

# Enzymatic Deinking of Old Newsprint with Alkalophilic Enzymes from *Coprinus cinereus* 2249

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## ABSTRACT

This study was to evaluate applications of the alkalophilic enzymes from *Coprinus cinereus* 2249 with old newsprint(ONP). A enzymatic deinking process based on alkalophilic enzymes was investigated. It was found that alkalophilic enzymes could effectively deink old newsprint. When applied on deinking of the old newsprint, it increases the freeness and brightness due to effect of hydrolysis at 0.1% enzyme concentration. Also, the physical properties of deinked pulp were improved.

## 1. Introduction

Recently, the demand for recycling of wastepaper has risen dramatically due to depletion of forest and energy resources. Conventional deinking process requires large quantities of chemicals such as surfactant and alkali, so its method is both capital and energy-intensive, and these chemicals were detrimental to environment.

The use of enzyme in deinking process can minimize these problems. Investigators have examined cellulytic enzyme such as cellulase and hemicellulase to improve the deinking performance of wastepaper. The author<sup>1,2)</sup> claimed that treating wastepaper with cellulytic enzymes results in a better pulp quality and effective in reducing the wastepaper disintegration time or energy, because the beating time required to attain a desired freeness is reduced by almost 50%

in the presence of cellulytic and hemicellulytic enzymes. Fukunaga<sup>3)</sup> and Prasad *et al.*<sup>4)</sup> and Wang *et al.*<sup>5)</sup> also examined enzymatic deinking of ONP and reported similar results, i.e., enzyme-deinked pulp showed higher brightness, the higher freeness, lower yellowing and greater paper strength, reduced chemical usage than conventional deinked pulp. But most fungal cellulases used in these experiments are active in the acid region. Thus, the cost to adjust to optimal pH is increased with the more use of recycled fiber. Under these surroundings, another application of enzymatic deinking technique with alkali-active or alkalophilic enzyme is suggested by several workers. Sreenath *et al.*<sup>6)</sup> reported deinking applications using alkaline-active cellulase from desert *Basidiomycetes* are advantages because neutralization of the recycled fiber is not necessary. Lion<sup>7)</sup> and Shraryo *et al.*<sup>8)</sup> claimed

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that alkaline lipases would facilitate the removal of lipid-based offset printing inks.

Although the use of enzymes to deink recycled fiber has been proposed, there is a fundamental lack of understanding of how these enzymes enhance the deinking of paper and what kind of enzyme type needed. Enzymatic deinking process is based on the principle of an enzyme with endoglucanase and cellobiohydrolase attacking the certain region where the ink is attached to the fiber. The dislodge ink particle can be removed by flotation. Zeyer *et al.*<sup>9)</sup> claimed the accessibility of enzymes to ink attached to the surface of the fiber was very important factor for deinking. Jeffries *et al.*<sup>10)</sup> stated the enzymes with the higher filter paper activity were good for deinking.

Although many enzymatic deinking studies appear in the literature, very few investigator have specifically studied the effects of the enzymatic deinking with alkalophilic enzyme.

In previous papers,<sup>11)</sup> we have produced the alkalophilic enzyme from *Coprinaceae* : optimum pH 9.0 and optimum temperature, 50°C, displaying the higher filter paper activity than CMCase. The objective of this paper was to evaluate applications of the alkalophilic enzyme with ONP.

## 2. Material and Methods

### 2. 1 Materials

#### 2. 1. 1 Raw material

Old newsprint of more than 6 month old was used as a raw material throughout this study. ONP was torn into small pieces by hand.

#### 2. 1. 2 Enzymes

We used alkalophilic enzymes from *Coprinus cinereus* 2249 (Table 1). Endoglucanase, filter paper and xylanase activities were assayed using 0.5%(W/V) CMC, Whatman No. 1 filter paper(50 mg) and 0.5%(W/V) birch wood xylan suspension, respectively, in the Tris-HCl buffer of pH 9.

Reducing sugars produced as the results of these assays were determined by using

**Table 1. Enzyme activity**

	IU/g protein at the pH 9
CMCase	41.2
Xylanase	60.0
Filter paper activity	72.9

the dinitrosalicylic acid method of Miller.<sup>12)</sup> Enzyme activity has been expressed in International Units(IU), as the amount of enzyme needed to release one mg mole of glucose and xylose.

### 2. 2 Experimental Methods

#### 2. 2. 1 Deinking procedure

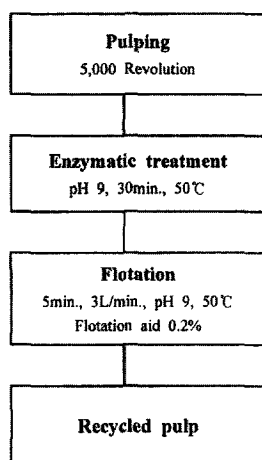
The deinking procedure used in these experiments is presented in Fig. 1.

#### 2. 2. 2 Repulping

The pulp was(40 g o.d. pulp) transferred to disintegrator and diluted with warm water to achieve 4% consistency and 50°C, and disintegrated for 25 min.(5,000 revolutions in the disintegrator).

#### 2. 2. 3 Enzymatic treatment

A batch of 4% pulp suspension was transferred to the enzymatic reactor which was subsequently heated to 50°C in a water bath. The pH was adjusted to 9.0 with reagent



**Fig. 1. Deinking method of ONP.**

grade 2 N NaOH. A series of treatments was performed using various amount of enzyme, and allowed reacting for 30 min. During the reaction period, the pulp was mixed properly and pulp suspension transferred into the flotation cell.

#### 2. 2. 4 Flotation process

The flotation was performed in a 6 L Laboratory flotation cell at a consistency of 1%. The standard conditions used for flotation were pH 9.0, temperature 50°C. The flotation chemicals used in these experiments were 0.2% collect soap on o.d. pulp. The pulp suspension was stirred at 600 rpm for 5 min. with an air flow of 3 L/min. Vacuum suction removed the floated ink.

The pulp treated the same way except no flotation step is the blank and control pulps were processed in a similar manner to those given the enzymatic treatments described above, except no enzyme additions were made.

#### 2. 2. 5 Handsheet preparation and testing

The pulp freeness was determined according to TAPPI Method T227. Handsheets of

the pulp samples were made with a standard TAPPI handsheet mold according to TAPPI Method T205. After flotation, Brightness pads were prepared according to TAPPI Method T218. The brightness of the pulp was measured according to TAPPI Method T452. The deinking effect was determined by measuring the brightness.

#### 2. 2. 6 Image analysis

For microscopic analysis of ink particle, we dilute the samples to a consistency of 0.01% and filter 100 mL this solution per pad. The dispersed ink particles were collected on cellulose acetate filter(Sartorius Ltd., 0.8 micrometer) from the filtrates of pulped samples. The mass of fiber plus ink is 10 mg and we made three slide glasses of each sample. Each slide glass sample was analyzed at 3 randomly selected areas with optical microscopy(Olympus Ltd., PM-20) equipped with camera(Olympus Ltd.; PM-C35DX). Obtained films were scanned by photoscanner(Hewlett packard Ltd., photosmart) with an optical resolution of 1200 dpi. Adobe photoshop version 3.0.5 software was used to interface with the scanner and to save the acquired images. Matrox inspector version 2.0(Matrox Electronic system Ltd.) was used to count ink particles in the saved images. The threshold of the dirt particles was set to a constant value of 250 to allow the direct comparison of data from different experiments. The data generated from the dirt count were analyzed using Microsoft Excel version 5.0. Particle size histograms represented the dirt size distributions within the sample. For this study, average values for the top and bottom for both brightness and residual ink area data were used.

### 3. Results and Discussion

#### 3.1 Effects of enzyme on the Freeness and fines content

Enzymatic treatment slightly affected the freeness and fines content of the pulp (Fig. 2). After treatment with each enzyme dose, there was an increase in pulp freeness by 6.9% compared to the control as well as a slightly decrease in fines content. The changes in freeness have been attributed to enzymatic hydrolysis of fines. Enzyme acts less aggressively on long fibers than on fines. Therefore, the enzyme seemed to increase the freeness of pulp. Jeffries *et al.*<sup>13</sup> claimed a mixture of cellulases and hemicellulase at low concentrations will markedly increase of recycled fibers without substantially reducing yield due to enzymes remove only fines that have a great affinity for water but which contribute little to the interfiber bonding potential.

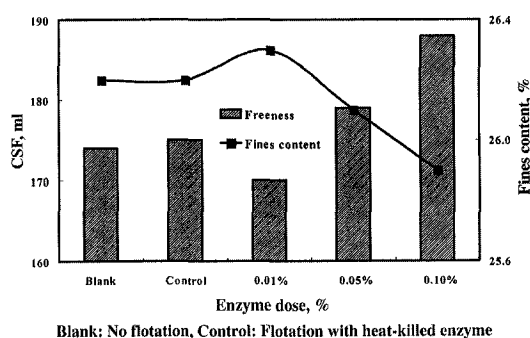


Fig. 2. Freeness and fines content.

However, these changes were minimal when enzyme dose was lower than 0.1%/o.d. g pulp. For 0.01% enzyme dose, the freeness was reduced by increasing of fines content. The reasons for the increase in the fines content have not been elucidated.

#### 3.2 Evaluation of deinking performance

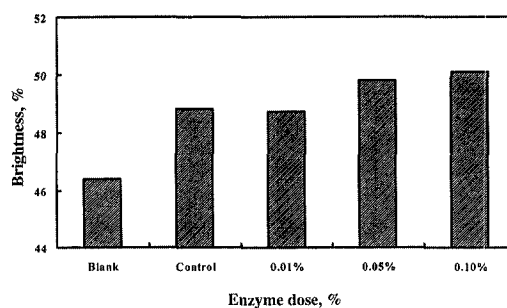


Fig. 3. Brightness.

Brightness was measured on enzyme-treated sheets made from the deinking experiments and the enzyme-treated pulps have a higher brightness than the control pulps (Fig. 3). Compared to Blank, Control and 0.1% enzyme dose were increased by 2.4 and 3.7 point respectively. For 0.1% enzyme dose, the maximum gain of 1.3 points brightness compared to the control. However, increasing the enzyme dose increases the reject content. It is possible that the higher brightness is related to the higher loss of the furnish.

Table 2. Residual ink area and ink removal

	Residual ink area(%)	Ink removal(%)
Blank	1.20	-
Control	1.04	13.7
0.01%	0.72	40.2
0.05%	0.61	49.5
0.1%	0.36	70.2

Table 2 shows residual ink area and ink removal efficiency determined by image analysis. The brightness (Fig. 3) does correlate with ink removal and residual ink area. The percentage of ink removal is based on the blank. The concentration of 0.01% enzyme of pulp increased the ink removal efficiency to 40.2%. Increasing the enzyme concentration to 0.1% increased the ink removal to approximately 70% and also

residual ink area reduced.

**Table 3. Ink distribution with enzyme dose**

	Blank	Control	0.01%	0.05%	0.10%
10 $\mu\text{m}$ <	1746	1347	1014	843	426
50 $\mu\text{m}$ <	105	131	82	64	36
80 $\mu\text{m}$ <	12	11	5	7	2.67
80 $\mu\text{m}$ >	1.44	2.55	2.1	0.55	0.66

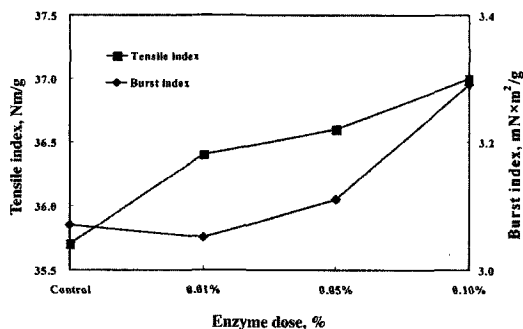
The enzymatic treatments also have a strong effect on reducing the ink particle size in addition to the effect on disintegration (Table 3). Increasing the enzyme concentration to 0.1% reduced the ink particle size and count.

In spite of reduction of ink particle count and efficiency of high ink removal, enzymatic treatment showed a slight increase of brightness than the control. It may be partly due to excessive pulping time, which make the small ink particle size. For blank, Image analysis observations have shown that almost ink particles are smaller than 10 micron after flotation at pH 9. These particles have the greatest adverse effect on brightness. Generally, flotation efficiency rises as ink particle size reaches 30 microns and above and then drops off quite rapidly as particle size reaches 150 microns. The pulping time ought to be controlled in order to maintain the ink size distribution within the desire range for an optimum flotation.

### 3.3 Physical properties of enzyme deinked pulp

The figure 4 shows the physical properties of laboratory handsheet made from the enzyme treated pulps. In the tensile index and burst index, enzyme treated pulps are stronger than the control pulp. It may be due to effect of enzyme hydrolysis. Earlier stated, the recycled pulp contains a rather

high amount of what may be called primary fines for repulping and secondary fines produced during beating. Primary fines not affect strength of paper than secondary fines. We carried out repulping with disintegrator which produced not the secondary fines but the primary fines. Therefore, enzymatic hydrolysis that either by acting directly on the fines or by changing the surface features of fiber increased the strength.



**Fig. 4. Physical properties with enzyme dose.**

## 4. Conclusion

Enzymatic deinking technology is newly used for deinking secondary fiber furnish. We studied the effect of alkalophilic enzyme to enhance flotation deinking of ONP by analyzing pulp properties and strength of handsheets and to investigated as a means to evaluated efficiency of deinking for ONP.

The results of this work provide some conclusions;

1. Using the alkalophilic enzyme at the alkaline region, deinking of ONP enables efficient flotation.
2. Increasing enzyme concentration to 0.1% can produce a deinked pulp with better physical properties and higher brightness and freeness than the control pulp.
3. The dispersed ink concentration remaining with the pulp after flotation was significantly lower with 0.1% concentration

compared to the control.

4. The use of enzyme at the pH 9 can reduce conventional chemical usage and subsequently decrease environmental problems.

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