Factors influencing enzyme deinking of recycled fiber

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ABSTRACT: Although there has been considerable research on enzymatic deinking, there is a fundamental lack of understanding of how mixtures of cellulases and hemicellulases can enhance the deinking of repulped paper products. In this research, cotton fabrics instead of paper were used as the substrate for enzyme deinking to understand better the influence of different conditions on the fiber surface. The results show that the cleavage of the cellulose polymers allows the deinking to take place. The accessibility of enzymes to the cellulose chains that bond the ink to the surface of the fiber was found to be a very important factor for deinking.

KEYWORDS: Cellulase, deinking, enzymatic activity, hemicellulase, models, reclaimed fibers.

For several years the use of hemicellulases, cellulases, or blends of hemicellulases and cellulases has been investigated for different purposes in papermaking *(1, 2)*. Better runnability of enzyme-treated stock on the paper machine and a reduction of the amount of bleaching chemicals are two established uses.

Recently, the use of enzymes to deink recycled fiber has been proposed and intensely studied *(1, 3–5)*. Many enzyme preparations have been investigated on different papers and inks, but there continues to be a lack of understanding of how these enzymes enhance the deinking of paper. To economically justify the use of relatively expensive cellulases and hemicellulases, a performance improvement has to be demonstrated; ultimately, this benefit has to be attributed to the specific activity of these enzymes. Since the main function of the enzyme is to cleave specific bonds between sugar units, an explanation as to how this activity can enhance deinking must be provided.

The objective of this research was to determine how enzymes enhance the deinking operation and under what conditions enzymes work best when performing this specific task. In this particular investigation, we de-emphasized the chemical conditions that best suit the activity of the enzymes, but tried to understand fully the fiber–ink–enzyme interaction.

Deinking can be divided into five different operations that may occur in partly consecutive, partly overlapping, stages *(6)*. These stages are disintegration, pre-cleaning, chemical or enzyme treatment, flotation and/or washing, and—finally—bleaching. A process analysis, however, cannot be done until the stock is made into a handsheet. The problem of understanding enzymatic deinking on paper lies in the fact that the enzyme treatment is preceded by disintegration and followed by a cleaning operation, which makes it very difficult to determine the exact role of the enzyme. The disintegration destroys the local arrangement of the printing ink on the fibers and leads to a complex mixing of fibers with residual ink, flakes of inks, and ink particles. The cleaning operation removes part of the ink that has previously been removed from the fiber. However, cleaning operations are sensitive to the conditions under which they take place, as well as the size of particles to be removed. Finally, the stock is reformed into a sheet, which leads to a complex filtration, where rotation in the axis of the fiber, the relative position in the z-direction of the fibers and ink particles, and the fil-

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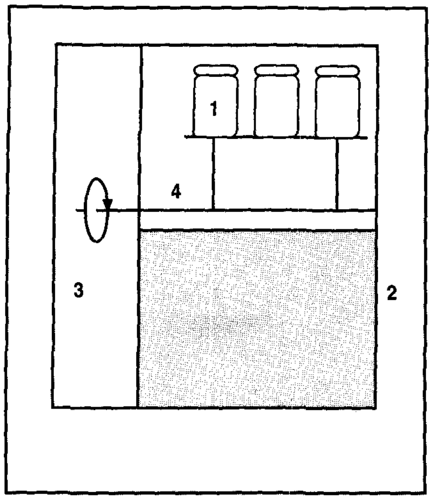
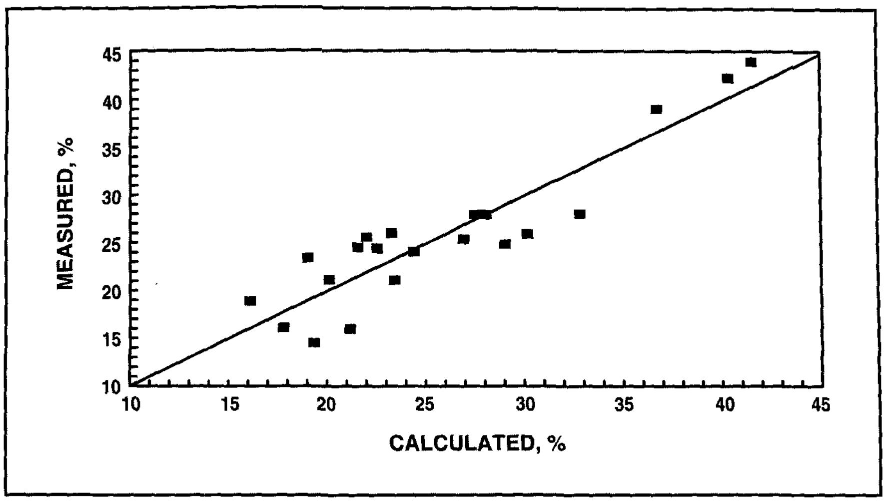
1. LAUNDER-OMETER: *1* Container with enzyme solution, fabric and steel beads, *2* Water bath (T = const), *3* Motor, *4* Axis



2. Ink removal calculated *vs* ink removal measured



tration conditions play an important role in determining whether an impurity affects the optical properties of the paper. An analysis of deinking efficiency, therefore, is reduced to a global measurement that can be influenced by many factors in the process.

We decided to work with printed cellulose fabric (cotton, rayon, or mercerized cotton) as a model because the disintegration, cleaning, and the filtration process in handsheet making can be avoided, and the local arrangement of the printing ink on the fiber is not destroyed. Thus, a measurement of the efficiency of the process can be obtained by image analysis by comparing the amount of points of a certain gray scale value before and after the treatment. We have shown previously *(7)* that the mechanical conditions under which deinking takes place are very important. The results published here show that there is a correlation between the amount of surface friction and the removal of ink. Beside this we investigated other factors that are relevant to enzymatic deinking.

Methods and materials

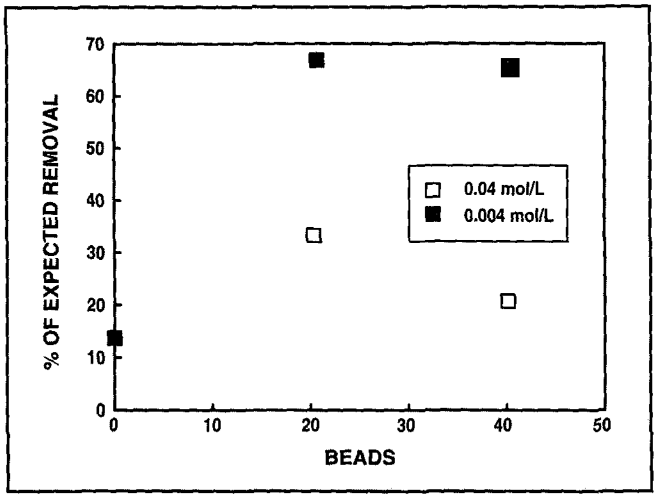
Most of the experiments were carried out with mercerized cotton because mercerization has some similarities to pulping. Recently, it was demonstrated that the rate of enzymatic hydrolysis is increased after mercerization *(8)*. All cotton samples weighed 0.9–1.4 g. The fabrics had a printed area of between 1% and 3%. The mercerized cotton was a woven fabric with a weight of 3.0 oz/yd^2 (102.4 g/m^2). The fabric had been desized, scoured, and bleached using standard procedures *(9)* for chemical preparation of woven cotton fabric prior to mercerization. Mercerization involves the treatment of cotton under tension with a concentrated (usually 25%) solution of NaOH at room temperature, and subsequent removal of the NaOH by rinsing with water while the fabric remains under tension *(9)*. This treatment produces cotton of high strength and luster as well as increased absorption power. A change in the crystalline structure from cellulose I to cellulose II accompanies mercerization, and the total amount of crystalline structures is reduced *(10, 11)*. To study the influence of the fiber structure, some experiments were carried out on bleached cotton. The bleached cotton fabric weight was 2.95 oz/yd^2 (100 g/m^2).

In some experiments, the enzyme was treated with $AgNO_3$ to inhibit its activity. Enzyme activity was determined using a standard procedure *(12)*. The ability of the enzyme to hydrolyze filter paper (Whatman No 1, Whatman, Kent, U.K.) was used as an overall measurement of its activity. This procedure was slightly modified in that the surplus Ag^+ was removed by either filtration or centrifugation after precipitation with $CaCl_2$ and before the color reaction used to detect the amount of released glucose was initiated. Both methods, centrifugation and filtration, resulted in a decrease of activity with increased concentration of $AgNO_3$.

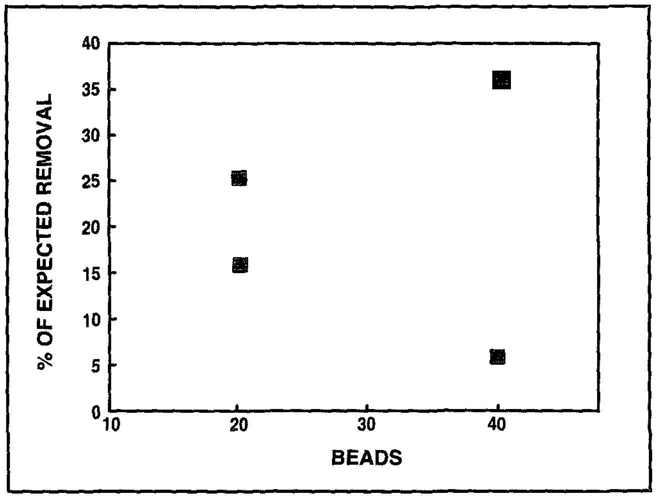
For another series of experiments, a surface sizing was applied prior to printing. A 6% starch solution of CATO 240, cationic waxy maize starch (National Starch, Bridgewater, NJ, USA), was used. The starch was applied using laboratory padder (McConnels, Rock Hill, SC), which is comparable to a size press, resulting in a pickup of 120% (7% dry).

For some samples of mercerized cotton, an internal sizing was applied. The internal sizing was applied using a similar process to the sizing of paper fiber. Three percent of an internal sizing agent(abietic acid, Hercules Inc.) relative to the fiber mass was dissolved in water and the fabric was added (consistency 1.5%). The solution was tumbled for 10 min in a

3. Deinking performance with enzyme deactivated by $AgNO_3$



4. Deinking performance with mechanical treatment before enzyme treatment



plastic container. Then, alum (Fisher Scientific) was added (6% relative to fabric weight) and the solution again tumbled for 30 min.

The fabric was then dried with blotting paper (James River Corp., Richmond, VA) and conditioned using TAPPI Method T 402 (standard conditioning and testing atmospheres for paper, pulp handsheets and related products). A Cobb test (TAPPI T 441) was performed to test the sizing. The absorbency of the fabric was dramatically reduced. Whereas the uptake of water by the sized fabric was about 10% of its weight in 2 min, the original fabric absorbed almost 20% of its weight in water in 1 min. With the unsized samples, the water was transported 3 cm beyond the wetted surface by capillary forces.

For some samples, a starch/latex coating was applied. The coating was made using standard procedures as outlined in *(13)*. The coating color had a solids content of 48%. It consisted of 100 parts clay (KO1 Huber Hydrafine 90, Huber Corp., Macon, GA), 18 parts starch (Penford 280, Penford Corp., Cedar Rapids, IA), 6 parts latex (General Latex 2V-3224), 0.3 part lubricant (HB 50, Union Carbide), and 0.1 part ammonia (Fisher Scientific). The coating was applied using Myron Rod No. 16. Due to the behavior of the fabric, it was difficult to apply the coating evenly. We decided therefore to reduce the changes in conditions and to increase the number of repetitions using the same conditions.

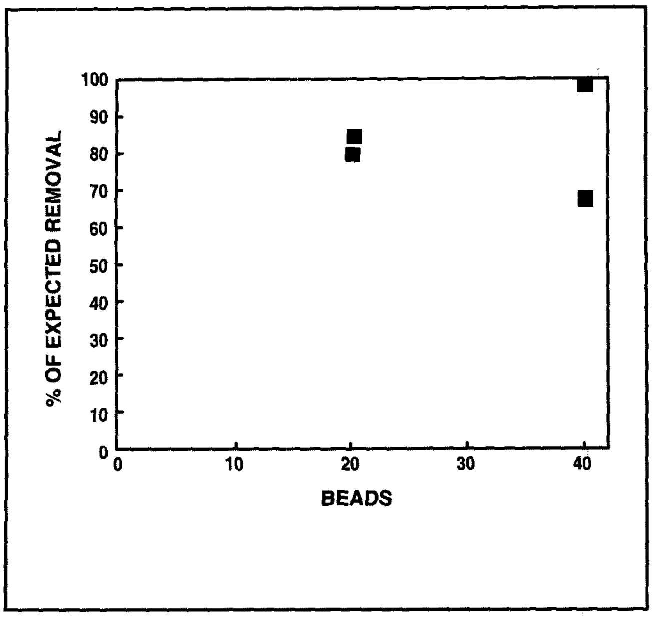
All samples were printed on a manual flat screen printing machine (Hopkins International Screen Printing Systems) with a commercially available, water-based, paper ink (Screen printing ink, Hunt Industries, Statesville, NC) using a vegetable gum carrier that, when dried, is water-insoluble. The pigment used is carbon black. The fabric was printed with repeated lines of alphabetical characters (Times Roman) with a size of 10 points. Each sample had an unprinted border of 5 mm around the periphery and four lines of characters. After the printing, the fabric was dried in one pass through an infrared oven (Precision Screen Machines, Inc., Hawthorne, NJ; power setting 100%, beltspeed 4). The samples were used within 4 days of printing.

For some experiments on mercerized cotton, the surface of the fabric was sueded prior to printing with a Wide Face Finishing Machine (Curtin Herbert & Co., Inc., Gloversville, NY). A 320-grit sandpaper was used at 1200-rpm cylinder speed and a fabric feed rate of 10.5 yd/min. The thickness of the fabric was 10 mil. For one sample, the gap was 5 mil; for two others, it was 6 mil. The smaller gap substantially disintegrated the fabric. Two samples were run using the wider gap. A subjectively, a noticeable difference in strength between these two samples could be observed.

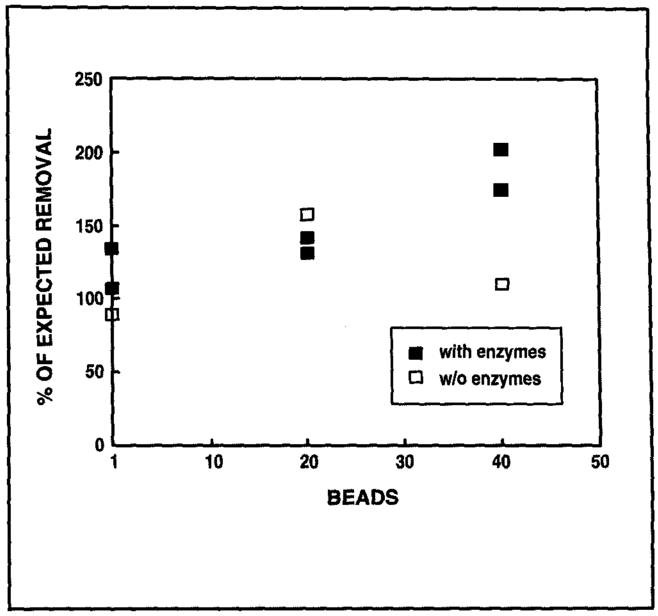
A LAUNDER-OMETER (Model LHT, Atlas Electric Devices, Chicago, IL) was used to perform the experiments (**Fig. 1**). A LAUNDER-OMETER is a device in which metal containers revolve around an axis at 40 rpm, thereby passing a water bath, set at a temperature of 40°C. Since the containers were only 50% filled, the process provided agitation, and fiber surface friction resulted. To increase the amount of friction, steel beads (weight: 1 g) were added to the containers.

The enzyme used was an experimental preparation containing both cellulases (3.9 U/mL CMCase activity and 28.7 filter paper units activity) and hemicellulases (385 U/mL xylanase activity) (see Ref. *5* for details of analytical procedures). All experiments were carried out at a concentration of 4 mL enzyme preparation/L distilled water. The temperature was maintained constant at 40°C and the total volume of the solution was 250 mL. The pH of these

5. Deinking performance with bleached cotton as substrate



6. Deinking performance with surface sized substrate



solutions was between 4.6 and 5.5. A series of experiments was also carried out with a commercially available enzyme preparation (Pergalase P40, CIBA-GEIGY Corp., Greensboro, NC).

Image analysis was performed on an HP Scan Jet Plus (Model 9195A) with an optical resolution of 300 dots/in. (dpi) interpolated to 600 dpi, and a custom-made file oriented image analysis program in the programming language C++. The image was thereby transformed into pixels having a gray scale value of 0 to 255, where 255 is white and 0 is black. The area covered by pixels with a gray scale value smaller than an arbitrarily set value (which we term the threshold) could then be calculated. The threshold in this research project was set at a gray scale value of 100. A comparison of measurements before and after treatment gives the number of black pixels removed. This can be considered as an indicator of the deinking efficacy. To lessen the variance of measurements and to obtain an estimate for the error boundaries of the image analysis procedure at the chosen resolution, duplicate measurements for each sample were carried out and the average was calculated. The generally applied principles for image analysis are outlined in *(14, 15)*.

Although not completely representative because of disintegration of the fabric at the borders, the weight loss of the fabric, as an overall indication of the enzyme activity, was measured. Before weighing the fabric, it was conditioned according to TAPPI Method T 402, Standard Conditioning and Testing Atmospheres for Paper Board, Pulp Hand Sheets, and Related Products.

Results

Deinking as a function of fiber surface friction and printing ink

As we reported previously in *(7)*, almost no deinking could be achieved in an experimental set-up with very little agitation. If, however, the LAUNDER-OMETER was used, an almost linear increase with time of deinking could be detected.

Next, a series of 21 experiments with mercerized cotton and an enzyme treatment time of 3 h was undertaken using the LAUNDER-OMETER. To provide additional friction, steel beads were added to the containers and the number of beads was varied from 0 to 40. The results could be correlated to a linear model in two variables (**Table I**). The regression was performed with a statistical data analysis program *(16)*. Both the amount of friction represented by the amount of beads and the reciprocal amount of ink on the fabric (measured by image analysis) proved to be significant variables. The resulting regression equation was:

$$Y = 35.77/X_1 + 0.25 * X_2 + 3.75 \qquad (1)$$

where

Y = amount of ink removed, % removal

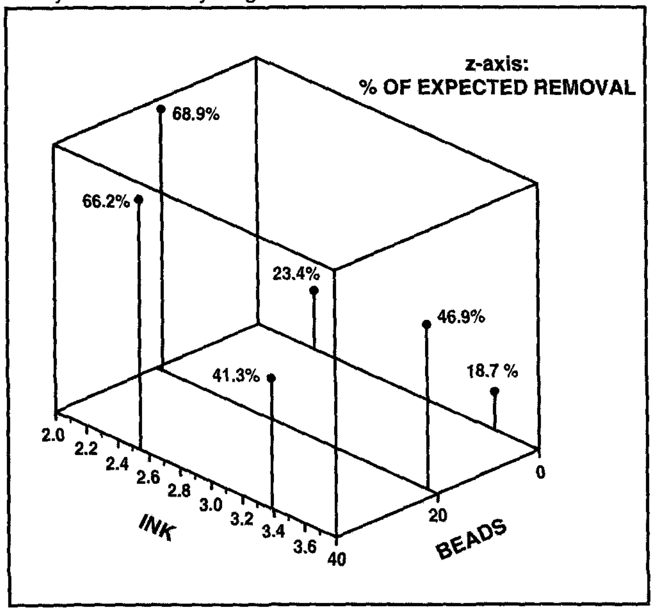
X_1 = initial amount of ink, % coverage

X_2 = number of steel beads.

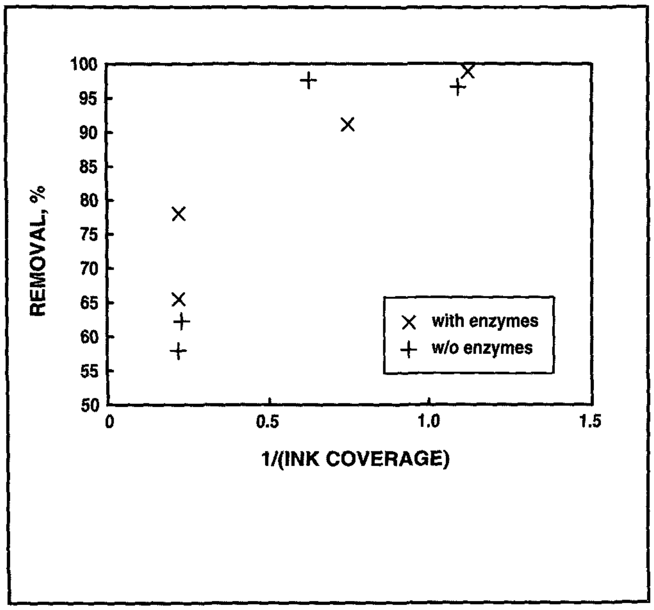
The parameters estimated by this regression of the enlarged data set agree well with our previous findings. The distribution of residuals for both variables and of measured points against calculated points (**Fig. 2**) does not cause the model to be rejected.

A statistical test of the model, such

7. Deinking performance of internally sized samples. Deinking efficiency is indicated by height in z-direction.



8. Deinking performance of coated fabrics



as an F-test, could not be performed because control of the amount of ink on the fabric was not good enough to allow reproducible experiments. However, the correlation coefficient of 0.84 leaves a probability of only 0.01% that the measurements are not in fact correlated. Therefore, we decided to adopt this model to test in further experiments. The model we developed indicates that both the amount of ink and the number of beads play an important role. This finding was also confirmed in subsequent experiments.

Table I shows that the expected error of the results is quite high. Although the error in the image analysis is quite high too, the error cannot be explained by simple measurement errors. We tried, therefore, to improve the model by also considering the shrinkage after treatment and the initial sample weight, but no correlation could be found. Although not correlated to the result, the different shrinkages on different samples, varying from 1% to over 10% for different samples, indicate that certain unmeasured properties of the fibers change within the fabric.

A few experiments, carried out with 60 beads, could not be correlated to the model as there was no increase in ink removal. This can be explained by the increasing interaction between the beads. The series of experiments using commercially available enzymes showed no significantly different results to the model base series.

Qualitative observations by microscope

Parallel to the quantitative image analysis made with the scanner, the printed characters were analyzed qualitatively with a microscope. The observations under the microscope revealed that the ink was removed only in locations exposed to fiber surface friction. The positions with locally high z-direction values (the direction perpendicular to the plane of the fabric) were almost totally deinked, whereas positions with locally lower z-direction values that were protected by fibers with higher z-direction values remained black. This is consistent with our general findings. The microscopic observations, however, were complicated by light reflection at the fiber surface.

Experiments using modified conditions: applied statistics

To test a number of possible factors which could influence the removal of ink, we designed an experimental strategy whereby the results of subsequent experiments could be compared to those of our model base series. Given the two variables, number of beads and initial coverage of ink, we calculated the ink removal we would most likely expect under the conditions of the model base series. The difference between this value and the actual measured value is likely to be distributed similarly to the residuals of the model, if the change in conditions had no effect on the system. Therefore, the sum of squares of this difference

$$SS_\Delta = \Sigma(Y_{mes} - Y_{cal})^2$$

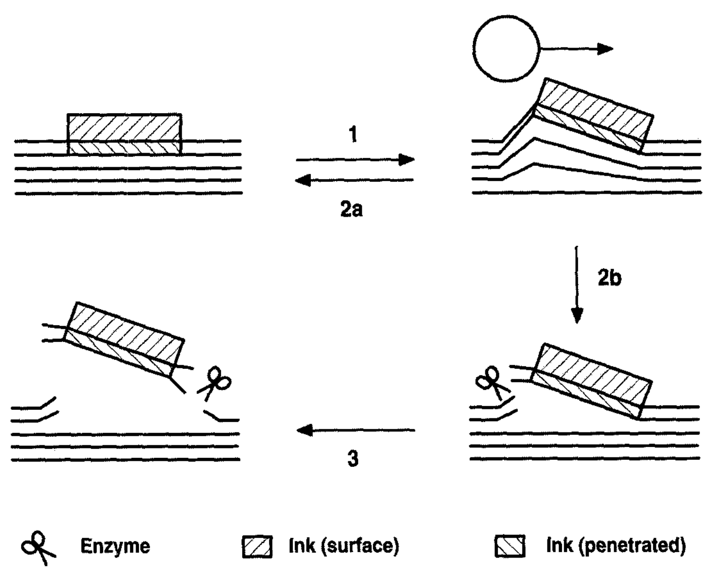
can be compared in an F-test to the variance of the model. The calculated F-value is then

$$F_{calc} = (SS_\Delta/m) * (SS_M/n)^{-1} \quad (2)$$

where SS_M is the sum of the squares of the residuals of the model, n is the degrees of freedom, and m is the degrees of freedom of the new series of experiments. This value is compared to a tabulated F-value of appropriate degrees of freedom. When F_{calc} is larger than the tabulated value, it is very likely that the variances differ *(17)*. This means that the probability is high that the new values do not represent the model calculated by the regression. Therefore, the changed condition had an effect on the results. Likewise, if the tabulated F-value is larger, no conclusion is possible. Additionally, the distribution of residuals was evaluated. If the new results fit the model well, the number of positive and negative residuals should be equal.

This procedure was used in all the experiments described below. The experimental conditions were the same as in a previous section (qualitative observations by microscope). All changes from these conditions are indicated. The results of the statistical analysis are summarized in **Table II.** The results are also presented in figures showing the measured removal in percent of the expected removal (as calculated by the model) versus the number of beads.

9. A model of enzymatic deinking: *1* Friction opens the fiber, *2a* Relaxation, *2b* Enzyme cutting, *3* Ink removed



I. Regression of data in Table I: parameter estimation, confidence intervals, and statistical data related to regression

	Coef.	*Std. err.*	*t*	*P>|t|*	*95% Conf. interval*	
1/ink (X_1)	35.77	4.44	8.061	0.000	26.15	45.08
Beads (X_2)	0.2500	0.0557	4.485	0.000	0.0174	0.4189
Constant	3.746	2.365	1.1584	0.131	-1.223	8.716

Number of obs. = 21
F(2,18) = 47.93
Prob > F = 0.0000

R-square = 0.84
Root Mean Square Error = 3.269
SS residual = 192.4

Reducing the enzyme activity

To determine whether the enzyme activity is the reason for the increased removal of ink, enzymes were inhibited with $AgNO_3$. Following the determination of the activity of the enzymes (filter paper activity) as a function of the concentration of $Ag+$, we decided to use two levels of inhibition, one in which the activity was reduced by roughly 50% (0.004 mol $Ag+$/L), and one in which almost no activity was expected (0.04 mol $Ag+$/L). For both series, the measured ink removal was much lower than expected (**Fig. 3**, Table II, experiment Ag). The experiments using the lower level of silver inhibition resulted in lower decrease of ink removal. It can, therefore, be concluded that the enzyme activity is responsible for the increase in removal of ink.

Enzymatic treatment after mechanical treatment

To investigate whether the surface friction and the enzyme treatment must be applied simultaneously to enhance deinking, the LAUNDER-OMETER was used without enzymes for the treatment of mercerized cotton. The enzyme treatment was then carried out after the LAUNDER-OMETER treatment in an experimental set-up providing little surface friction. The ink removal is significantly lower (**Fig. 4,** Table II, experiment EAT). Thus, it can be concluded that the enzyme activity must be applied at the same time as the surface friction.

Bleached cotton substrate

In the next series of experiments, bleached cotton was used instead of mercerized cotton. These experiments showed no statistically different result from our model (**Fig. 5,** Table II, experiment BC). However, the distribution of the residuals indicates a slightly smaller ink removal. To observe no differences between fibers was an unexpected result since there are considerable differences in the physical structures such as the crystallinity of the fiber. The fraction of crystalline cellulose decreases from bleached cotton to mercerized cotton. There are two possible explanations for the observed effect: a more open structure not only allows better access of the enzyme, but also a deeper penetration of the ink into the fiber; on the other hand, the interior of the fiber is more crystalline than the outer, amorphous surface. Since the attachment of ink mainly takes place at the surface of the fibrils, the differences in the crystallinity might not be of importance. This hypothesis is supported by Buschle-Diller *et al. (8)*. Their results show that the hydrolysis of bleached cotton proceeds as rapidly as the hydrolysis of mercerized cotton at the beginning, but later slows down.

A regression carried out with the experimental results of this series confirmed the linear correlation between ink removal and level of fiber friction, but correlation with the amount of ink was less obvious. Since the number of experiments in this series is quite small, the importance of this finding should not be overstated.

Surface sized substrate

Focusing now on sized fabrics and papers, a series of samples was used to study the deinking of a sized substrate where the printing ink is applied on top of the sizing. The resulting deinking was much higher than that expected (**Fig. 6**, Table II, experiment SS). There was a significant difference between the basic model series and the experiments with sized samples. An additional regression, contrasting the sized samples treated with enzymes to that of sized samples not treated with enzymes, showed no significant difference. It can, therefore, be concluded that the use of enzyme for the deinking of sized furnish does not lead to an improvement. This is consistent with the findings of others *(18)*, who found that desizing with amylases has to take place to allow the enzymes to remove protruding fibers from cotton in biopolishing.

Internal sized substrate

The enzymatic deinking of the internally sized samples was significantly lower than the removal of the model base series (**Fig. 7**, Table II, experiment IS). The Cobb test demonstrated that the wettability of the fiber is significantly reduced. It is, therefore, not surprising that deinking is reduced since the penetration of the enzymes into the sized surface and scission of the cellulose chains is hindered. Figure 7 shows that the difference between the expected value and the actual value appears to increase with the initial coverage of ink.

Surface coated substrates

As mentioned above, it was difficult to apply the coating evenly to the fabric. All experiments, therefore, were made using 20 beads to achieve significant results by replicating the experiments. However, deinking was much faster and the experiment had to be stopped after 1 h to be able to measure a substantial amount of remaining ink. **Figure** 8 shows a plot of the actual removal of ink for different samples. The coating resulted, therefore, in a very significant increase in the removal of ink. As in the case of surface sizing, there was no difference between experiments with and without enzymes. This again demonstrates that the accessibility of the actual fiber to the enzyme is a major factor in enzymatic deinking.

Substrate with sueded surface

The results in this section are ambiguous because the reproducibility of the sueded surface is difficult to achieve due to the small size and relative fragility of the fabric samples. The results, however, tend to indicate that a rougher sueding leads to a higher removal of ink (Table II). The reason for this could be that the ink is deposited mainly on the relatively easy to remove protruding fibers. It is worthwhile mentioning that, in one series, the removal was strongly negatively correlated to the size of the fabric sample. In a second series, the correlation was noticeable, but less obvious (**Table III**). This correlation could indicate that there is a competition between the removal of protruding fibers and deinking. Since there was no correlation to the fabric sample size in the model base series, it can be concluded that this competition is only important when the amount of protruding fibers is high. However, the existence of a competition between ink removal and cleavage of easily accessible chains is likely from biochemical considerations of enzyme/substrate binding as well as from our observations *(19)*.

Nevertheless, the amount of protruding fibers does not account for the experimental error. A series of experiments, where the protruding fibers were first removed by a 2.5 h enzyme treatment, showed no reduced error. It can be speculated that the error is largely due to the noncontrollable variables such as applied pressure and speed in printing.

Discussion

Overall, the experiments demonstrated that the enzyme preparations were able to improve ink removal. Experiments made with inhibited enzymes showed that the enzyme activity plays a key role in deinking. The accessibility of the cellulose chains for cleavage seems to play an important role, too. Shielding the fiber from enzyme attack reduces the deinking effect, whereas mechanical action drastically increases the deinking.

The experiments show that fiber surface friction plays an important role in the deinking of cotton fibers by enzymes. Experiments under conditions with very little or no fiber surface friction show no measurable ink removal. Our empirical model suggests that the efficiency of deinking increases linearly as the fiber surface friction increases.

General biochemical considerations suggest that there is an optimum fiber surface friction because of denaturation of the enzyme due to shear *(20, 21)*. However, practical experience in enzyme treatment of jeans fabric suggests that rather high shear stresses have to be applied to denature the enzyme.

The mechanical forces applied to the fabric in our experiments are much smaller than the force applied in a refiner. Still, the friction at the moment of the impact might be higher than that achievable by stirring. It is conceivable that a parallel refiner treatment might damage the enzymes, and thus reduce their beneficial effect. It seems, therefore, to be a crucial, yet difficult task to achieve friction of the appropriate magnitude on the surface of fibers.

Our findings concerning fiber surface friction are consistent with the research done on stone washing of indigo dyed cotton fabrics with enzymes and general surface treatment of cotton fabrics *(18, 22, 23)*. All three research groups reported that enzyme treatment has to be accompanied with mechanical action. The explanation for this is that small fiber ends protruding from the cotton yarn are weakened by the action of the enzyme, but mechanical action is needed to complete the process *(22)*. However, this does not explain why in our case, after up to 3 h of treatment with enzymes, no significant ink removal took place in a setting with little friction *(7)* although the weight loss indicated an almost linear removal of cellulose. In deinking, however, there is a slightly different situation in that the fiber is covered by a relatively thick layer of ink (d > 1 μm). This layer shields the fiber from enzyme attack. Thus, mechanical action must play a role in opening the cellulose structure and allowing the enzyme to cleave cellulose chains. It has been reported previously *(18, 22)* that additional friction can be provided by adding "tuff" stones to the enzyme treatment for denim stone washing. We found that the result of treatments with stones was comparable to our experiments made with beads; however, the results were highly unpredictable because of the irregular size and shape of the stones.

Figure 4 shows that fiber surface friction and enzyme cleavage of the cellulose chains by the enzyme have to be performed simultaneously to achieve ink removal. Evaluating all evidence on the behavior of enzymes found in our experiments, we suggest a model of elastic opening of the contact between the ink and the fiber followed by cutting of the anchoring fibers by enzymes (**Fig. 9**). The mechanical action leads to a distortion of the arrangement of the chains and increases the accessibility of cellulose chains that attach the ink particle to the fiber. At that point, the enzyme is able to cleave the glycosidic bond. This is consistent with the general finding *(24)* that only easily accessible cellulose chains can be cleaved by enzymes. When a sufficient number of these chains is cleaved, the ink particle is able to break loose from the surface and can then be removed by either mechanical action or additional enzyme activity.

The qualitative observation that only easily accessible parts of the cotton fabric were deinked raises, however, the question of whether complete deinking of paper is achievable in enzymatic deinking. Since the surface of the fiber is not completely smooth and the printing takes place using a liquid under pressure, it has to be expected that some of the ink will be attached in small grooves and,

II. Statistical tests for additional experiments

EXP	*DF*	F_{calc}	F_{tab}	α	N_n	N_p	
Ag1	3	49	5.01	99%	3	0	reject
Ag2	3	14.6	5.01	99%	3	0	reject
EAT	4	41.7	4.5	99%	4	0	reject
BC	6	2.43	2.63	95%	6	0	accept
SSE	6	22.1	3.94	99%	0	6	reject
SSN	3	5.1	5.01	99%	1	2	reject
IS	6	12.36	3.94	99%	6	0	reject
SU1	8	0.9	2.48	95%	I	7	accept
SU2	9	26.1	3.52	99%	2	7	reject

DF: Degrees of freedom of experiments
F_{calc}: F-values calculated
N_n: Number of negative residuals
Degrees of freedom of general model: 19
F_{tab}: Tabulated F-value with (DF,19)
N_p: Number of positive residuals

Experiments (EXP):
Ag1: $AgNO_3$ 0.04 mol/L
Ag2: $AgNO_3$, 0.004 mol/L
EAT: Enzymatic after treatment
BC: Bleached only cotton
SSE: Surface sized, with enzyme
SSN: Surface sized, no enzyme
SU1: Sueded surface, 6 mil
SU2: Sueded surface, 5 mil

Table III. Regression of data for model base series and series of sueded fabric including fabric weight

	MBS[a]	SW1[b]	SW2[c]
A (X1, beads)	0.251	0.181	0.69
Std Dev.	0.058	0.022	0.095
B (X2, 1/ink)	35.79	47.03	150.28
Std Dev.	4.57	14.47	32.12
C (X3, 1/weight)	**1.24**	**11.17**	**8.36**
Std Dev.	**10.2**	**3.13**	**14.07**
D (const.)	2.66	-5.37	-44.62
Correlation	0.902	0.973	0.971
ε^d	3.36	0.953	4.63

Regression equation: $Y = AX1 * BX2 * CX3 * D$

Variables:

Y: ink removal

X1 (beads): number of beads

X2 (1/ink): reciprocal value of ink coverage

X3 (1/weight): reciprocal value of sample weight

[a]MBS: Regression of the experimental results of the model base series (including weight)

[b]SW1: Regression of the experimental results with sueded samples, setting 6/1000 in.

[c]SW2: Regression of the experimental results with sueded samples, setting 5/1000 in.

[d]ε: Estimation of error

Note that weight (bold) does not play a significant role in MBS but in SW1 and SW2.

thus, be protected from the combined attack of mechanical surface friction and enzymes. The low brightness gain achieved by enzymatic deinking could be due to these residual ink patches on the surface of the fiber. On the other hand, redeposition of ink could also lead to a decrease in brightness. In both cases, process modifications should be investigated. Parameters that have to be investigated are the mechanical conditions under which the enzyme treatment should take place and the use of chemicals that can help prevent the redepositon of removed ink particles.

Any coating of the fiber had an adverse effect on the performance of enzyme deinking. Whereas internally sized samples were not deinked completely, probably due to the repulsion of the enzyme as a result of a more hydrophobic fiber surface, externally sized and coated samples showed an increase in deinking, but failed to demonstrate a difference between experiments using enzymes and experiments with mechanical action only. The reason for this might be that the starch was washed away with increasing treatment time, thus removing the ink and making the enzyme unnecessary. At the same time, these surface coatings might have also shielded the fiber from the enzyme attack.

Conclusions

The research herein provides additional evidence that only easily accessible cellulose chains are subject to enzymatic cleavage. Our data also suggest that mechanical action, such as surface friction on the fiber, is able to alleviate this restriction by opening the outermost layers of the fiber to expose fully the cellulose chains. Only then is the removal of a significant amount of ink possible. Our findings may ultimately lead to a novel process design producing a high quality recycled fiber by enzymatic deinking. ■

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