper, carboxymethylcellulose (CMCase), and xylanase activities as well as preferred temperature and pH ranges were provided by Genencor International (Table 1). GCC was stored at 4°C until used in experiments at various weights per 50 g of ovendry waste paper (%w/w).

Table 1: GCC activities (optimal pH range 4.8-6.0, optimal temperature range 45-55°C).

Enzyme Preparation	Selected Activities		
	Filter Paper [U/ml DNS]	CMCase [U/ml DNS]	Xylanase [U/ml DNS]
GC Cellulase	96	4100	2900

2.2 Pulp and Paper Furnish

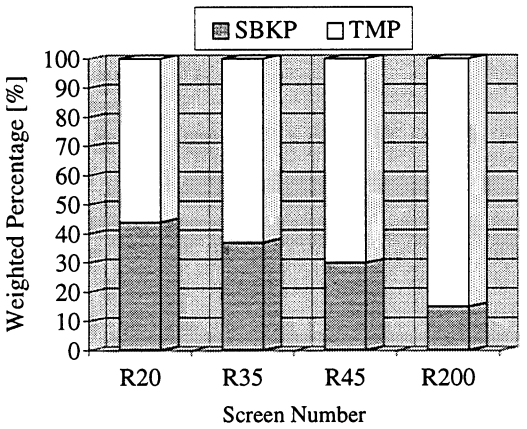
Newsprint, provided by Bowater Inc. (Catawba, SC), consisted of 17% semibleached kraft pulp (SBKP) and 83% thermomechanical pulp (TMP). Up to 29% of the fiber was "broke," i.e., waste made and reused during papermaking. Chemicals added during manufacture were a dual-polymer retention aid, alum for pH and pitch control, and red and blue dyes for shade adjustment. Reported basis weight was 48.8 g/m^2. This paper, printed and unprinted, was used as "waste paper" throughout the research described herein.

Fiber length distributions (TAPPI Method T 233 cm-82) and weighted percentages (TAPPI Method 401-om 93) of SBKP and TMP fibers are given in Table 2 and Figure 1, respectively.

Table 2: Bauer-McNett fiber fractions of experimental pulp and paper materials.

Screen Number	R20	R35	R45	R200	-R200
Waste Paper (%)	23.2	10.9	14.8	22.3	28.9
SBKP Paper (%)	68.0	11.2	10.8	7.4	2.6
TMP Paper (%)	31.6	14.4	16.2	18.7	19.1
SBKP (%)	64.7	11.3	10.1	8.3	5.7
TMP (%)	23.5	8.6	13.7	19.3	34.9

Figure 1: Pulp distribution of the two pulps in the waste paper as a function of Bauer-McNett fiber fractions as measured in accordance to TAPPI Method 401-om 93.



Samples of pure SBKP and TMP were also provided by Bowater Inc. These were used to make 100% SBKP and 100% TMP papers on a Formette Dynamique sheet former, with subsequent wet pressing and drying using a laboratory rotary dryer can. Target basis weights were 48.8 g/m^2. Fiber length distributions are given in Table 2.

2.3 Ink

Soybean oil-based offset ink (Process Black, SC-3152, Wikoff Color Corp., Atlanta, GA) was used due to growing worldwide usage of such inks (10, 11). Unlike mineral oil-based inks, vegetable oil-based inks can dry rapidly and thoroughly, giving significant rub and smudge resistance (12). Dryers in the formulation promoted crosslinking and may have fostered formation of covalent bonds with cellulose (13, 14). The ink produced high contrast, rub-resistant print, a challenging venue for gauging the effectiveness of and mechanisms underlying GCC deinking.

Unprinted waste paper was handcut into 28x43.2 cm sheets, and printed with the aforementioned ink on a sheet-fed Heidelberg GTO offset press (Georgia Southern University (GSU), Statesboro, GA). Ink application per sheet approximated amounts used on commercial newspapers. Each printed sheet was covered with the same image, a random text sample from GSU. Printed and unprinted samples were stored for 3 months (15) under TAPPI standard conditions (23°C and50% relative humidity) to limit age-related variations.

2.4 Equipment

Paper samples were disintegrated in a laboratory standard pulp disintegrator (NORAM) at 3000 rpm. Temperature and pH were measured before and after disintegration, with adjustments made as needed. Consistency was 3%; higher consistencies gave poor disintegration.

Flotation experiments were performed in a laboratory-scale device (Eimco/Wemco Process Equipment Center, Salt Lake City, UT). Operating conditions were air flow 5 l/min.; stirrer speed 900 RPM; sample weight 29.5 g O.D.; consistency 0.8%; time 7 min.;

temperature 40°C; and pH 5.0. Froth accumulating on the surface of the fiber suspension was constantly removed. Pulp suspension lost with the froth was replaced with water, preadjusted to pH 5 and to 40°C temperature.

2.5 Enzymatic Deinking

Factors likely to influence the effectiveness of enzymatic deinking were identified from the literature, the GCC supplier, and preliminary research. These were evaluated in a series of fractional factorial-designed experiments. Included were enzyme concentration, reaction time, disintegration time, time of surfactant addition (DI-600; nonionic surfactant), consistency, pH, and temperature. Response variables included brightness before and after flotation; ink count and area before and after flotation; yield after flotation; and reducing sugars produced by enzymatic hydrolysis.

The statistical significance of main effects and interactions was evaluated via analysis of variance. Linear regression was used to identify factor levels useful and practical for effective deinking. Analysis of variance was performed with SAS software (16), and all tests of significance were made at the 0.05 level of probability.

Process steps for enzyme deinking are outlined below and summarized in Figure 2. For each experiment, approx. 50 g of O.D. printed paper (9 printed sheets) were torn into 2 to 3 cm square pieces by hand and placed into the pulping container. Consistency was adjusted to 6% with distilled water preheated to the selected temperature. Dilute sulfuric acid (O.2N) or sodium hydroxide (0.2N) was used to meet the pH requirements of the particular experiment. All trials involved a 30 min. soak in water at the selected temperature and pH. Before enzyme addition, the pH was measured and, if necessary, readjusted to the desired level. Predetermined weights of GCC were diluted 1:100 and, depending on the experiment, added to a solution containing the surfactant. This solution was then immediately poured into the pulping container, thereby decreasing consistency to 3%. The suspension was mixed for 1 min. and GCC then allowed to react for varying time periods before disintegration for various times. A small pulp sample was taken from the suspension after disintegration and immediately filtered. The filtrate was frozen and later used to document the reducing sugar amounts produced by enzymatic hydrolysis. Approximately 10.5 g O.D. of the remaining pulp were diluted with water at 4°C to minimize enzyme activity and stored in a cold room for approx. 30 min. Consistency was checked before handsheets of 1 g were formed for brightness measurements and ink particle count. Another 29.5 g O.D. pulp was diluted, and the nonionic surfactant was added, if not already previously added together with the GCC preparation. The pulp suspension was then flotated under standardized conditions. After flotation, consistency was determined to ensure constant handsheet grammage of 1.0 g. Then, handsheets for brightness measurements and ink particle count were prepared.

2.6 Control Deinking Experiments

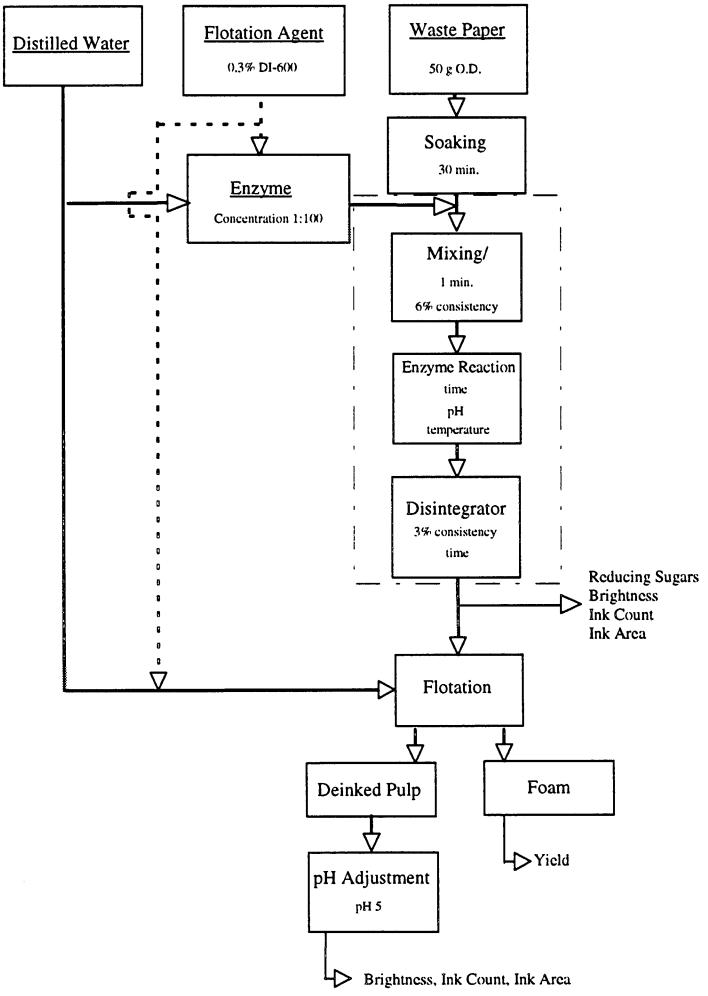
Control experiments were conducted in the same manner, except that GCC was not added. Also studied were effects of any enzyme-stabilizing additives in GCC and the potential surface activities of the enzyme protein. GCC was denatured by boiling for 20 min., and then

added as described above. Reducing sugar concentrations were quantified to determine extent of GCC activity in all experiments as well as to confirm that boiling had denatured GCC.

2.7 Chemical Deinking

Results from chemical deinking trials were used as standards against which to compare the effectiveness of enzymatic deinking. Procedures were similar to those for GCC (Fig. 2). Temperature, reaction time, and disintegration time were the same as for GCC. Disintegration was done before chemical reaction time, however, as deinking chemicals react more effectively with fibers (17). After soaking approx. 50 g O.D. paper for 30 min. at pH 4.5 and 50°C, chemicals were added as follows: 1% sodium hydroxide; 2% sodium silicate; 0.3% nonionic surfactant (DI-600); 0.3% DTPA (chelating agent); and 1% hydrogen peroxide. Percentages are on dry weight of fibers. Resultant pulps were sampled and tested in the same manner as those from GCC deinking.

Figure 2: Flowchart illustrating the course of enzymatic deinking experiments.



2.8 Deinking Effectiveness Based on Handsheets

Deinking effectiveness was evaluated by measuring brightness values, ink areas, and ink particle counts of sheets formed using a vacuum-assisted fritted glass KIMAX funnel and filter paper. Suspensions were mixed with a perforated stirrer (TAPPI Method T 205 om-88) during dewatering. Five sheets were formed from samples collected before and after flotation. Wet sheets were pressed between a blotter and one filter paper for 2 minutes at 420 kPa. After pressing, filter papers were removed, sheets placed on dry metal plates, and covered with new filter papers. Each plate was placed into a drying ring and dried according to TAPPI Method T 218 om-83.

Brightness measurements were performed on both sides due to differences between the top and bottom sides. Brightness values are the average of the two sides. Brightness was measured as per TAPPI Method T525 om-92.

Residual ink spot size, ink spot number, and ink areas were assessed with image analysis software Spec*Scan® (Version 1.2, Apogee Systems, Inc., Powder Springs, GA). Spec*Scan® is a windows-based system utilizing a flatbed scanner to digitize images. Scanner optical resolution was set to 600 dots per inch. Threshold value (100), white level (75), and black level (65) were chosen manually after comparing computer images to handsheets.

An area of 0.005232 m^2 was scanned on both sides of five handsheets. Ink particles were categorized according to size. Size intervals were preset with the software according to TAPPI methods T213 and T437. Only ink specks larger than 0.04 mm^2 (225 μm diameter) were counted because these represent the lower limit of the TAPPI method. Thus, all ink spots detected were within the visible range. Data on ink particles larger than 0.30 mm^2 were grouped for reporting purposes.

2.9 Yield

Yield was defined as the quantity of pulp obtained after deinking relative to starting mass. Froth overflowing during flotation was collected and filtered through preweighed filters on a vacuum-assisted Buchner funnel. The filtered fiber and ink were then dried and weighted. Yield was calculated as the difference between the starting and filtered masses divided by the starting mass, and then expressed as a percentage.

2.10 Reducing Sugar Determination - Somogyi-Nelson Method

Filtrates from all trials, whether enzymatic or otherwise, were analyzed to determine if treatment had hydrolyzed cellulose to reducing sugars. Filtrate samples were kept frozen until assay via the Somogyi-Nelson Method (18). Thawed filtrates were centrifuged at 5000 RPM for 30 minutes to minimize suspended solids. Sufficient supernatant was collected for triplicate assays of each sample. A standard curve was constructed using known quantities of glucose, and used to calculate glucose equivalents from reducing sugars in sample filtrates.

2.11 Scanning Electron Microscopy

A scanning electron microscope (SEM) was used to characterize the effects of various treatments on fiber surfaces. Samples were prepared for microscopy via critical-point drying. Intermediate solvents were ethanol and amyl acetate. The transitional fluid, liquid carbon dioxide (CO_2), was used to replace the intermediate solvent amyl-acetate. Critical-point dried samples were stored under vacuum in glass bottles until examined via SEM. Samples were sputter-coated on a Hummer V coater before inserted into the SEM. Coating time was set to provide approximately 20 nm thickness of gold/palladium.

Photomicrographs were generated with a JEOL JSM 6400 scanning electron microscope. Beam penetration was selected by setting the acceleration voltage to 5 kV. Working at a low accelerating voltage in the secondary electron mode allowed for visualization of fine fiber surface structures. The working distance was varied to optimize the depth of field.

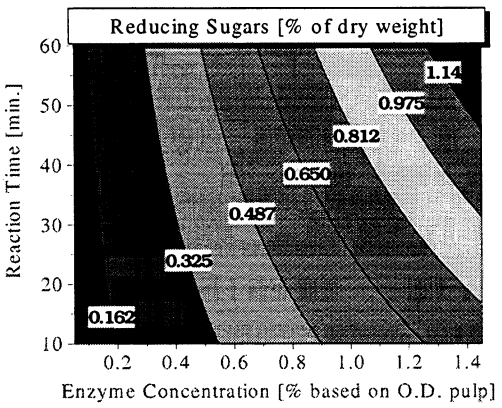
3. Results and Discussion

3.1 GC Cellulase Effectiveness

3.1.1 Cellulose Hydrolysis

Gauging GCC effectiveness in deinking first involved identifying factors most affecting the hydrolysis of cellulose to reducing sugars. GCC concentration, GCC reaction time, disintegration time, consistency during GCC reaction, and reaction temperature produced statistically significant effects. Significant interactions were also observed between GCC concentration with reaction time, disintegration time, consistency, and temperature. A particularly strong interaction was noted between GCC concentration and reaction time (Fig. 3), clearly demonstrating that these factors must be carefully controlled to prevent excessive fiber degradation. This seems especially critical in that the Somogyi-Nelson method measures only quantities of reducing sugar endgroups (19). Since cellulase mixtures are likely to produce monomers, dimers, and oligomers, actual hydrolysis of cellulose and thereby fiber degradation may be greater than indicated by values in Figure 3.

Figure 3: Reducing sugar production as a function of GCC concentration and reaction time.



Increasing GCC concentrations would be expected to promote formation of enzyme/substrate complexes (20, 21), thus leading to greater sugar production. Similar effects would be predicted, and were observed, for other significant factors, especially at higher GCC concentrations. Increased reducing sugar production across lengthening disintegration times indicates that shear forces during disintegration did not denature GCC.

Surfactant addition, before or after GCC reaction, did not produce significant effects. This outcome, however, may have been caused by the large statistical error term noted in the analysis of variance. Thus, the possibility of some small effect cannot be excluded. Surfactants have been shown to influence cellulase activity (22, 23).

3.1.2 GC Cellulase Deinking Effectiveness

Factors having significant effects on appearance of handsheets made before and after flotation were fewer in number than those affecting cellulose hydrolysis. GCC concentration, time of surfactant addition, and disintegration time significantly affected most variables used to quantify ink removal: brightness, residual ink count, and ink area.

As an example, the residual ink area declined with increasing enzyme concentration (Fig. 4). The effect was relatively small but real at disintegration times up to 20 minutes. Evidence from related trials indicates that further reduction would occur at concentrations beyond those shown in Figure 4. Using concentrations greater than 0.75%, however, seemed impractical in view of the potential for damage to fiber and paper properties (24). Occurrence of such effects seemed unlikely to aid in discerning mechanisms. Moreover, the GCC supplier suggested a concentration of 1% as the economical limit. Thus, a concentration of 0.75% was used in most subsequent trials.

Disintegration time produced the largest positive effects. Brightness gain and ink count reduction by flotation increased, while residual ink areas after flotation (Fig. 4) decreased with increasing disintegration time. Although this factor did not interact significantly with others, the joint impact of increasing enzyme concentration and disintegration time is noteworthy. At low concentrations and short times, handsheets made after flotation contained both numerous and large areas of ink (Fig. 5). Increasing levels of both factors gave not only lower ink counts of large ink particles but also large reductions in residual ink areas. Such findings suggest that a major effect of cellulase activity is separation of fibers covered by large masses of ink.

Figure 4: Effect of GCC concentration and disintegration time on residual ink area after flotation (main effect plots).

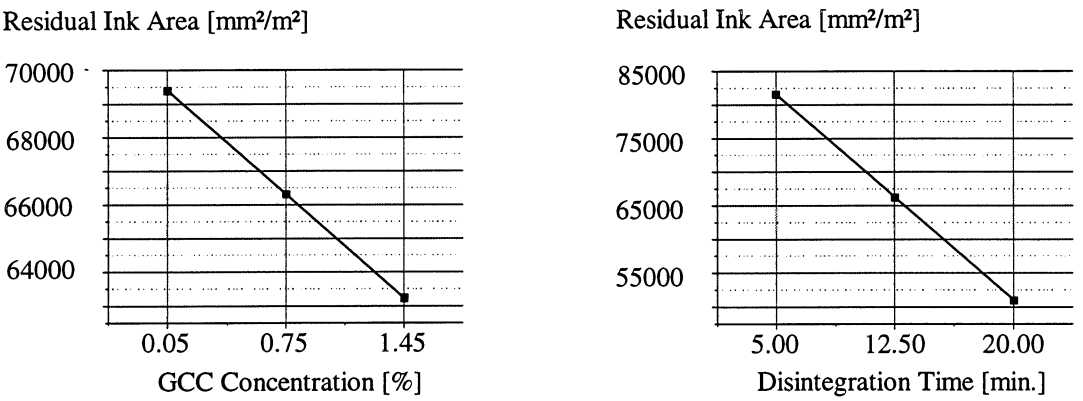
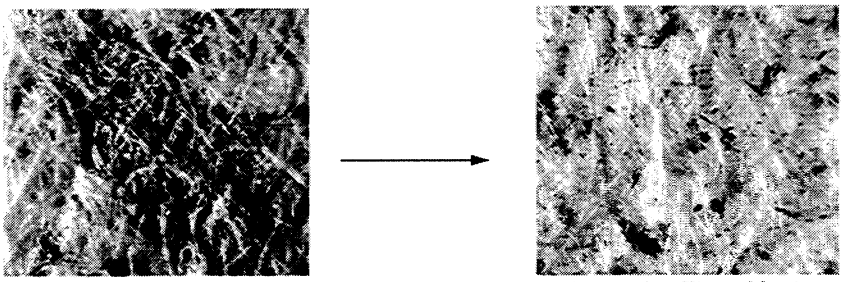


Figure 5: Effects of GCC concentration and disintegration time on visual appearance of handsheets made after flotation (40X).



Disintegration Time: 5 min.
GCC Concentration: 0.05 %.

Disintegration Time: 20 min.
GCC Concentration: 1.45 %.

The only significant interaction was that between enzyme concentration and reaction time. The ink area before flotation further decreased with increasing reaction time at higher enzyme concentration.

Yield reduction during enzymatic deinking was expected to be affected by both cellulose hydrolysis and loss of fibrous material during froth removal. Cellulose hydrolysis, as measured by production of reducing sugars, varied directly with GCC concentration, but accounted for only minor fiber loss. That is, reducing sugar production averaged only 0.56% of the starting mass. Most yield loss, an average of 18.1%, occurred during flotation. The magnitude of this loss component also increased slightly with GCC concentration.

Yield was also significantly affected by the timing of surfactant addition. Losses were lowest when the surfactant was added with GCC considerably in advance of flotation. Addition shortly before flotation, on the other hand, caused higher losses. Under these conditions, the surfactant may not have had sufficient time to interact with ink, thereby favoring foaming and aggravating fiber loss (25).

Comparing results from enzymatic deinking to those of chemical treatment showed that GCC effects were not as good as chemical. The final brightness of chemically deinked pulp (51.8% brightness) was 1.5 percentage points higher than that following GCC treatment. Ink count and ink area were 23.5% and 47.5% lower, respectively, after chemical deinking. Such findings demonstrate the difficult challenge posed by the ink and paper used in the present research.

3.2 Importance of GCC Activity

Experiments comparing responses to GCC with those of heat-denatured GCC and a water control showed that hydrolysis and reduction in the residual ink area occurred only in the presence of active GCC (Fig. 6). The minor differences between the effects of denatured GCC treatment and those of the control further indicate that any additives present in GCC or surface-active properties of the enzyme protein could not be responsible for the large reductions in ink area. Parallel findings were obtained in terms of brightness changes before and after flotation (Fig. 7).

Figure 6: Residual ink areas after treatment with GCC, heat-denatured GCC, and a water control (disintegration time 55 min.).

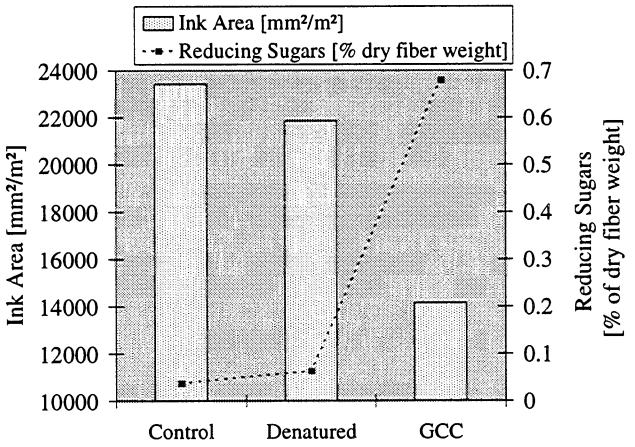
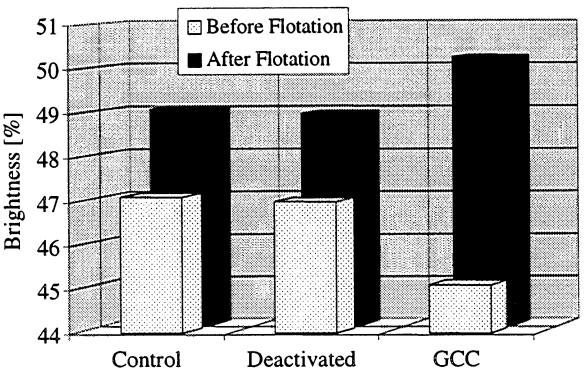


Figure 7: Brightness values before and after flotation following treatment with GCC, heat-denatured GCC, and a water control (disintegration time 55 min.).



3.3 Improved Effectiveness

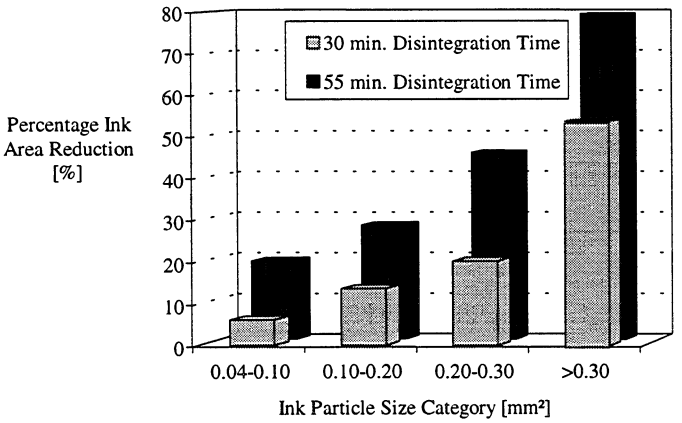
As noted above, GCC treatment produced statistically significant effects, but removal of the highly crosslinked ink was far below that produced by conventional chemical deinking. Since GCC concentration and disintegration time had the greatest impact on deinking effectiveness, their effects were further explored in subsequent trials. Primary emphasis was placed on disintegration time.

Other factors were maintained at levels deemed best in the aforementioned experiments. Reactions were performed at 50 °C, the temperature considered most favorable for enzymatic hydrolysis. Varying pH between 4.0 and 5.0 had produced only a nominal effect. Since the pH of waste paper suspensions routinely was 4.5-4.7, further evaluation and/or adjustment was not undertaken. The surfactant was added together with the enzyme preparation to limit fiber loss during flotation. Increasing consistency during GCC reaction improved hydrolysis, but increases beyond 3% caused problems with other aspects of processing. As a result, consistency was maintained at this level. Further testing of consistency levels during disintegration was also abandoned. Disintegration at levels exceeding 3% was ineffective in the disintegrator. Not being able to disintegrate at higher consistencies may explain why disintegration time was so important in the present research. Mechanical action is essential for effective deinking (26), and increasing disintegration time was the only means of obtaining sufficient mechanical action.

Increasing disintegration time from levels used initially to 30 and 55 min. yielded significant improvement. Not only were those extended times better than shorter ones (e.g., 20 min.), but 55 min. treatment significantly reduced ink areas below those of 30 min. (Fig. 8). Such findings underscore the importance of mechanical action as mentioned above and reported elsewhere (9, 27, 28).

Figure 8: Effect of disintegration time on residual ink area as a function of ink particle size.

$$\text{(Percentage Ink Area Reduction after flotation} = \frac{\text{Control} - \text{Enzyme}}{\text{Control}} * 100\ )$$



Reaction Conditions:
Reaction time: 40 min.
GCC concentration: 0.75 %
pH 4.5
Temperature: 50 °C

Also apparent in Figure 8 are distinct differences in the magnitude of effects among ink particle size classes. Differences between effects of 30 and 55 min. disintegration times were much greater in larger size classes. This pattern of differences may explain the enhanced

appearance of handsheets shown in Figure 5. That is, GCC treatment facilitates disaggregation of ink-fiber complexes during disintegration. Large areas of ink, e.g., print covering or overlapping several to many fibers, are dislodged and broken into smaller particles as fibrous materials are separated by enzymatic activity. Such findings also agree with past research in which cellulase activity decreased average residual ink particle size and yielded cleaner pulps (29, 30).

The differential reductions noted above did not cause increases in other particle size classes confirming that large ink areas were freed, broken into microscopic particles, and removed by flotation. Indeed, brightness measurements taken before flotation suggest that microscopic particles were generated (Fig. 7). Regardless of disintegration time, brightness values before flotation were lower after GCC treatment than in controls without GCC. Similarly, brightness values before flotation decreased with increasing GCC concentrations. Dependence of brightness on ink particle size has long been recognized (31). Moreover, treatment of unprinted waste paper with GCC had no effect on brightness.

That smaller differences occur in the smaller particle size classes (Fig. 8) further suggests that ink associated with only one or relatively few fibers is much less likely to be removed by enzymatic action. Collectively, these findings support the thesis advanced above that ink removal by cellulases is primarily associated with fiber separation.

Increased disintegration times, especially the 55 min. interval, clearly improved overall GCC deinking effectiveness. Nevertheless, ink removal efficiency characterized by final ink count, residual ink area, and brightness was still less than that obtained in parallel chemical deinking trials. Even with the improvement occasioned by lengthened disintegration time, enzymatic hydrolysis at acidic pH levels was no substitute for the major advantages of chemical deinking, e.g., fiber swelling and ink saponification due to alkaline conditions, as well as fiber bleaching due to the presence of hydrogen peroxide.

3.4 Likely Mechanisms

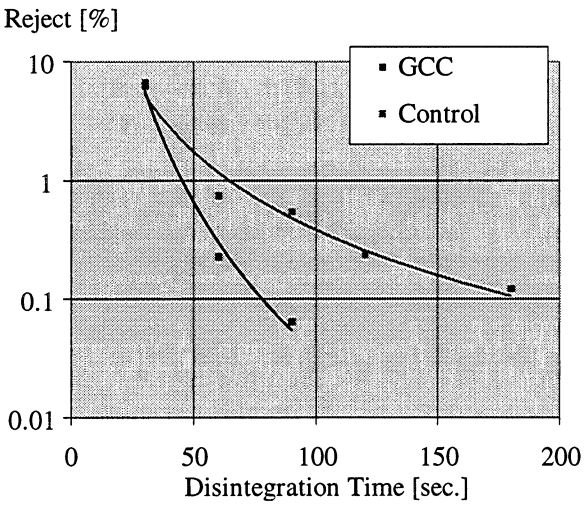
As described above, the major role of cellulases in deinking may be the weakening of bonds between fibrous materials. Ink particles, especially large ones, then might be dislodged and/or broken into smaller particles as fibrous masses separate in response to mechanical action during disintegration. That such a mechanism underlies removal of ink by enzymes has been suggested by other authors (8, 32). Also, a correlation between fiber separation and ink detachment is believed to occur in chemical deinking (33). To date, however, definitive evidence has not been published.

Consequently, experiments involving unprinted waste paper were performed to determine if GCC treatment increased and/or hastened fiber separation. Samples treated with and not treated with GCC were collected on 0.7 mm slotted screen after various disintegration times. GCC treatment clearly accelerated disintegration; the unprinted waste paper was reduced to individual fibers or very small masses within 100 sec. (Fig. 9). Samples not treated with GCC still contained significant numbers of fiber bundles after the same disintegration

time (Fig. 9 and 10). This effect of enzymes on disintegration time in deinking applications was also reported by Kim et al. (34).

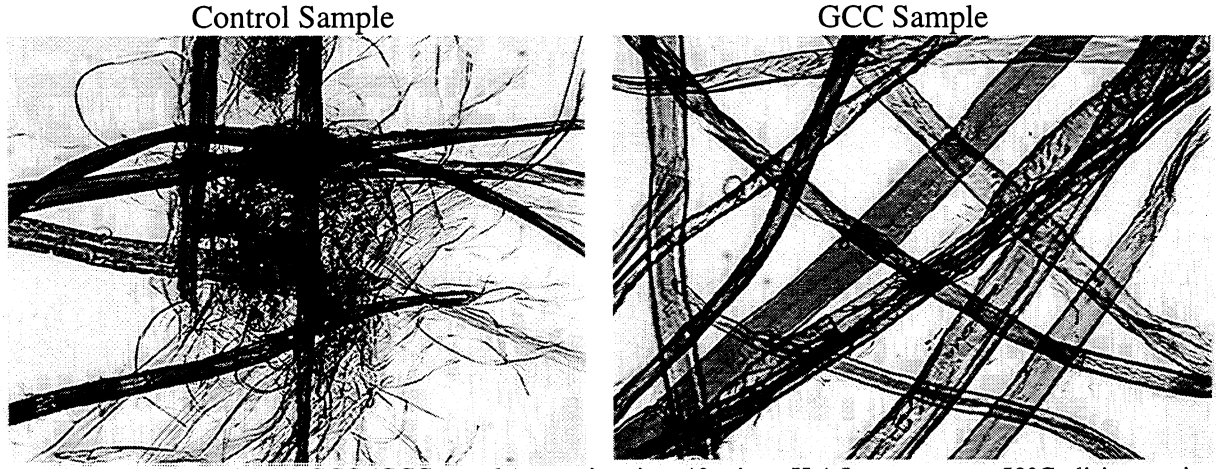
The ease with which unprinted waste paper disintegrated contrasted markedly with the difficulty observed with printed paper. Lengthy disintegration times (55 min.) were required for significant ink removal. Presumably, the highly crosslinked ink holds numerous fibers in place even in the presence of water and mechanical action.

Figure 9: Effect of GCC treatment on disintegration time of unprinted paper.



Shive Analyzer: 0.7 mm slotted screen
unprinted paper
GCC concentration: 0.75%
Reaction time: 40 min.
Temperature: 50°C
pH 4.5

Figure 10: GCC-enhanced disintegration of fiber agglomerates in unprinted papers (100X).



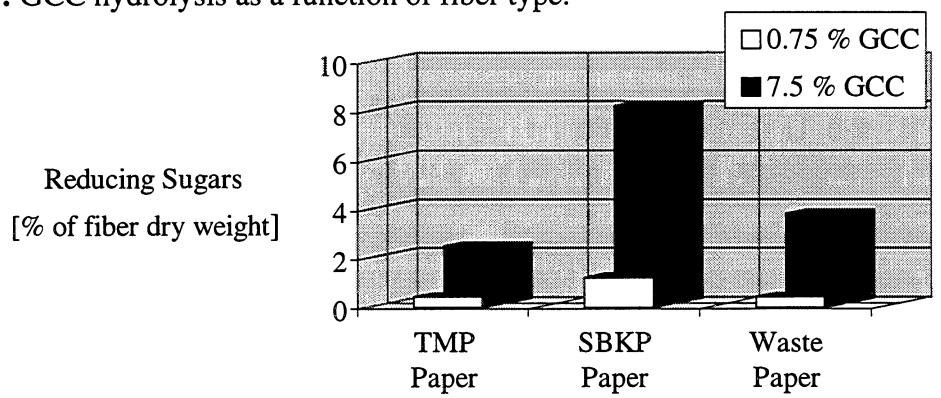
Reaction conditions: 0.75% GCC (GCC sample), reaction time 40 min., pH 4.5, temperature 50°C, disintegration time 100 sec.

To better elucidate causes of fiber separation, paper samples made from 100% SBKP and 100% TMP were prepared and individually treated with GCC. Disintegration times were 5 min., a duration more than sufficient to disintegrate unprinted waste paper (Fig. 9). Two

GCC concentrations were employed. The lowest (0.75%) had yielded significant deinking effects in initial trials. The highest (7.5%) was chosen to ensure contrasting results. Outcomes were assessed via measurements of reducing sugar production and SEM observations of fiber surface characteristics.

The degree of cellulose hydrolysis, as measured by reducing sugar production, varied directly with GCC concentration, but more importantly, also with paper type (Fig. 11). SBKP paper clearly was more susceptible to GCC attack than TMP paper. At the low GCC concentration, 1.25% of the SBKP paper mass was degraded as compared to 0.46% for TMP paper. At the high GCC concentration, reducing sugar production increased to 8.0% for SBKP paper as compared to only 2.3% for TMP paper. As expected, cellulose in the SBKP paper was much more accessible to GCC than in the lignin-rich TMP paper (35, 36). The importance of cellulose substrate accessibility in deinking was previously demonstrated in tests using various sizing agents (37). More research is necessary to clarify if the greater GCC effect on SBKP fibers during the deinking of printed waste paper will cause more efficient ink removal from chemical fiber surfaces than from mechanical pulp fibers.

Figure 11: GCC hydrolysis as a function of fiber type.



Reaction conditions: reaction time 40 min., pH 4.5, temperature 50°C, disintegration time 5 min.

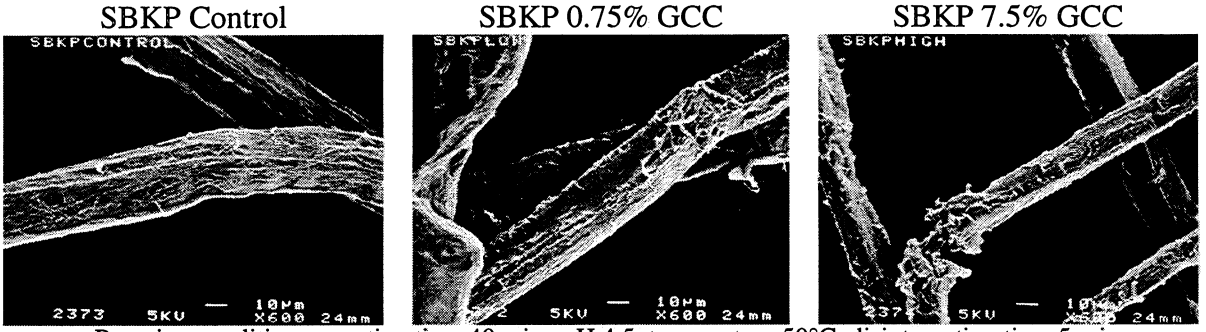
SEM observations confirmed results obtained from measurements of reducing sugar production. Fibers in SBKP paper treated with GCC were more affected relative to nontreated controls (Fig. 12) than those from TMP paper (Fig. 14).

Distinct changes to SBKP paper fiber surfaces occurred even at the lower GCC concentration. Fibrils on the surface of fibers, however, were not removed. Indeed, external fibrillation was noticeable on all fiber surfaces examined in this research (Fig. 12). Increase of fibrillation in response to cellulase attack was also reported by Grinberg et al. (38) due to partial digestion of fiber surfaces. Contradictory to an earlier hypothesis (39), these findings indicate that removal of surface fibrils by cellulases does not contribute to fiber separation in enzymatic deinking.

Photomicrographs of SBKP paper fibers also showed localized degradation along individual fibers (Fig. 12). Such degradation may be caused by preferential attack by

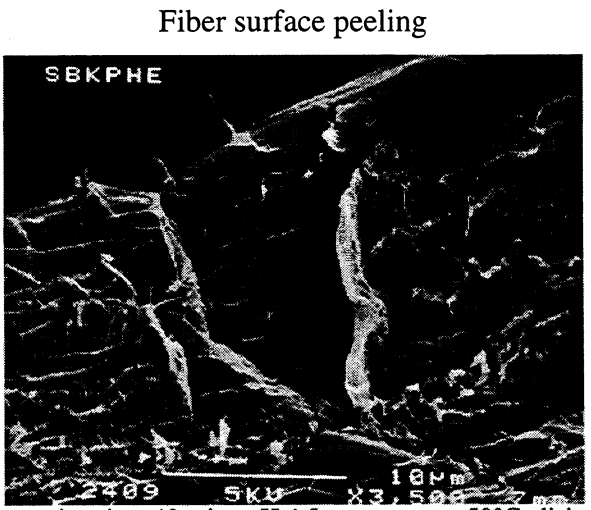
cellulases at structurally irregular zones, kinks, or nodes in fiber walls (40). Structural imperfections of this nature have been shown to occur during the blowing of pulp from digesters after kraft cooking. Preferential attack by endo-glucanase II has been postulated in studies of never-tried kraft pulp fibers (41). Some peeling of surface layers was also apparent at the high GCC concentration (Fig. 13). These structural changes may facilitate ink removal by increasing fiber flexibility and, correspondingly, efficiency of mechanical action. The localized fiber degradation phenomenon warrants further research not only from the standpoint of deinking effectiveness but also from that of potential reductions in paper strength (41, 42). Clearly needed are cellulases that facilitate deinking without causing serious localized degradation.

Figure 12: Effect of GCC hydrolysis on fiber morphology of SBKP.



Reaction conditions: reaction time 40 min., pH 4.5, temperature 50°C, disintegration time 5 min.

Figure 13: Detail of SBKP fiber surface damages caused by enzymatic hydrolysis.



Reaction conditions: reaction time 40 min., pH 4.5, temperature 50°C, disintegration time 5 min.

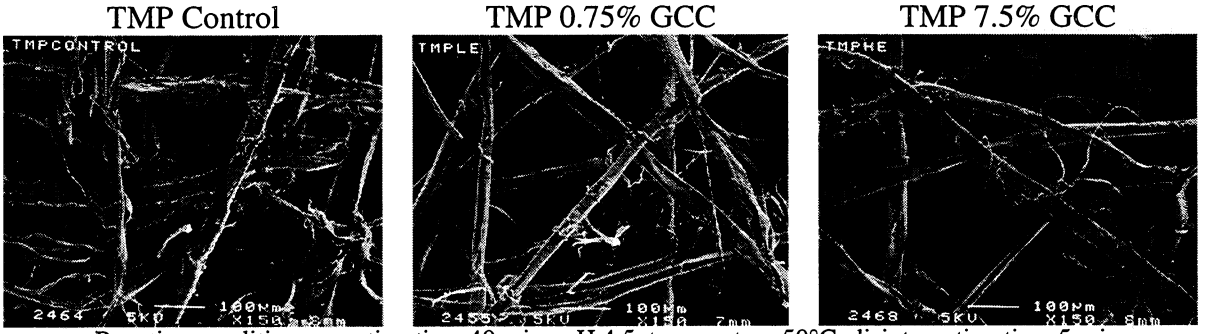
As noted above, GCC treatment caused less obvious changes to TMP paper fiber surfaces (Fig. 14). Regardless of GCC concentration, however, some fibrils were removed from fiber surfaces, and the effect was most noticeable at the high GCC concentration. In contrast to findings for SBKP, removal of fibrils from TMP fibers can therefore contribute to

enzymatically enhanced separation of fibrous materials. Smoothing of TMP fiber surfaces by cellulase activity has been demonstrated (43).

Low GCC concentration did not degrade TMP fiber surfaces as was observed for SBKP fibers (Fig. 13 and 14). At the high concentration, some areas of localized damage were apparent. Damage of this nature may explain reductions in tear strength observed in previous enzyme research (42).

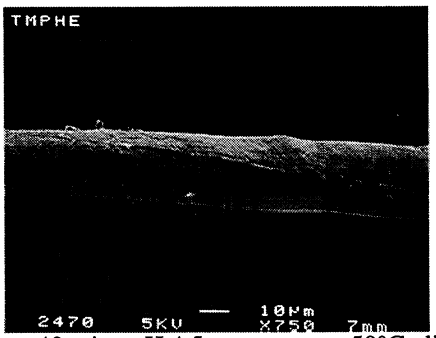
Hydrolysis of cellulose, as measured by reducing sugar production (Fig. 11), may also have occurred via preferential degradation of fines. Previous research on secondary fibers has shown that cellulases tend to attack fines in addition to surface fibrils due to the high specific surface area of such materials (44). Assuming that fines constitute a significant component of ink-fiber complexes based on the waste papers fines content of 28.9%, degradation of fines, whether from SBKP or TMP pulps in the present research, could play a significant role in the enzymatically enhanced separation of such complexes.

Figure 14: Effect of GCC treatment on morphology of TMP fibers.



Reaction conditions: reaction time 40 min., pH 4.5, temperature 50°C, disintegration time 5 min.

Figure 15: Effect of GCC hydrolysis on fiber morphology of TMP (7.5% GCC).



Reaction conditions: reaction time 40 min., pH 4.5, temperature 50°C, disintegration time 5 min.

Parallel experiments with unprinted waste paper yielded similar results. Surfaces of SBKP fibers were noticeably affected by GCC, but somewhat less severely than those from pure SBKP paper. Some removal of fibrils from TMP fibers was also observed. Thus, findings from tests with waste paper were consistent with those from papers made with the two pure pulp types.

4. Summary and Conclusions

In the present research, a commercial cellulase preparation was used to remove a highly crosslinked soybean oil-based ink from a newsheet consisting of 17% SBKP and 83% TMP. Investigation of various process variables indicated that enzyme concentration and disintegration time had the greatest impact on the effectiveness of enzymatic deinking. Brightness values and reductions in residual ink count and area obtained in response to cellulase treatment did not approach those achieved with conventional chemical deinking. Optimizing process conditions, however, resulted in significant improvement relative to water controls.

Enzymatic activity greatly reduced the number and size of residual ink spots, presumably by separation of large ink-fiber agglomerates. Much of the ink remaining after enzymatic treatment appeared to be smaller particles attached to individual fibers. Trials with unprinted paper confirmed that cellulase treatment significantly accelerated and eased disintegration. Thus, the major role of cellulases in deinking apparently involves weakening of bonds among fibrous materials. Ink particles, especially large ones, then are simply dislodged and/or broken into smaller particles as fibrous materials separate in response to mechanical action during disintegration.

Trials using a denatured enzyme preparation indicated that neither additives present in the preparation nor surface active properties of the enzyme protein had any significant effect.

Reducing sugar measurements and SEM micrographs collected during experiments with pure SBKP and TMP papers showed that SBKP fibers were a major site of cellulase attack. Enzyme activity caused some local degradation of SBKP fibers, but did not remove surface fibrils with any great frequency. Visible effects on TMP fibers were lesser, but some shortening and removal of fibrils were evident. Similar, though less dramatic effects, were observed in photomicrographs of fibers following enzymatic treatment of the unprinted newsheet.

Based on these observations, removal of fibrils from SBKP fibers cannot account for the observed enzymatic enhancement of disintegration. Cleaning of fibrils from TMP fibers, though limited in extent, may nevertheless play an important role. Clarifying mechanisms underlying the effectiveness of enzymatic deinking clearly requires understanding the effects of cellulases on each type of fiber present in waste paper.

Localized degradation of SBKP fibers may contribute to fiber flexibility and thereby facilitate disintegration. Further research is necessary, however, to determine the likelihood and extent of involvement.

Though not examined in the present research, fines fractions may be the primary determinant of deinking effectiveness. Regardless of pulp type, fines are constituents of ink-fiber agglomerates, and cellulases are known to preferentially attack fines due to their high specific surface area.

While this research accounts for the enzymatic removal of large ink particles, the role of cellulases in detaching smaller ink particles from individual fibers remains uncertain. Ongoing research is addressing this topic as well as seeking to clarify the role of fines.

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