Journal of Materials Science and Engineering A 13 (10-12) (2023) 83-95
doi: 10.17265/2161-6213/2023.10-12.001

Investigation and Benefits of the Use of Enzymes for Unbleached Kraft Recycling Wet Strength Paper at Lab and Industrial Scale

Audrey Bajul-Baradon, Terence Nguema-Nkili, Anais Sanfaute and Gilles Bajul
CELODEV SAS, 9 rue des Verriats,51500 CHAMPFLEURY, France

Abstract: The deterioration of recycled fibers especially unbleached kraft with high wet strength resin content due to the irreversible structural changes caused by drying and added chemicals makes the raw material difficult to repulp. The mechanical effect in the pulper over time with chemical treatment has a negative impact on the recycled fibers. At lab scale, different compositions of enzymatic treatment C022L were under investigation to select the most efficient laccase Lacc1, Lacc2 or Lacc3 and to observe the impact of lipases during repulping at low and high consistencies. Pulp disintegration at different times was evaluated to define the level of rejects and to analyze the morphology of fibers after treatments. These results were more significant for Lacc2, by increasing the pulp consistency to 15% in the pulper. Combining lipases with CELODASE 022L appeared to decrease the efficiency of enzymes. The results showed a high reduction of energy power with the enzymatic treatment and a significant reduction of fines level in fibers' suspension. The most efficient version of C022L was used at industrial scale to compare directly with the standard conditions used in a paper mill.

Key words: Energy, enzymes, recycling, unbleached kraft paper, wet strength resin.

Nomenclature

C022L	enzymes formulation of CELODASE 022L
conc.	consistency
HC	high consistency
Lacc	laccase
LIP	lipase
ORP	oxydo reduction potential
PAE	polyamide-epichlorohydrin
R (%)	level of reject
RP	recovered paper
WS	wet strength
χ	conductivity

1. Introduction

Actually, the pulp and paper industry faces several problems such as a global pressure to reduce water consumption, to use more recycled fibers and to lower environmental impacts. Sustainability and environment become one of the main challenges in the last decade. Meeting these challenges calls for innovative approaches to increase the recyclability of paper while ensuring product quality. This requires higher standards for the quality of the recycled pulp and it is agreed that pulp properties deteriorate during recycling. The deterioration of the paper-making properties of recycled fibers is mainly due to the irreversible structural changes in the fiber wall caused by drying section during paper production which impact the hydrogen bonding between cellulose microfibrils [1] resulting in inferior strength properties and bulkier sheet. After drying section, fibers lose their conformability and swelling capacity, which cannot be recovered by rewetting the fibers. The deterioration is also generated by other mechanical and chemical treatments such as pulping, refining and bleaching. The fibers become less flexible and shorter than virgin fibers causing lower strength and less bonding between

Corresponding author: Audrey Bajul-Baradon, Ph.D. from University of Toulouse, research fields: pulp and paper.

the fibers which force the papermakers to use virgin pulp most of the time.

In its search for solutions to the above-discussed challenges, the pulp and paper industry is increasingly turning to enzymes technology [2, 3]. This enzymes technology also offers an environmentally friendly method for modifying solid wood, pulp, or other lignocellulosics by biografting of phenols and other molecules. In this rapidly emerging field of research, properties such as increased paper strength were obtained by the use of enzymes [4-7]. The use of an appropriate cocktail of enzymes has been defined to address the challenges with highly efficient, biorenewable, mild, nonpolluting, selective, and inexpensive solutions, and to improve paper quality [8].

The use of enzymes in upgrading secondary fiber has also been extensively studied in recent years [9-13]. In most of the studies, commercial mixtures of different cellulases and hemicellulases have been used to treat recycled pulps and the treatments have resulted in improved drainage properties. Other studies were focused on the bleached recycled paper [14, 15]. Enzymatic oxidation of lignin with the oxidoreductases such as laccases has been shown to increase the bonding strength of fibers in fiberboards [16-18]. As well as lignin content and according to the literature, other contaminants can be treated by enzymes especially for the recycled paper. In fact, one of the major consequences of fibers recycling from unbleached kraft pulp is dealing with stickies containing various components not always well-known which make them difficult to control. Stickies coming from wood extractive of other lipophilic components can cause problem with quality of the final product or runnability of the machine. Biological reduction of pitch using enzymes is a very effective method and intensively highlighted in the literature. Lipase enzymes are mostly used in pitch removal [8]. Different studies were focused on the use of esterases to break down the stickies to get them smaller and less tacky [15, 19]. Enzymes are very specific and the hardest work is to define the right enzymes to break down the right substrate [15]. In this perspective and according to the expected application, enzymes formulations can be Taylor-made to take all the different substrates into account.

The studied raw material for this work deals with kraft unbleached paper with a high lignin content as RP (recovered paper). The paper contains also a high level of wet strength resin, PAE (polyamideamine-epichlorohydrin). In fact, polyamides used as wet strength agents in paper and paperboard products are commonly derived from adipic acid and diethylenetriamine followed by a cross-linking with epichlorhydrin [20]. The cationic azetidinium groups and protonated secondary amine groups promote PAE adsorption onto pulp fiber surfaces. During heating and drying the reactive azetidinium forms covalent grafts with carboxyl groups on fibers, and crosslinks within and between PAE chains [21-23]. The crosslinked polymer networks between contacting fiber surfaces make the paper hydrophobic with a tendency to swell or hydrolyze in water [24]. RP containing this polymer requires extreme conditions such as high pH, high temperature and strong oxidizing agents [25]. High pH can cause damage of the fibers structure and has to be adjusted after all as the temperature which increases the global price of the treatment at the end. Besides, the chemistry is never enough to get fibers suspension. The RP needs to be under pulping with high shear agitation. The high level of chemicals is combined with the mechanical effect on the fiber's suspension release salts from the degradation of the PAE and fines. Both fines and salts have a direct impact on the water consumption, the energy demand and the quality of the effluents. The fines level can cause production issues especially for the retention and the salt content can generate an increase of the fresh water consumption to obtain a better stability of the paper machine.

The alternative of using enzymes is to be focused on each issue such as the reduction of salts from chemical reactions, pH stage adjustment, repulping time, energy demand, fines generation.

In this work, recycled kraft papers were treated with different enzymatic cocktails made by CELODEV to define the right synergy between enzymes and its efficiency regarding the application. The unbleached kraft paper was recycled in the laboratory and the effects of recycling on the pulp properties were evaluated by fractionating and by the analysis of the fiber's morphology with the CELOFIBRE tool developed by CELODEV. Two different consistencies of the pulping stage and the introduction of lipases were also investigated to go further on the enzymatic optimization process before scaling-up at industrial scale.

2. Materials and Methods

2.1 Recycling Procedure

RP, grade 4.08 according to the European Standard EN 643, with different layers (virgin unbleached pulp, recycled unbleached pulp, ink, starch, wet strength resin and other compounds) considered as unbleached kraft paper with high wet strength resin content was used in the experiments. And 200 g of RP pieces of about 1 cm^2 was placed in a lab HC pulper with 3 L of chlorine free tap water and kept 10 min without agitation. The conditions of the repulping were set at 45 °C with a constant rotor speed at 20 Hz. Two consistencies were investigated: the first at 6.5% and the second at 15.0% with 450 g of RP.

2.2 Enzymatic Treatments

Commercial enzymes were used for the experiments under the name CELODASE 022L (C022L). Different versions of the enzymes were studied with three types of laccases supplied for pulp and paper industry and dedicated to catalyze the lignin (Lacc1, Lacc2 and Lacc3). Lipases were introduced in the formulation C022L-Lip. In order to observe the efficiency of the lipases, a previous work was made with lipases to avoid inhibition regarding the other enzymes formulated in C022L. The current lipases for the work were carefully selected for the experiments. The main components of the formulations gather cellulases, hemicellulases, esterases, laccases, amylases and lipases. The enzymatic treatments during repulping were carried out at a dosage of 1.8 mL per pulper corresponding to a dosage of 9 L/ton of dried paper. Samples were taken after 30 min and 60 min of treatment under agitation. A sample at 90 min was also taken for the fibers analysis. The different versions of C022L under investigation were mentioned in Table 1.

2.3 Fiber Analysis

After repulping, 40 g of dry pulp were screened in a fiber classifier (Somerville) for 8 min through a 0.2 mm slot screen at a water flow rate of 15 L/min. The system was modified with two control valves. When, the first valve was opened (configuration 1, Fig. 1), the flow 1 went through two different screens in series (sieve 1 at 140 μm and sieve 2 at 71 μm). The rejects (fraction of fibers retained in the flat screen) were collected, dried at 105 °C for 4 h and weighed. Repulping and classification were performed at 23 °C in triplicate and the average was reported.

The level of rejects was defined as:

$$R\ (\%) = m_{rejects}/m_{initial} \quad (1)$$

with $m_{initial}$ corresponding to the initial mass of pulp (40 g), $m_{rejects}$ corresponding to the mass retaining on the 0.2 mm screen. The index represents the percentage of fibers satisfactorily not repulped and not recovered. A lower value of R (%) indicates easier recycling process of the sheet.

Table 1 Composition of the different C022L for the experiments.

	Laccase	Lipase
Blank (no enzymes)	None	None
C022L(0)	None	None
C022L(1)	Lacc1	None
C022L(2)	Lacc2	None
C022L(3)	Lacc3	None
C022L LIP	Lacc 1, 2 or 3	1% to 5%

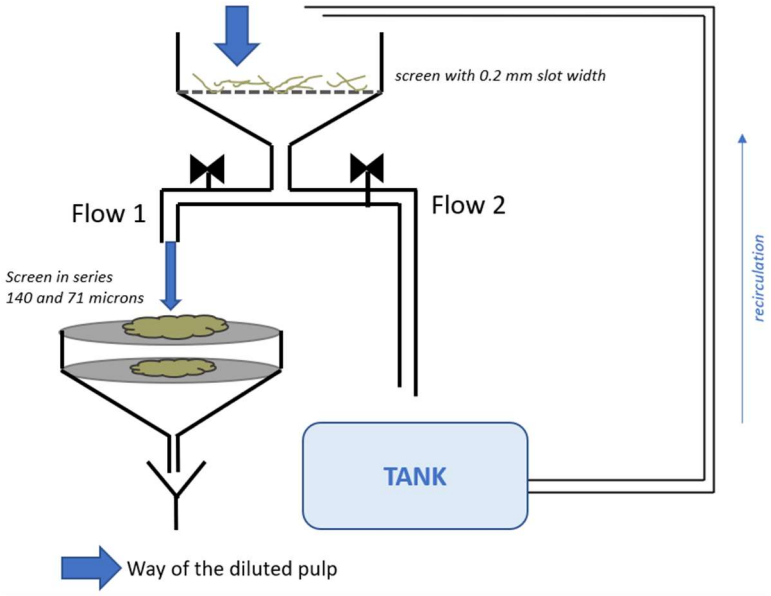

Fig. 1 Somerville system in configuration 1 for rejects evaluation.

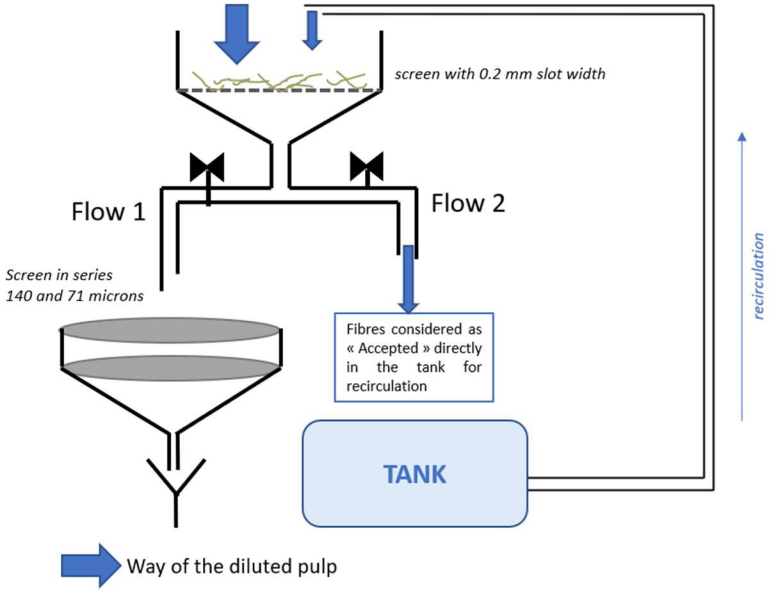

Fig. 2 Somerville system in configuration 2 for fibers morphology analysis.

When the second control valve was opened, the flow 2 went to a tank. After the 8 min of washing stage, a recirculation was made for 90 s (Fig. 2).

Samples were taken directly in the tank at the end of the recirculation and analyzed with a fiber morphology analyzer CELOFIBRE. The size of the fines for the tool was defined between 5 and 100 µm. The device enabled to determine the average length of fibers by weight, the width of fibers by weight and the level of fines by weight. Samples were taken after 30, 60 and 90 min of repulping in triplicate.

2.4 Chemistry Analysis

Measurements on the pulp were made on the different samples after enzymatic treatments with the multimeter ORION Versa Star. The parameters such as pH, conductivity, ORP (oxydo reduction potential) and temperature were following to compare the blank with the different versions of C022L.

2.5 Handsheet

After 30 min and 60 min of enzymatic treatments, isotropic handsheets (Handsheet former Rapid Köthen) were made according to TAPPI Method T-205 om-88, with some modifications. The target mass was changed to 120 g/m^2. Handsheets were made from untreated pulp (blank) for control measurements. The handsheets were dried between two hot metal plates at 150 °C for 3 min in a Rapid Dryer (Lorentzen and Wettre, Kista, Sweden).

2.6 Industrial Trial

The most appropriate formulation of enzymes was chosen to be used at industrial scale. A paper mill in Spain is producing packaging paper with dried post-consumer unbleached kraft pulp with a high level of WS resin from packaging (grade 4.08). The standard repulping conditions and chemical treatment used were: high consistency pulper (14%-15%), the capacity of the pulper is one ton of 100% of WS unbleached kraft paper with multilayer, using a chemistry treatment, steam directly in the pulper to reach 80 °C during repulping and the pulping time was between 50 and 60 min. The procedure for enzymatic treatment became: high consistency pulper (14%-15%), the capacity of the pulper is one ton of 100% of WS unbleached kraft paper with multilayers, using enzymatic treatment, no adjustment of the temperature and a reduction of repulping time. The dosage of enzymes was adapted to the conditions of the process and set at 1 L/ton instead of 9 L/ton at lab scale.

3. Results and Discussion

3.1 Fiber Fractionating

As there is no widely accepted standard test method to evaluate the recyclability of paper, in that case, the first screen of the Somerville device and the high flow of the dilution water enabled to separate as much as possible the fibres forming flocs or flakes to the fibers isolated from the slurry.

The experiments showed the values of rejects at 30 and 60 min in order to evaluate the evolution of the enzyme's efficiency regarding the raw material during repulping. The results according to Eq. (1) were directly compared with a blank without any treatment (Table 2).

The first experiments were performed in order to select the most efficient laccase to introduce lipases and increase the consistency during repulping with the most appropriate formulations of enzymes. Without any treatment, the level of rejects was higher due to the important presence of flakes highlighted by the results in Table 2.

After the enzymatic treatments, the level of rejects decreased significantly especially for the C022L(3) at 30 min of repulping. In the beginning the Lacc3 enabled to accelerate the action of other types of enzymes in the blend and then after 60 min the efficiency tended to decrease. With the C022L(1), the blend reached a steady state and no evolution of the raw material appeared. Besides, the C022L(2) was more appropriate because the enzymes worked constantly and helped to have a better access to the fibres network which implied a decrease of rejects at the end. For the introduction of lipases, the Lacc2 was kept (Table 3). Since the results showed that without lipases the enzymes were slowed down. An inhibition of efficiency of enzymes occurred during repulping with the released components from the enzymatic treatment. The case C022L(2) without lipases seemed to be the most relevant formulation to study the effect of the consistency during repulping (Table 3).

Table 2 Rejects after Somerville configuration 1 with the 3 types of laccases.

	Level of reject (%) after 30 min	Level of reject (%) after 60 min
Blank (no enzymes)	28.8 ± 0.3	18.8 ± 0.3
C022L(0)	19.2 ± 0.2	11.5 ± 0.1
C022L(1)	16.8 ± 0.1	16.6 ± 0.2
C022L(2)	10.0 ± 0.1	6.5 ± 0.1
C022L(3)	9.3 ± 0.1	8.3 ± 0.1

Table 3 Rejects after Somerville configuration 1 with lipases and higher consistency during repulping.

	% of reject (30 min)	% of reject (60 min)
C022L(2) LIP	13.8 ± 0.2	9.2 ± 0.1
C022L(2) - 15%	3.0 ± 0.1	1.8 ± 0.0

The results suggested that increasing the consistency enabled to improve the repulping stage since the friction between fibers was more intense. The evolution of the different formulations enabled to confirm that the type of laccases can have a real impact on the raw material.

3.2 Fiber Morphology Analysis

Measurements on fibers morphology were made on pulp samples without enzymes (blank) and with the C022L containing the different laccases Lacc1, 2 and 3. The interest of using the lipases in the formulation was rejected due to its limited efficiency regarding the rejects. The measurements were made by using the CELOFIBRE.

The length of the fibers, the width and fines level and the fibrillation were measured in weight. First, the average length and also the repartition of the length in the samples were determined.

Table 4 showed that the measurements for the blank were not performed due to the size of the flakes in the pulp suspension. For the blank, at 60 min without enzymes results showed that the long fibres formed flocs or flakes and were not considered during measurements. The long fibers were kept on the screen (0.2 mm) and only small fibers were measured by CELOFIBRE. With repulping time, the average did not increase significantly. Small fibers and fines were released with the mechanical effect due to the high WS resin content. For C022L(1), the length tended to increase due to the previous observation. The morphology analysis did not consider the flakes as fibers and tended to false the average. By consequences, the repartition of the length was necessary to observe the effect of enzymes on the length. Besides, the cutting effect of the different bonds between fibers occurred and enabled to keep only the long fibers for analysis. C022L(1) did not reduce the flakes content and the enzymes worked only on the suspension easily available. C022L(2) proceeded more on the separation of fibers in the first place and the cutting effect became the second effect when the cellulases had access to the fibers suspension. The efficiency of the other enzymes contained in the cocktail revealed the positive synergy in the cocktail. When the enzymatic treatment became effective, the length of fibres tended to decrease over time. For C022L(3), the results showed that the enzyme worked preferably on the flakes and was able to separate fibers from each other more than the C022L(1). In fact, the cutting effect was easier after all for the "free" fibers in water. Lacc1 and Lacc3 reacted on the lignin after the action of cellulases which created more cutting effect and then the separation of fibers could occur. Lacc2 reacted synergistically with the other enzymes in the cocktail which could improve the recyclability of the raw material by limiting the cutting effect from cellulases. The first comparison enabled to conclude the efficiency: (2) > (3) > (1).

The repartition of the fiber's length gave a clear picture of the situation (Fig. 3). The blank at 60 and 90 min after repulping contained only small fibers length at 200 μm with 26% of fibers and 23% of fibers respectively. Since most of the flakes after repulping were considered as rejects, only "free" fibers were analyzed by the CELOFIBRE. With C022L(1), the part of smallest length stayed high with 30, 60 or 90 min

Table 4 Average length of fibers (in weight) after enzymatic treatment and for the blank.

l (μm)	C022L(1)	C022L(2)	C022L(3)	Blank
30 min	824.0 ± 31.1	1,260.9 ± 27.3	417.3 ± 3.5	-
60 min	921.2 ± 10.1	1,009.2 ± 73.5	1,172.4 ± 18.7	357.9 ± 1.3
90 min	1,064.0 ± 75.1	987.6 ± 64.5	1,023 ± 67.3	406.2 ± 2.1

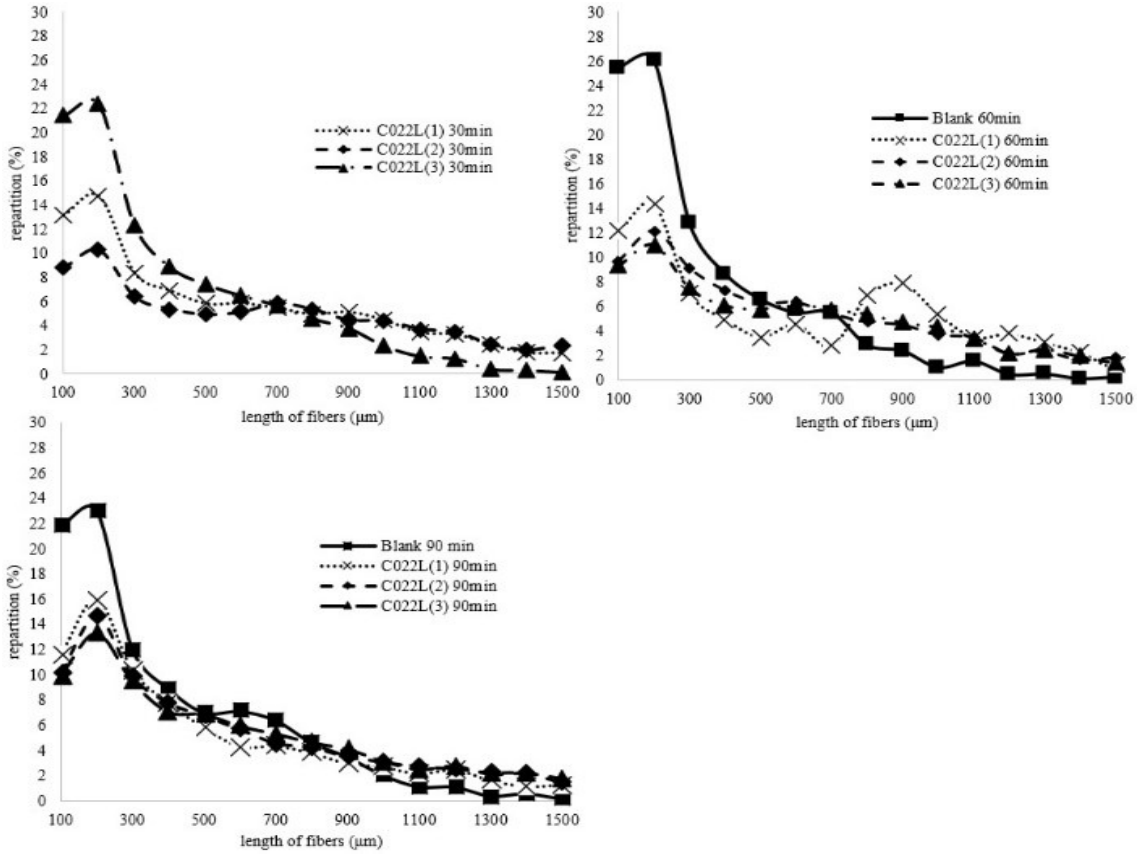

Fig. 3 Fibers length repartition (in average in weight).

compared with other enzymatic treatments. The formulation was more focused on the cutting effect along the fibers and its action gave more variations due to the presence of small flakes even after 60 min of treatment. The curve at 60 min with two peaks reflected the difficulty of the flakes to be disintegrated which explained the variation observed in Table 4. C022L(2) gave the most appropriate results regarding the fibers length repartition as it was expected in the previous data. Most of the fibers were longer since the enzymes were working together by synergy to make more fibrillation and removed the PAE adsorbed on the fibers surface. The repartition with this treatment was more homogeneous to compare with others. Results with C022L(3) at 30 min showed that small fibers were released from the catalysis. Since the flakes were very small and considered as "accepted", once they went through the analyzer they are not considered as "fibers". The cross-linked fibers forming flakes cannot be counted by the analyzer. After 60 min of treatment, the flakes were completely dispersed and the effect of enzymes along fibers suspension occurred.

Concerning the width of the fibers, the conclusion concurred with the analysis of the length (Fig. 4).

The fibers were thinner without enzymatic treatment. By adding enzymes during repulping, the width tended to increase which was also seen directly with the average of the width per samples (Table 5).

Enzymes especially the cellulases improved the hydration and the fibrillation effects on fibers. The difference between the formulations came from the type of laccases and the synergy they can have on the cellulase enzymes.

The fibrillation effect along the fibers can be observed especially after 30 min of treatment (Fig. 5). C022L(1) and (3) tended to cut the fibers length (Table 4) and the fibrills (Fig. 5). The laccases or the synergy with the other enzymes for these both cases, confirmed that the

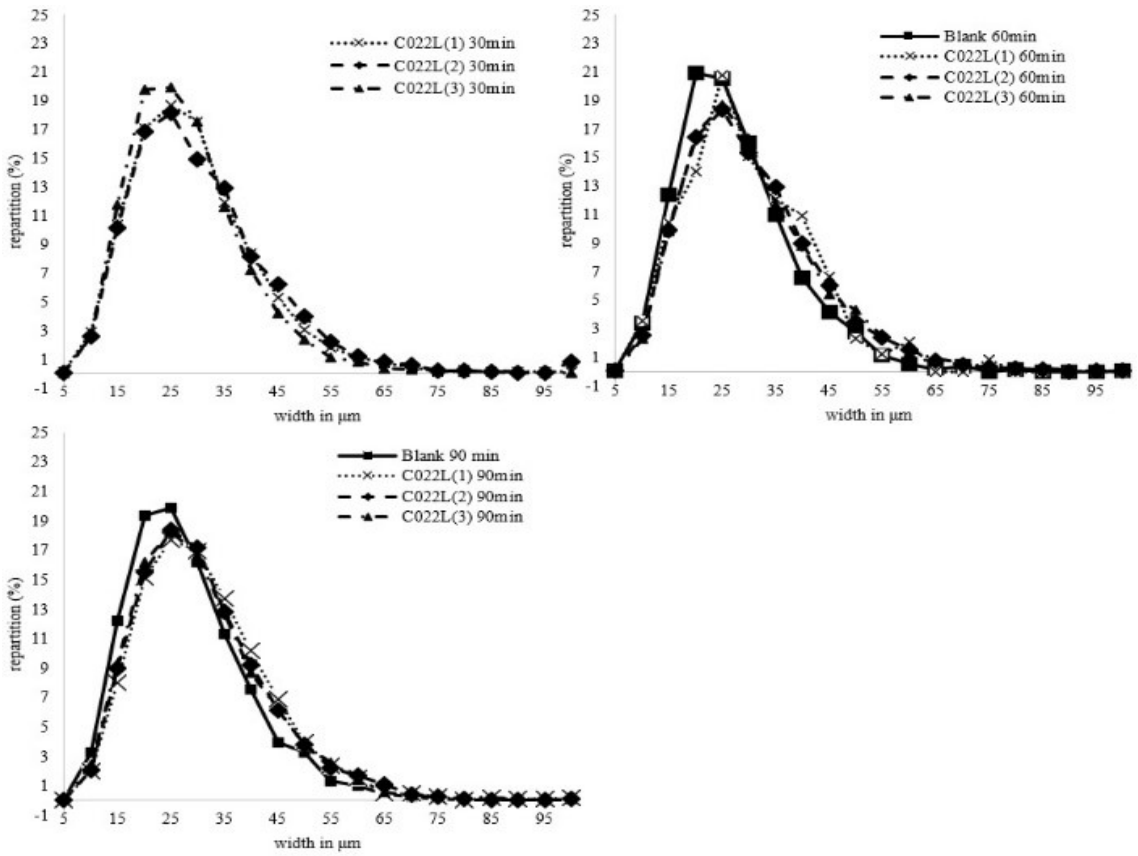

Fig. 4 Fibers width repartition (in average in weight).

Table 5 Average width of fibers (in weight) after enzymatic treatment including the blank.

l (µm)	C022L(1)	C022L(2)	C022L(3)	Blank
30 min	25.3 ± 0.1	25.8 ± 0.0	24.4 ± 0.1	-
60 min	25.3 ± 0.0	25.9 ± 0.0	26.0 ± 0.1	23.8 ± 0.1
90 min	26.4 ± 0.1	26.5 ± 0.1	26.1 ± 0.1	24.1 ± 0.1

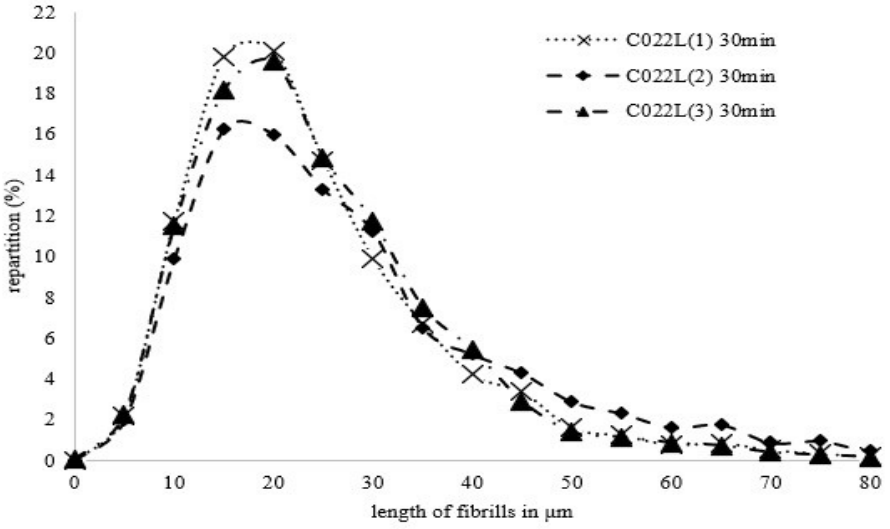

Fig. 5 Fibrills length repartition (in average in weight).

Table 6 Average of the fines ratio (in weight) after enzymatic treatment including the blank.

Fines (%) in weight	C022L(1)	C022L(2)	C022L(3)	Blank
30 min	16.2 ± 0.4	9.5 ± 0.2	25.7 ± 0.9	-
60 min	17.3 ± 0.2	9.3 ± 0.1	10.2 ± 0.6	38.4 ± 0.3
90 min	10.6 ± 0.7	9.3 ± 0.3	9.7 ± 0.7	31.6 ± 0.5

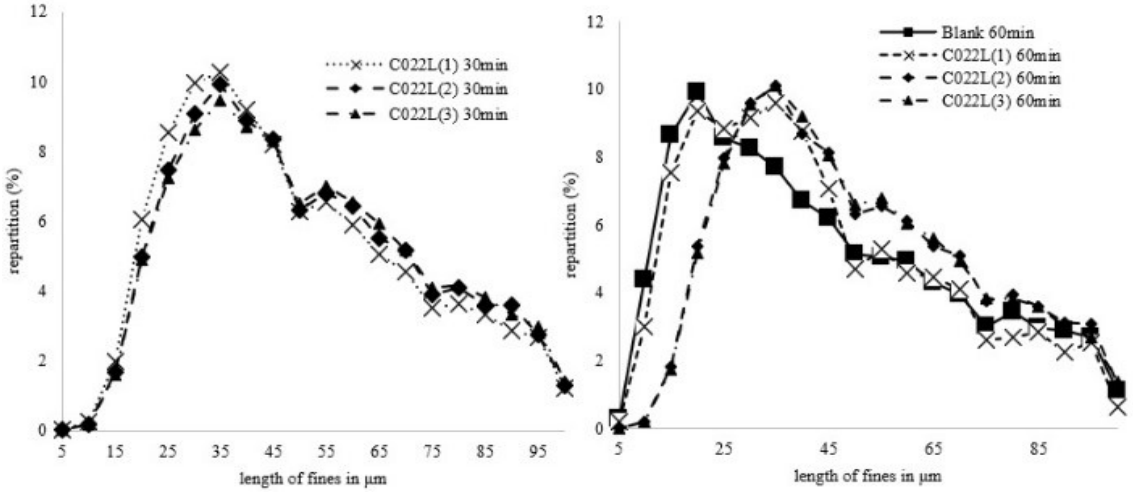

Fig. 6 Fines length repartition (in average in weight).

cellulases reacted faster than other enzymes in the system due to the availability of substrates for enzymes action. The most appropriate cocktail of enzymes for the application was the C022L(2) at 30 min. At 60 min the trend for all enzymes became similar due to the saturation of enzymes and also their efficiency regarding the raw material.

Regarding the fines level showed in Fig. 6, without any enzymatic treatment, the level of fines was higher due to the flakes content. Only the small fines and fibers were released in the tank where the measurements with the CELOFIBRE were done.

The conclusions regarding the previous results and Table 6 emphasized the cutting effect of fibers and fibrills with both formulations C022L(1) and (3). According to the graphs, the curves followed the previous conclusions.

3.3 Chemistry Analysis

Since the Lacc2 was the most relevant laccase from the investigation, the chemistry parameters were under control to observe a possible evolution at industrial scale. The objectives of this section were to know the limits and compare the enzymatic treatment with a standard chemical treatment used at industrial scale. The parameters were taken directly from the pulper during sampling to keep the temperature at 45 °C (Tables 7 and 8).

The repulping stage without enzymatic treatment involved over time a slight increase of the conductivity in the medium since the shear and the fibers' friction liberated the contaminants retained in the fibers network. The same effect was observed with the pH.

Table 7 Parameters of the pulp suspension after 30 min of enzymatic treatment under repulping at a consistency of 6.5%.

Treatment	Blank	C022L(0)	C022L(2)	C022L(2) Lip
pH	7.77	7.13	7.27	7.21
T (°C)	44.9	45.0	45.0	45.0
χ (µS/cm)	990.2	1,027.0	987.6	1,091.0
Orp (mV)	-100.1	-115.3	-101.3	-102.8

Table 8 Parameters of the pulp suspension after 60 min of enzymatic treatment under repulping at a consistency of 6.5%.

Treatment	Blank	C022L(0)	C022L(2)	C022L(2) Lip
pH	7.85	7.38	7.41	7.26
T (°C)	45.1	45.1	45.0	45.1
χ (µS/cm)	1,018.0	988.9	748.7	1,046.0
Orp (mV)	-109.3	-132.2	-113.8	-110.7

Table 9 Study of the degradation of heated PAE films at 40 °C for 40 min [31].

Chemical	Conc. (mmol)	pH initial	χ (µS/cm) initial	pH final	χ (µS/cm) final	Δm_{PAE} (%)
H_2O	100 mL	5.8	2.87	3.4	6,970	7.95
NaOH	2.500	10.7	170	4.4	16,060	8.57
H_2SO_4	1.020	1.9	656	2.5	4,680	8.27
K_2SO_4	0.866	3.9	1,817	3.5	4,880	9.40
H_2O_2	1.000	5.4	6.38	3.2	2,714	9.71

Table 10 Study of the degradation of heated PAE films at 80 °C for 180 min [31].

Chemical	Conc. (mmol)	pH initial	χ (µS/cm) initial	pH final	χ (µS/cm) final	Δm_{PAE} (%)
H_2O	100 mL	5.9	1.64	3.2	12,980	7.48
NaOH	20	11.0	7,800	10	44,400	17.4
H_2SO_4	1.04	2.8	921	3	13,200	7.31
K_2SO_4	1.04	3.9	2,162	3	8,160	17.8
H_2O_2	1.000	5.4	6.4	2.6	5,990	20.7

However, with enzymatic treatment the pH was slightly lower and kept the pH more neutral. The ORP value kept stable during the repulping process and was not impacted by the use of enzymes. The use of enzymes did not perturb the chemistry balance in the medium compared with the blank. Without laccases or by adding lipases in the formulation C022L, the conductivity increased between 0 and 30 min of treatment which indicated that both formulations were more aggressive than the C022L(2). The release of contaminants and by-products from the enzymatic reactions approved the fact that the cocktail of enzymes reacted faster. After 60 min of treatment, the conductivity of the formulation containing lipases became steady and stable.

Enzymatic treatment improved the fibrillation effect which created more anionic sites from carboxylic acid groups along the fibers. These sites reacted with cations from the medium which implied a reduction of free ions content in the medium. The effect was more intense for the C022L(2) where the conductivity decreased by 24.4% compared with the blank after 60 min.

The experiments were not performed with the chemical treatment used at industrial scale. According to the literature, the covalent bonds between the carboxyl groups of cellulose or hemicelluloses and the polymer's azetidinium group result in inter-fiber non soluble in water which make the repulping stage very difficult [16, 22]. Most of the papermills are forced to use a combination of mechanical energy and oxidizing agents with soda for repulping [27-30]. Siqueira [31] studied different chemical treatments usually used at industrial scale regarding the degradation of the wet strength resins. The conditions were set at 40 °C for 40 min and 80 °C for 180 min and the results were as mentioned in Tables 9 and 10.

Siqueira's work [31] mentioned that the efficiency of the chemistry used for repulping was based on the pH which needed to be set at 11 and had to be maintained until the end of the reaction. The first table (Table 9) showed that the conditions at 40 °C for 40 min was not enough to degrade PAE and needed to be increased at 80 °C [31]. The use of the chemistry showed also a huge impact on the conductivity which can have a direct impact on the paper production at the end and on the fresh water consumption.

Siqueira [31] suggested a mechanism of the PAE degradation where primary, secondary and tertiary amines and N-substituted amides of the PAE were susceptible to oxidation. Besides, the use of enzymes allowed avoiding the salt generation and limiting the use of fresh water in the system. Enzymes hydrolyze directly the cellulose cross-linked to the PAE and can be recovered by flocculation coagulation easily or recovered directly as a fine.

3.4 Observation of the Handsheets

The handsheets after repulping were made for a visual observation (Fig. 7) on the blank and formulations containing different laccases. The observation confirmed the previous results concerning the flakes content. Also, depending on the type of laccases used, the treatment induced paper yellowing.

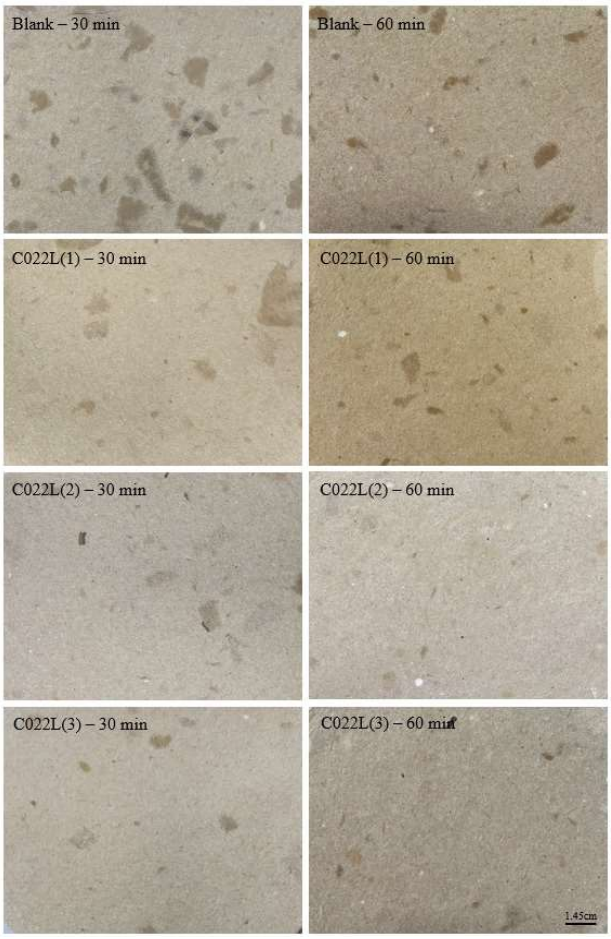

Fig. 7 Observation of the handsheets after sampling.

The Lacc1 used for the experiment proved that the formulation did not help to repulp properly. The conclusion concerning this formulation showed that some laccases dedicated to the lignin removal can have a negative effect on the unbleached kraft pulp especially with the yellowing effect and with the suspicion to limit the efficiency of the other enzymes contained in the formulation. However, the two other laccases (Lacc2 and Lacc3) showed that the formulations helped the repulping stage and the results suggested that a bleaching effect can be observed for the case of Lacc2.

3.5 Industrial Scale

The time of repulping according to the conditions mentionned in the Section 2.7 approved by the paper mill was reduced to 30 min during the trial. The reduction of time could be made since the consistency was higher at industrial scale and the shear in the pulper was more important due to the design of the rotor. The consistency was one of the key parameters during trial and was under investigation in the first part of this study.

The pH needed to be adjusted with soda at 10 with the use of chemicals. After repulping, it was down to 7 in order to avoid issue with the production. With enzymes, no adjustment was made and pH kept stable at 6.9. The conductivity of the water with chemistry was more than 5 mS/cm and was down to 2 mS/cm after few pulpers with enzymes. The trial suggested a reduction of the conductivity in the global process. Concerning the temperature, since the diluted water for repulping came from the machine, the temperature reached 51 °C at the end with enzymes against 80 °C with chemistry. The savings with the enzymatic treatment were from the fresh water consumption, the chemistry cost, the energy of repulping and steam. No flakes were observed at the end of the repulping.

4. Conclusions

The role of cocktail enzymes for bio-repulping of unbleached kraft pulp with a high WS resin content was

investigated to select the appropriate synergy between enzymes to respond to the challenges regarding the sustainability of the pulp and paper industry.

Enzyme-based ecofriendly and greener bio repulping technologies are essential and productive. These biotechnologies are intended to stop the use of chemicals, which are toxic and pollute the environment, especially after paper mill effluents are discharged into the environment. It also enables to reduce the energy costs and highlight the possibility of improving the runnability of the machine for production. Less chemicals use means less fresh water is used to reach the goal of the quality of the paper, which is particularly important in developing countries where water scarcity is a major issue.

The lab scale investigation enabled to select the best choice for the industrial scale and understand the synergy between all the enzymes. This study was also focused on the fact that enzymes can be formulated together but they reacted differently between each other. The choice of enzymes in the cocktail had to be appropriate for the application itself. The present study approved that the selection of the laccases on an enzymatic blend was important to avoid inhibition or an unappropriated effect on the raw material despite the fact that the laccases were sold as an enzyme dedicated for the delignification. Large-scale application of enzymes for bio-repulping was approved by the papermill and their production becomes greener and more stable.

References

[1] Laivins, G. V., and Scallan, A. M. 1993. "The Mechanism of Hornification of Wood Pulps." *Transactions of the 10th Fundamental Research Symposium* 2: 1235-60.

[2] Bajpai, P. 1999. "Application of Enzymes in the Pulp and Paper Industry." *Biotechnol. Prog*. 15 (2): 147-57.

[3] Viikari, L. 2002. "Trends in Pulp and Paper Biotechnology." In *Biotechnology in the Pulp and Paper Industry* (vol. 21), edited by L. Viikari and R. Lantto. Amsterdam: Elsevier, pp. 1-5.

[4] Chandra, R. P., and Ragauskas, A. J. 2002. "Evaluating Laccase-Facilitated Coupling of Phenolic Acids to High-Yield Kraft Pulps." *Enzyme Microb. Technol.* 30 (7): 855-61.

[5] Chakrabarti, S. K., and Ragauskas, A. J. 2002. "Elucidating of Effects of Laccase on the Physical Properties of High-Kappa Kraft Pulps." *Prog. Biotechnol.-Amst.* 21: 165-72.

[6] Chandra, R. P., Lehtonen, L. K., and Ragauskas, A. J. 2004. "Modification of High Lignin Content Kraft Pulps with Laccase to Improve Paper Strength Properties. 1. Laccase Treatment in the Presence of Gallic Acid." *Biotechnol. Prog.* 20 (1): 255-61.

[7] Chandra, R. P., Felby, C., and Ragauskas, A. J. 2005. "Improving Laccase-Facilitated Grafting of 4-Hydroxybenzoic Acid to High-Kappa Kraft Pulps." *J. Wood Chem. Technol.* 24 (1): 69-81.

[8] Kumar, A., *et al.* 2022. "Chapter 14: Enzyme Cocktail: A Greener Approach for Biobleaching in Paper and Pulp Industry." In *Nanotechnology in Paper and Wood Engineering*, edited by R. Bhat, A. Kumar, T. A. Nguyen, and S. Sharma. Amsterdam: Elsevier, pp. 303-28.

[9] Pommier, J.-C., Goma, G., Fuentes, J.-L., and Rousset, C. 1990. "Using Enzymes to Improve the Process and the Product Quality in the Recycled Paper Industry. II: Industrial Applications." *Using Enzym. Improve Process Prod. Qual. Recycl. Pap. Ind. II Ind. Appl.* 73 (12): 197-202.

[10] Bhat, R., Heitmann, J., and Joyce, T. 1991. "Novel Techniques for Enhancing the Strength of Secondary Fiber." *Tappi J.* 74 (9): 151-7.

[11] Bhardwaj, N. K., Bajpai, P. K., and Bajpai, P. 1995. "Use of Enzymes to Improve Drainability of Secondary Fibres." *Use Enzym. Improve Drainability Second. Fibres* 48 (5): 378-80.

[12] Bhardwaj, N. K., Bajpai, P., and Bajpai, P. K. 1997. "Enhancement of Strength and Drainage of Secondary Fibres." *Enhanc. Strength Drain. Second. Fibres* 50 (3): 230-2.

[13] Sarkar, J. M., Cosper, D. R., and Hartig, E. J. 1995. "Applying Enzymes and Polymers to Enhance the Freeness of Recycled Fiber." *Tappi J.* 78 (2): 89-95.

[14] Oksanen, T., Pere, J., Paavilainen, L., Buchert, J., and Viikari, L. 2000. "Treatment of Recycled Kraft Pulps with *Trichoderma reesei* Hemicellulases and Cellulases." *J. Biotechnol.* 78 (1): 39-48.

[15] Bajpai, P. K. 2010. "Solving the Problems of Recycled Fiber Processing with Enzymes." *BioResources* 5 (2): 1311-25.

[16] Felby, C., Pedersen, L. S., and Nielsen, B. R. 1997. "Enhanced Auto Adhesion of Wood Fibers Using Phenol Oxidases." *Holzforschung* 51 (3): 281-6.

[17] Kharazipour, A., Huettermann, A., and Luedemann, H. D. 1997. "Enzymatic Activation of Wood Fibres as a Means for the Production of Wood Composites." *J. Adhes. Sci. Technol.* 11 (3): 419-27.

[18] Yamaguchi, H., Maeda, Y., and Sakata, I. 1994. "Bonding among Woody Fibers by Use of Enzymatic Phenol Dehydrogenative Polymerization: Mechanism of Generation of Bonding Strength." *Mokuzai Gakkaishi* 40 (2): 185-90.

[19] Jones, D. R., and Fitzhenry, J. W. 2003. "Esterase-Type Enzymes Offer Recycled Mills an Alternative Approach to Stickies Control." *Pulp Pap.* 77 (2): 28.

[20] Crisp, M. T., and Riehle, R. J. 2018. "Regulatory and Sustainability Initiatives Lead to Improved Polyaminopolyamide-Epichlorohydrin (PAE) Wet-Strength Resins and Paper Products." *Tappi J. USA* 17 (9): 519-32.

[21] Espy, H. 1995. "The Mechanism of Wet-Strength Development in Paper: A Review." *Tappi J. USA* 78 (4): 90-9.

[22] Wågberg, L., and Bjőrklund, M. 1993. "On the Mechanism behind Wet Strength Development in Papers Containing Wet Strength Resins." *Nord. Pulp Pap. Res. J.* 8 (1): 53-8.

[23] Lindström, T., Wågberg, L., and Larsson, T. 2005. "On the Nature of Joint Strength in Paper—A Review of Dry and Wet Strength Resins in Paper Manufacturing." In *Trans. of the XIIIth Fund. Res. Symp. Cambridge*, FRC, Manchester, 2018, pp. 457-562.

[24] Sharma, S., and Deng, Y. 2016. "Dual Mechanism of Dry Strength Improvement of Cellulose Nanofibril Films by Polyamide-Epichlorohydrin Resin Cross-Linking." *Ind. Eng. Chem. Res.* 55 (44): 11467-74.

[25] Yang, R., and Luettgen, C. 2020. "Repulping of Wet Strength Paper Towel with Potassium Monopersulfate." *Tappi J.* 19 (9): 463-70.

[26] Devore, D. I., and Fischer, S. A. 1993. "Wet-Strength Mechanism of Polyaminoamide-Epichlorohydrin Resins." *Wet-Strength Mech. Polyam.-Epichlorohydrin Resins* 76 (8): 121-8.

[27] Espy, H. H., and Geist, G. W. 1993. "Persulfates as Repulping Reagents for Neutral/Alkaline Wet-Strength Broke." *Persulfates Repulping Reag. Neutralalkaline Wet-Strength Broke* 76 (2): 139-42.

[28] Balos, B., Clungeon, N. S., Patterson, J. V., Rodriguez, J. M., and Fischer, S. A. 2023. "Process for Repulping Wet Strength Paper." US5593543A, 14 janvier 1997 https://patents.google.com/patent/US5593543A/en.

[29] Bhardwaj, N. K., and Rajan, V. 2004. "Wet Strength Paper Repulping: Effect of Process Variables." *Appita Technol. Innov. Manuf. Environ.* 57 (4): 305-10.

[30] Adams, J. J. 2017. "Wet Strength Paper Repulping." M.Sc. thesis, Monash University.

[31] Siqueira, E. J. 2012. "Polyamidoamine Epichlorohydrin-Based Papers : Mechanisms of Wet Strength Development and Paper Repulping." Ph.D. thesis, Universidade da Beira Interior.