USE OF ENZYMES IN DEINKED PULP BLEACHING

CÉLINE LEDUC, LISA-MARIE LANTEIGNE-ROCH and CLAUDE DANEAULT*

Université du Québec à Trois-Rivières, Centre de Recherche sur les Matériaux Lignocellulosiques, 3351, Des Forges Blvd., Trois-Rivières, Québec, G9A 5H7
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To comply with government's environmental policies and to satisfy the requests of the consumers, recycling of waste paper is essential for reducing the consumption of virgin fibers in pulp and paper industry. However, the poor optical properties of recycled fibers restrict their use in value-added papers. Enzymes, such as lipase and laccase, represent an interesting alternative. The detachment of contaminants and ink particles, as well as the chemical modifications induced by these enzymes, could increase brightness and reduce residual ink concentration. The main objectives of the present study were to assess the effectiveness of deinking and bleaching with lipase and laccase. The optimum process conditions selected for lipase were of 10 IU/g (International Unit/gram of oven-dry pulp), without pH adjustment.

Keywords: enzyme, lipase, laccase, mediator, deinked pulp, bleaching, brightness, residual ink, ERIC

INTRODUCTION

Recycling of old papers has become mandatory for the pulp and paper industry, since the Coulombe report[1] in 2005, which defined new rules for forest management in the province of Quebec. Social pressures for a sustainable development, increasing landfill costs and regulations from the United States are some of the factors that contributed to an increased consumption of recycled fibers. Furthermore, the amount of recycled fibers added to different paper grades has been regulated; manufacturers are expected not only to comply with these rules, but also to meet the requirements of their customers and become more and more respectful of the environment. To satisfy all these requirements, the industry must import old papers from the United States.

Secondary fibers require deinking, to ensure a quality that could compete with that of virgin fibers. Recycled fiber quality is still an issue to be solved and remains the main goal of many research projects.[2-6] Ink removal constitutes the major technical obstacle for converting this raw material into quality products. Many attempts have been made using enzymes, such as lipase, laccase, cellulase and xylanase.

Table 1 summarizes the results and comments reported in the literature on the use of lipase, laccase, cellulase and xylanase in deinking and bleaching processes.[7-39] In particular, Morkbak *et al.*[7] showed that the deinking effect of lipase was caused by a partial degradation of the binder of soy bean oil-based inks, thereby releasing the ink particles from paper. Furthermore, Xu *et al.*[22] demonstrated that old newsprint could be deinked with a laccase-violuric acid system (LVS). Also, several authors observed that the use of cellulase facilitates ink removal.[26-30]

Some previous results[4] indicate that fines are contaminated with residual ink, which limits improvements in paper brightness. The hypothesis on which the present investigation is based is that the hydrolysis of ink vehicles composed of vegetable oil, by lipase, or the oxidation of pulp lignin, by laccase, allow the detachment of ink particles from the fiber during the enzymatic treatment (ET), and also that these detached ink particles are probably removed by the flotation process (F). Possibly, this will promote the peroxide bleaching efficiency (P) of the floated pulp. The main objective of this study is therefore to evaluate the potential of enzymes, such as lipase and laccase, to improve the quality of deinked pulp.

MATERIALS AND METHOD

Deinked pulp from a Canadian mill, composed of 70% old newsprints (ONP) and 30% old magazines (OMG), was selected for the experiments. The pulp was pretreated with 0.2% DTPA (Diethylene Triamine Pentaacetic Acid) at 60 °C, for 15 min, at 3% consistency, after which the pulp suspension was thickened. Lipase was provided by the Innu-Science company, laccase (from *Rhus vernicifera*) and the mediator (methyl 3,5-dimethoxy-4-hydroxybenzoate) were bought from Sigma-Aldrich. The enzymatic treatment (ET) with lipase or laccase/mediator was conducted at 50 °C, for 3 h, at 5% consistency, after which the pulp was washed at 1% consistency and thickened. Flotation of the resulting pulp was conducted in a Leeds flotation cell, at 50 °C for 5 min. Pulp suspension consistency was of 0.55%, and 0.3% oleic acid was added. Pulp pH was adjusted to 9.0, and 80 ppm of calcium chloride was added. Following the flotation step, the pH of the floated pulp was adjusted to 5.0 and the pulp suspension was thickened. The floated pulp was further bleached with 3.0% peroxide, 3.0% sodium silicate and 2.36% sodium hydroxide. The bleaching stage was conducted at 70 °C, for 3 h at 12% consistency. The bleached pulp was diluted to 1% consistency and neutralized at pH 5.5. The standard method C.5 of PAPTAC was used for the formation of handsheets. ISO brightness (PAPTAC E.1) and ERIC – Effective Residual Ink Concentration (TAPPI 567) were measured in each step of the process.

Table 1
Literature review on the use of different enzymes

Enzyme	References	Comment
Lipase	[7]	The deinking effect of lipase was caused by the partial degradation of the binder of the soy bean oil-based inks, thereby releasing the ink particles from the paper
	[8]	The use of lipases could help control pitch in mills using TMP (Thermomechanical Pulp) at high water temperatures
	[9]	By modifying the hydrophobicity of fine materials from the deinking stock, through lipase pretreatment, flotation selectivity can be controlled and production costs reduced
	[10]	The addition of cellulase and lipase to the pulper improved contaminant removal, pulp freeness and pulp brightness
Laccase	[11-19]	The use of laccase or of a laccase/mediator could be useful in delignification, biobleaching and colour removal
	[20]	The capability of laccase to oxidise surface lignin from mechanical pulp fibers offered a means to functionalize fibers for customised papers and board products
	[21]	The laccase treatment of TMP from two newsprint mills resulted in a significant degradation of extractives and had an even greater effect on lowering the extractives content present in a paper machine white water
	[22]	Old newsprint could be deinked with a laccase-violuric acid system (LVS)
	[23]	The xylanase pretreatment facilitated the access to cellulose fibers, thereby boosting the effect of the laccase-mediator system in reducing the content of residual lignin
	[24]	A synergistic deinking effect is manifested between cellulase/hemicellulases and LVS. The enzyme-combining deinked pulp gave higher brightness and physical properties and lower ERIC (Effective Residual Ink Concentration) compared to the pulps deinked with each individual enzyme
	[25]	Newsprint white waters contained a significant amount of colloidal substances. Laccase treatment resulted in the degradation of most of the extractives, while lipases specifically hydrolyzed the ester-bonded extractives present in the colloidal fraction
Cellulases	[26-30]	Facilitated ink removal
	[29]	Caused brightness increase and reduction in ink counts
	[31-32]	Cellulase deinking was less efficient than in either alkaline or sulphite chemistry, but contributed to paper strength
Xylanases	[27-28], [33-36]	Used in prebleaching of kraft pulp to minimize consumption of chlorine chemicals in subsequent bleaching
	[37-39]	A mixture of cellulases and xylanases was found to be optimum for the treatment of laser printed paper or newspaper

RESULTS AND DISCUSSION

Lipase results

Lipases are enzymes which catalyse the hydrolysis of ester chemical bonds, possibly acting on liposoluble compounds, such as the numerous contaminants contained in old papers, especially in some ink samples containing vegetable oil (which is the case of some typographic or lithographic inks used for printing of newspapers). The general reaction of lipases is to catalyse the hydrolysis of triglyceride in glycerol and fatty acids. Enzymatic deinking with lipases can be more efficient, if considering the partial degradation of either carrier or binder in vegetable oil-based inks. Furthermore, lipases could potentially act as a surfactant, due to their amphiphilic properties and their association with oil-aqueous interfaces.[40]

The lipolythic activity of lipase was determined using a spectrophotometric dosage of para-nitrophenol (pNP) liberated by the hydrolysis of the para-nitrophenyl palmitate (pNPP) substrate. The pNP is a yellow chromogene with maximal absorption[41] at a wavelength of approximately 405 nm. The enzymatic activity of lipase was of 93.5 IU/g. Figures 1 and 2 show the relative enzymatic activity of lipase as a function of temperature and pH. Temperature levels ranging from 45 to 55 °C and pH above 8 seemed suitable for preserving the maximum activity of lipase. A temperature of 50 °C was selected for the enzymatic treatment, as an optimum enzymatic activity of lipase was observed. The pH of pulp suspension during the enzymatic treatment was adjusted to different alkaline pH values, to preserve a maximum lipase activity.

Figures 3 and 4 show brightness and effective residual ink concentration (ERIC) as a function of different lipase concentrations, ranging from 0 to 10 IU/g, for pulps subjected to enzymatic treatment (ET), when the pH of the pulp suspension was adjusted to 9, following flotation (F) and peroxide bleaching (P). The abbreviation "REF" represents only the deinked pulp floated and further bleached. The trial with 0 IU/g signified that the pH of the pulp suspension was adjusted to 9, but no lipase was used. The adjustment of pulp pH to 9 for the enzymatic treatment induced alkali darkening, which resulted in a brightness loss of 1.4 point, from 58.7, to an average value of 57.3% ISO. According to the literature,[42-43] alkali darkening is caused by the reaction of mechanical pulp lignin with sodium hydroxide. Following the flotation step, the ERIC values were significantly reduced, inducing a slight improvement in brightness. The flotation step was efficient in eliminating some ink particles, as the ERIC values were reduced from an average of 277 to 185 ppm. Furthermore, the use of lipase seemed to be beneficial, if considering the slight reduction of the ERIC values from 184 to 151 ppm, for a lipase concentration of 10 IU/g. According to Morkbak *et al.*[7,40] hydrolysis of triglycerides induced by lipase allowed sufficient detachment of ink for an efficient flotation step, and even the partial hydrolysis of the carrier of vegetable oil-based inks having an impact on the deinking process. Brightness results of the enzymatic treatment and flotation were equivalent to those obtained without lipase. However, a final pulp brightness of 67.6% ISO was achieved for a lipase concentration of 10 IU/g, resulting in a slight improvement of 0.9 point compared to the reference, which is probably due to the fact that a lower ink concentration was beneficial to the overall bleaching efficiency.

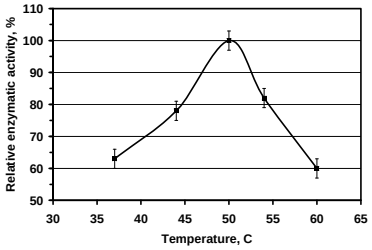

Figure 1: Relative enzymatic activity of lipase as a function of temperature

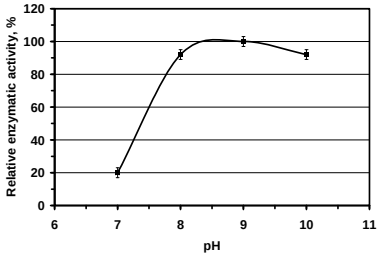

Figure 2: Relative enzymatic activity of lipase as a function of pH

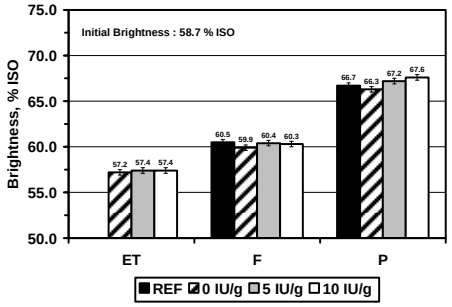

Figure 3: Brightness as a function of lipase concentration (pH adjusted to 9)

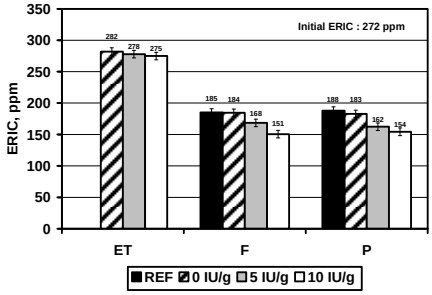

Figure 4: ERIC as a function of lipase concentration (pH adjusted to 9)

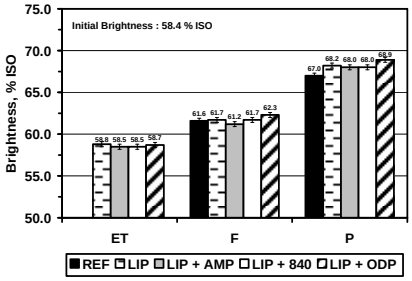

Figure 5: Brightness resulting from the use of lipase and different surfactants added in the Enzymatic Treatment (ET), (pulp pH 6.2)

Table 2
Brightness and ERIC for trials with lipase (concentration of 10 IU/g) at different pH values

pH	Sequence	Brightness (% ISO)	ERIC (ppm)
---	F-P	66.7	188
pH adjusted to 8.5	ET-F-P	67.6	148
pH adjusted to 9	ET-F-P	67.6	154
pH adjusted to 10	ET-F-P	68.0	143
---	F-P	67.0	160
No pH adjustment	ET-F-P	68.2	160

Table 2 shows that the use of lipase had a slight impact on brightness, when the pH levels were adjusted between 8.5 and 10. Nevertheless, an experiment was conducted with lipase, without any pH adjustment. Even at a pH of the pulp suspension of 6.2, when the results of the relative enzymatic activity indicated that it would be reduced, a slight improvement in the brightness level, of 1.2 points, was noticed. Since one of the priorities of the pulp and paper industry is to reduce the total production cost, the authors decided no pH adjustment in further experiments. Avoiding the use of sodium hydroxide to increase the pH was expected to have a beneficial effect on the effluent quality, preventing a higher chemical oxygen demand from alkaline dissolution of the organic compounds.[44]

Figure 5 illustrates the brightness results obtained on pulps treated with lipase and different surfactants that had been added in the enzymatic treatment. Surfactant AMP was an amphoteric type capryl/capramidopropyl betaine, while surfactant 840 was a non-ionic type alcohol ethoxyle with short chains and the ODP surfactant was an amphoteric type octyl dipropionate. All were biodegradable in aerobiose and anerobiose and did not bioaccumulate. The ODP had the lowest aquatic toxicity. Surfactant concentration was fixed at 0.01%. The assumption was made that the surfactant action in the enzymatic treatment might allow a decrease in the surface energy of the fiber and promote a better diffusion of lipase within the fiber. The only surfactant combined with

lipase having a slight effect on the brightness level was ODP, the brightness of which varied from 68.2 to 68.9% ISO. The results illustrated in Figure 6 also show that ODP had a slight effect on ink concentration reduction, values from 160 to 141 ppm being obtained. In this case, the pH of the pulp suspension was not adjusted and the value was 6.2. Another observation was that the lipase treatment allowed a brightness gain, but not an ink concentration reduction, just like the one obtained at pH 9. Possibly, the reaction of lipase with the vegetable oil of the vehicle induced the release of some ink particles; also, the decrease in ink concentration was probably responsible for the slight brightness improvement.

Laccase results

Laccase enzyme is a lignanase-type enzyme with the capacity to degrade lignin. Laccase is a copper-based oxidative enzyme, which reacts specifically with different phenolic residues of lignin in the presence of oxygen as an electron acceptor. The substrate specificity of laccase is that it uses molecular oxygen as an electron acceptor instead of hydrogen peroxide (as peroxidase does), which might be useful for industrial applications and beneficial for the environment.[14] When laccase is used together with a mediator, it will allow oxidation of the non-phenolic groups. Our assumption is that laccase can induce detachment of the ink particles linked to lignin.

The activity of laccase was determined by spectrophotometric dosage of the oxidized syringaldazine liberated by the reaction of substrate syringaldazine with the enzyme.[45] The oxidized syringaldazine is a quinone with a fuschia color, and with maximum absorption at an approximately 530 nm wavelength. The enzymatic activity of laccase was of 116 ± 7 IU/mg.

Figure 7 shows that the relative enzymatic activity of laccase will be over 60%, when varying the temperature levels from 30 to 55 °C. A temperature of 50 °C was selected for the enzymatic treatment in all experiments with laccase. Figure 8 illustrates that the relative enzymatic activity will be over 80% when the pH levels varies from 6.5 to 8.5. At a pH of 5.5 or higher (pH 9.5), no activity was detected.

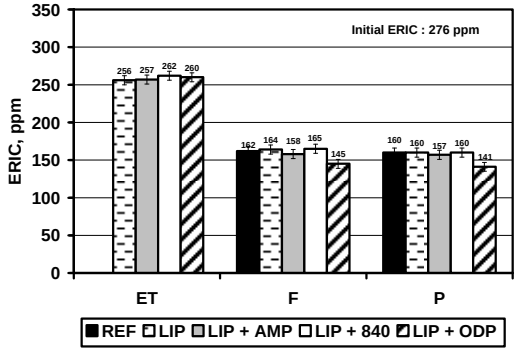

Figure 6: ERIC resulting from the use of lipase and of different surfactants (pulp pH 6.2)

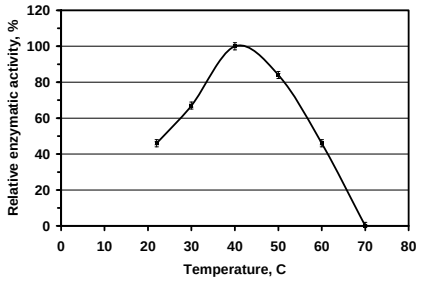

Figure 7: Relative enzymatic activity of laccase as a function of temperature

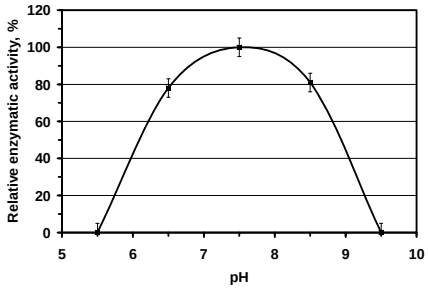

Figure 8: Relative enzymatic activity of laccase as a function of pH

Table 3
Brightness and ERIC variations for trials with laccase and mediator

pH	pH adjustment	Brightness variation (% ISO)	ERIC variation (ppm)
5.8	No	+ 0.1	- 21
6.2	No	+ 1.1	-4
7.5	Yes	+ 0.1	+ 9
8.0	No	+ 0.8	- 6
8.5	Yes	+ 0.1	+ 3

None of the experiments conducted with laccase in different concentrations, with or without pH adjustment, improved the brightness level, comparatively with the trial without laccase, possibly because laccase had no sufficient accessibility to the fiber, or because the presence of molecular oxygen was absolutely necessary for achieving the enzymatic reaction.

Table 3 illustrates the brightness gain obtained when laccase was combined with 0.5% of the mediator (LMS). The results obtained demonstrate that the LMS system allowed a slight brightness gain when the pH of the pulp suspension was not adjusted. Brightness gains of 1.1 and 0.8, respectively, were achieved at pH levels of 6.2 and 8.0, indicating that a slight portion of lignin was modified by the LMS system, as no significant reduction in ink concentration was recorded. The trial conducted at pH 5.8 had no impact on brightness, probably because the pH was too low for inducing sufficient enzymatic activity from the part of laccase. No brightness gain was registered when the pH was adjusted to 7.5 or 8.5. Probably, the presence of hydroxyl ions provided by the addition of sodium hydroxide for pH adjustment led to the inhibition of laccase.[46]

CONCLUSIONS

In this research project, the potential of enzymes, such as lipase and laccase, was evaluated in deinking and bleaching processes. The addition of lipase in the enzymatic treatment at pH values varying from 8.5 to 10 had a slight impact on pulp brightness. At pH 9, a brightness gain of 0.9 point was obtained, resulting in a final brightness of 67.6% ISO compared to the 66.7% ISO recorded as a reference. A slight reduction in the values of effective residual ink concentration (ERIC), from 184 to 151 ppm, was observed after the flotation step. However, a brightness gain of 1.2 point was observed when the pH of the pulp was not adjusted, remaining at 6.2. Also, the combination of lipase with a surfactant (ODP) allowed a slight brightness improvement and a reduction of the ERIC value. The results of laccase addition at different concentrations or at different adjusted pH values, or without pH adjustment, did not improve the brightness level or ERIC. Nevertheless, the use of laccase and of a mediator (LMS) without any pH adjustment allowed a slight brightness increase (pH 6.2:1.1-8.0:0.8). The lack of efficiency of these enzymes seemed to be related to the fact that the ink particles were strongly tied up to the fiber or they were less accessible.

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