

Improved Fiber Separation and Energy Reduction in Thermomechanical Pulp Refining Using Enzyme-Pretreated Wood

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Black spruce (*Picea mariana*) wood chips were subjected to enzymatic pretreatments, using an enzyme formulation containing cellulolytic and hemicellulolytic enzymes, combined with light mechanical treatments including downsizing and/or compression/decompression. These pretreatments were followed by 3 stages of mechanical refining in a lab-scale disc refiner. Depending on the treatment, the overall energy savings obtained could be as high as 15%. Most of the savings were obtained during the first stage of refining. Pulps collected after this stage were imaged using TEM and SEM. Differences were observed between differing treatment types. The most significant differences were seen when macerated chips were compressed and allowed to decompress in enzyme solutions. Enzyme treatments are able to change the rupture pattern during refining, thus lowering the overall specific energy consumption (SEC) as evidenced by improved pulp freeness and direct energy measurements. Increasing enzyme penetration helps to improve the overall SEC savings and also improves the distribution of energy savings throughout the refining stages by moving initial fiber separation from the middle lamella into the secondary wall.

Keywords: Mechanical pulping; Enzymatic treatments; Specific energy consumption; Fiber modification; Lignocellulosics

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INTRODUCTION

Thermomechanical pulps (TMP) are still in demand despite the recent tumble in the pulp and paper market. This is mainly due to very high yields (~95%) that limit the need for vast quantities of raw material. These pulps also have certain attractive qualities such as bulk and opacity that lend themselves well to certain types of end products. These selling points coupled with advancements that have allowed for better recyclability have permitted the sector to remain viable for the time being; however, this is a very energy-intensive process and with increasing energy costs, which will likely only continue to increase in the near future. These mills will need to continue researching and implementing novel innovations in order to remain viable.

One such area of interest is biopulping. This process involves the use of different strains of fungi that have the ability to degrade certain components in wood structure. This degradation in turn makes for an easier separation of fibers from whole wood. The main focus has been on lignin-degrading basidiomycetes called white rot fungi. These fungi are capable of selectively degrading the lignin in woody substrates. They do this in order to feed on carbohydrates exposed from the delignification process. Some studies

have shown significant specific energy consumption (SEC) reductions (as high as 40%) when chips were inoculated with strains of white rot fungi (Akhtar *et al.* 1993, 1998; Ferraz *et al.* 2008).

Biopulping does have its disadvantages. Since the organisms used in these types of applications need to be grown, chips must be relatively sterile. They also must be stored for long periods of time, sometimes weeks, and kept at specific temperatures and humidity in order to optimize this growth (Akhtar *et al.* 2000). The implementation of a mill that would be equipped to handle these demands would have a much larger footprint than a traditional mill of similar capacity. This would in turn require much more capital. To help overcome these drawbacks, focus has shifted towards the enzymes produced by these fungi.

Industrial-scale enzyme production and purification methods have come a long way. Protein engineering and genetic engineering of new organisms capable of producing novel enzymes in sufficient quantities have made significant advances in the last decades. These man-made enzymes are able to withstand the more severe conditions found in industrial process streams. As more active enzymes are discovered and/or created, treatment times will continue to drop and efficiencies will continue to increase. Some studies report significant energy savings with relatively short treatment times (as low as 30 minutes) (Meyer *et al.* 2008). Since sound wood has a median pore size of 20 to 40Å (Stone *et al.* 1968) and on average hydrolytic enzymes are greater than 50Å (Cowling *et al.* 1976; Walker *et al.* 1991), decreases in enzyme size, while maintaining activity, will likely play a key role in these improvements (Chen *et al.* 1988; Maiti *et al.* 1997).

Even though the enzyme mechanisms and the reactions that they catalyze are becoming better known, the exact process that allows these reactions to lower the amount of energy required to reach a given degree of refining is still not very well understood. During purely mechanical refining (refiner mechanical pulping, RMP, and pressurized ground wood, PGW) fiber separation occurs between the primary and secondary wall and more preferably between S1 and S2 layers, but in TMP, the initial separation usually occurs at the middle lamella, leaving lignin behind that can interfere with interfiber bonding and increase the amount of energy needed for fibrillation (Shao and Li 2006). A study by Lei *et al.* (2008) suggested that the use of a xylanase pretreatment prior to refining could loosen the fiber wall allowing ruptures to occur predominantly at the preferable S1-S2 boundary and lowering refining energy.

Compression/decompression has been shown to facilitate penetration of aqueous solutions by a few studies (Watanabe *et al.* 1998; Abe *et al.* 2001) and one in particular by Hart *et al.* (2009) investigated the impregnation of enzymes for the reduction of energy in alkaline-peroxide mechanical pulping (APMP). In the present study, a complex enzyme mixture containing both cellulolytic types (endoglucanase and cellobiohydrolase) along with a hemicellulase (mannanase) was used to pretreat black spruce chips. The wood chips were first macerated using an Impressafiner-type chip maceration device. This device provides both shear and compressive forces. The macerated chips were then recompressed prior to being allowed to decompress in the enzyme solution. Samples of hydrolysed macerated chips were imaged by scanning electron microscope (SEM) and transmission electron microscope (TEM) to see the effects of enzymatic hydrolysis, the rest was refined in a KRK Ltd. single disc atmospheric refiner coupled to an energy monitor which was used to determine specific energy consumption (SEC). Samples were taken from the first stage of refining and again imaged using SEM and TEM to examine

whether the changes in energy observed corresponded to changes in the fiber separation location predicted by Lei *et al.* (2008).

EXPERIMENTAL

Materials

Woody material: 100% Black spruce (*Picea mariana*) wood chips were collected after washing and screening from Irving Pulp & Paper, Saint John, NB. Some chips were compressed with an Andritz Impressafiner providing macerated wood chips. This process consumed, on average, 75 kWh/oven dried ton (ODT). The macerated chips were tested for moisture (TAPPI method T210 cm-03) before further treatments were applied.

The enzyme solution, Celluclast 1.5L, used was provided by Novozyme Inc. (USA). The applicable pH for this enzyme solution is from 4.0 to 5.0, and the temperature can range from 40 to 60 °C. Soaked pulp initial pH was roughly 5.5. Diluted reagent grade HCl or NaOH was used to adjust the pH accordingly. All other chemicals and reagents were purchased from Sigma Aldrich or Fisher.

Methods

Enzyme activity determination

Standard enzyme activity determination methods were applied in this study (Adney and Baker 2008). In this method, we applied enzymes to hydrolyze standard substrates, then used a spectrophotometer (Milton Roy, Spectronic 1001 Plus) to test the absorbance of the coloured solution obtained after applying the 3,5-dinitrosalicylic acid (DNS) method as described by Miller (1959). The enzyme activities obtained are listed in Table 1. CMCase, filter paper, and mannanase activity were determined using carboxymethyl cellulose, Whatman #1 filter paper, and locust bean gum as substrates, respectively. The reducing ends of the sugar chains released are measured following the methods mentioned above.

Table 1. Enzyme Activities

Activity Type	Enzyme Activity (AU/mL)
CMCase	1850
Filter paper	150
Mannanase	285

Hydrolysis conditions

According to preliminary work, the enzyme dosage was chosen to be 10 Filter Paper Units per gram of oven dried wood (FPU/g ODW) and applied in each enzyme treatment. Two hundred grams of oven dried wood (ODW) was made into a solution of 10% consistency with tap water at 50 °C. The solution was brought to the proper pH with

a dilute HCl solution or a dilute NaOH solution when necessary. This was prepared within a plastic bag. After adding the appropriate amount of enzyme solution, the plastic bags were thermosealed and placed in a hot water bath at a given temperature. Initial hydrolysis time was chosen to be one hour. During this time the bags were massaged every 15 min to ensure proper heat and enzyme distribution throughout the hydrolysis. A control was prepared in the same way with the exception of enzyme addition in order to eliminate the possible effects of pH adjustments alone.

At the end of the hour, bags were removed from the hot water bath. Chips were then dewatered (a 100 mL sample of filtrate was kept for reducing sugar analysis) and rewatered to the refining consistency.

Recompression and decompression of macerated wood chips in hydrolysis liquor

Wood chip maceration was done at PAPTAC's Pointe-Claire, Qc in an MSD Impressafiner. Macerated wood chips were either used as obtained or recompressed in a laboratory scale compression device capable of delivering a maximum of 100 atm of compressive force. After compression, an enzyme solution of given strength was added to the compression chamber while the chips were expanding. Hydrolysis was then allowed to occur following the stated conditions.

Refining conditions

The refiner was first run for an appropriate amount of time (1 hour) to ensure proper shaft thermal balance, thus maintaining correct gap measurements throughout the entire process. Once this was achieved, the plate gap was set to 0.50, 0.30, and 0.15 mm for first, second, and third stage refining, respectively. Feed rate of chips to the refining zone was controlled using a set speed of 2 on the feeder motor control as well as by hand feeding to the hopper to avoid plugging. This amounted to a feed rate of approximately 75 g OD material per minute. The main motor was not stopped between stages. The pulp collected was weighed and strained and/or rewatered to adjust the consistency to the correct value. Pulp that remained in the refiner, stuck in the gaps, was measured previously and was found to be on average 50 g OD pulp. This loss was accounted for when adjusting consistency, and the feed time was also modified accordingly. Consistencies were chosen to be 15 and 20% for the first and last two stages, respectively. This was done to limit the amount of steam generated during refining. An ION 7330 energy meter was coupled to the motor to allow for accurate energy consumption monitoring.

CSF testing

Following hydrolysis and refining, CSF was determined as a measure of degree of refining. In this case, pulp was collected after the final pass in the refiner and was sealed in airtight bags to balance moisture and prevent mould growth. The moisture content was checked before CSF was measured following the same basic procedures described in TAPPI standard method T 227 om-09.

Imaging

SEM images were taken on a JEOL JSM6400 Digital SEM. TEM images were taken on a JEOL 2011 STEM. Sample preparation was done at the Microscopy and Microanalysis Facility at the Fredericton campus of the University of New Brunswick. Enzyme precipitation was done following a method described by Donaldson (1988). In this method, enzyme-treated substrates are dewatered and then soaked in a solution of

10%/10% (v/v) phosphotungstic/hydrochloric acids. Their group showed that this solution was able to precipitate cellulase enzymes. The precipitate formed was described as being a complex since the precipitate formed was white, while heat-denatured protein precipitate was brown.

RESULTS AND DISCUSSION

Structural changes of macerated chips as observed by TEM

Donaldson used the technique described in the Experimental section to observe enzyme penetration within holocellulose and alpha-cellulose. Figure 1 shows control samples stained with either KMnO_4 /lead citrate (top images), as used by Donaldson, or with uranyl acetate/lead citrate (bottom images). It was decided that the lighter images provided by the uranyl acetate/lead citrate allowed for better visualization of the various cell wall layers and was thus chosen for all subsequent images. In the images provided in Fig. 1, there are clear indications of mechanical-type disruptions caused by shear and compression forces produced during maceration. The fibers are being pulled and torn apart, mainly in the middle lamella (ML) and between it and the primary layer (P).

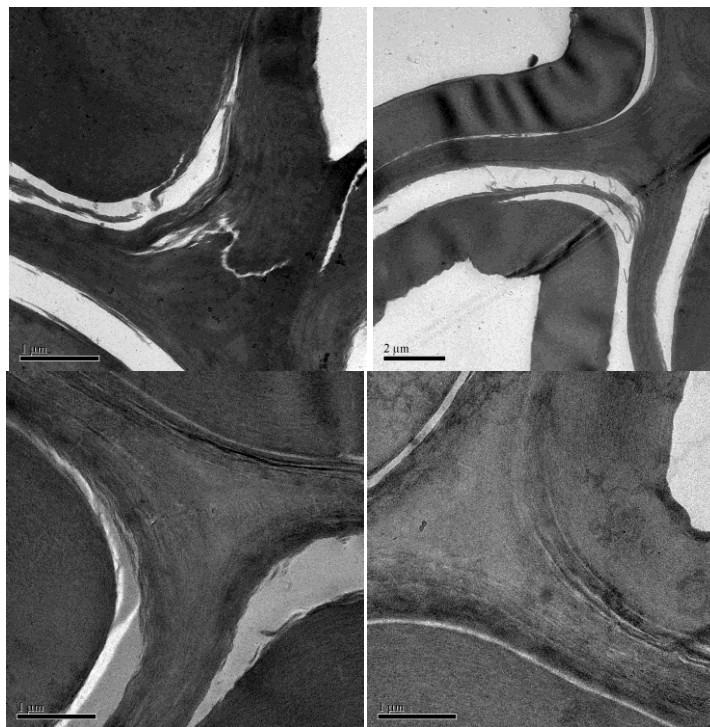


Fig. 1. Examples of control fibers. The top images were stained with KMnO_4 /lead citrate while the bottom images were stained with uranyl acetate/lead citrate.

Figure 2 shows several images taken of a matchstick from the macerated chips treated with 5.0 FPU/g ODW of the enzyme solution and stained using the uranyl stain. Some precipitates can be seen on the lumen side, as well as on most accessible surfaces. According to Donaldson, these are enzymes fixed by the PTA solution. Several areas of the fibers shown in the left image contain some areas weakened by enzymatic attack. One such area is highlighted by a small black square. The other images are further

magnifications of the same area. The damage to the fibers shows clear indications of fiber material “dissolution” as the enzymes are working. The larger black precipitates, as mentioned earlier, are large agglomerations of protein from the enzyme. The enzymes that are penetrating deeper into the hydrolysis site are too small to create larger agglomerations and are thus not seen here. Figure 3 shows another area depicting some more enzyme attack.

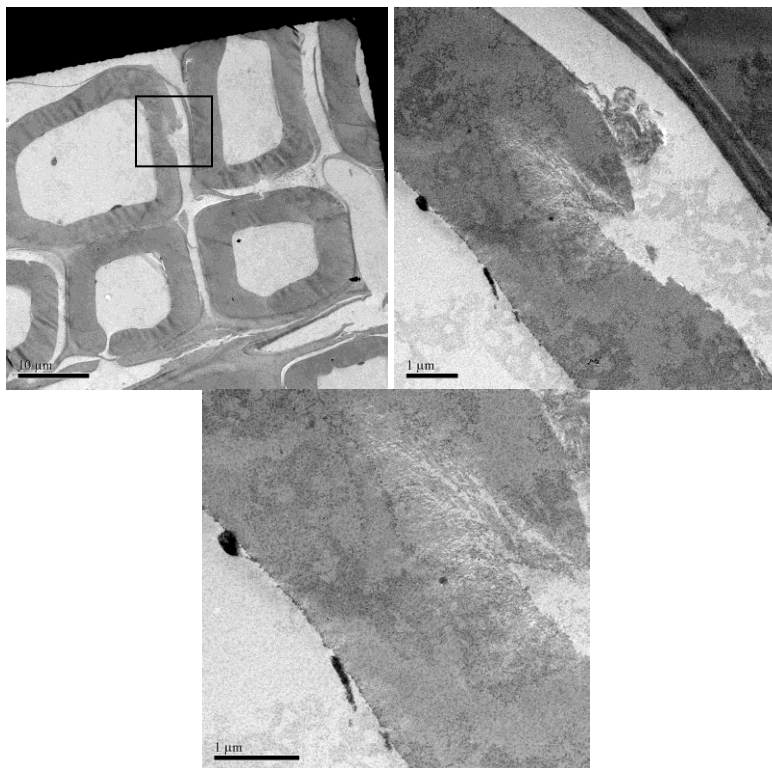


Fig. 2. The top left image shows part of a macerated wood chip decompressed in an enzyme solution. After dewatering, the chips were treated in a PTA solution to fix and deactivate the enzyme. There is clear evidence of wood cell wall hydrolysis. One such area is magnified and shows precipitated enzymes.

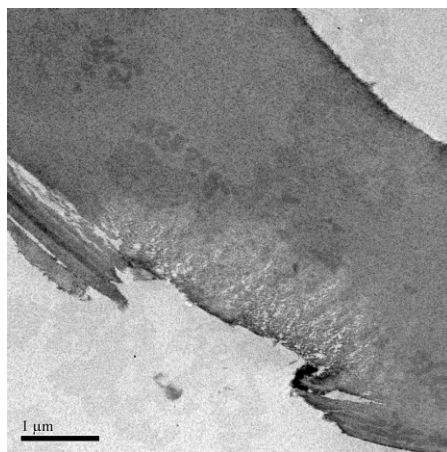


Fig. 3. Another area from the treated sample showing more enzyme activity and precipitation

The bigger precipitates are more likely larger agglomerations of enzymes (considering they are close to 250 nm across, while a typical enzyme is around 3 to 11 nm across). Donaldson also observed this. Some smaller precipitates can also be seen along the area where the hydrolysis is occurring. Energy-Dispersive X-ray spectroscopy was done in an attempt to confirm the formation of a tungsten complex; however, the concentrations were either too low or there was no complex formation and any Tungsten associated with the protein was washed away.

Specific Energy Consumption

In a previous article (Pelletier *et al.* 2013), the authors presented work showing that macerated chips that were compressed and allowed to decompress while in the enzyme solution resulted in greater energy savings (~15%) than either chips that were compressed\decompressed or macerated without compression\decompression. Figure 4 shows, on the left, a graph taken from Pelletier *et al.* 2013, and presents the overall SEC after three stages of refining. Data are averages of two separate runs and error boars are standard deviations (SD). The graph on the right in Fig. 4 shows the final CSF obtained as a method of comparing degree of refining. The CSF was slightly different but fell within +/-10 mL, which is typical for mechanical pulps (T 227 om-99), especially considering that the SD for compressed values are larger than for controls. These graphs show that the different pulps had been refined to comparable levels.

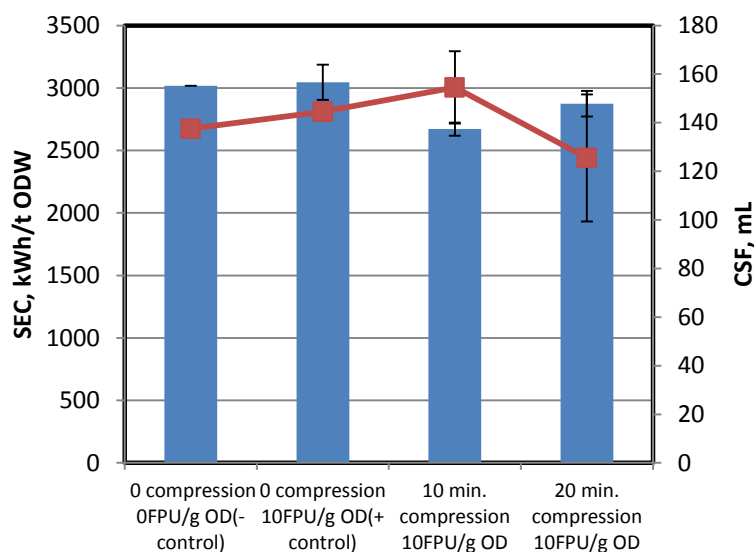


Fig. 4. Overall SEC reductions (Pelletier *et al.* 2013) and freeness of finished pulp (triplicate of each refining run for a total of 6 measurements with SD)

Various treatment conditions were compared for the maceration of chips. The energy usage was broken down in order to determine which refining stage saved the most energy. Figure 5 clearly shows that in all cases most of the energy saved occurs during the first stage. This is to be expected, since the majority of the weak points created by the treatment should be broken. An important observation made in the previous paper (Pelletier *et al.* 2013) was that the compression\decompression treatment could improve enzyme penetration under certain conditions, and this could also explain why its energy consumption distribution between the stages was slightly more balanced than the others. Extending compression time seemed to have a negative impact on penetration but could

be caused by a number of factors including the low number of replicates (2), as well as difficulties in accurately reproducing decompression speeds. This last possibility could also hint at changes in material properties of the macerated chips; it is possible that extending compression time affected the chips ability to decompress as fast as with lower compression times.

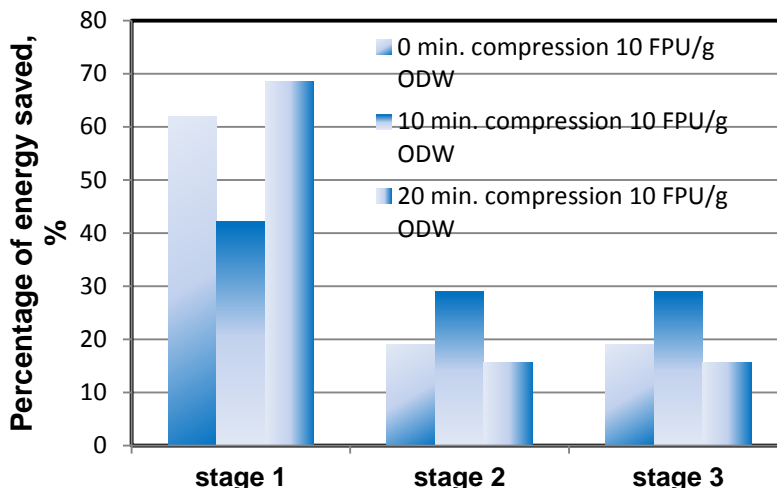


Fig. 5. Percentages of overall energy savings attributed to each stage of mechanical refining depending on treatment conditions.

SEM and TEM of 1st Refined Pulps

The energy savings distribution obtained indicated that some interesting changes to wood structure and fiber separation were occurring during the first stage of refining. Pulps collected from this stage were then used to collect both SEM and TEM images in the hopes of finding some differences in fiber separation location. First, whole chips (untreated and enzyme treated) were refined in order to compare with the macerated (untreated and compression enzyme treated). Figure 6 shows various SEM images of untreated whole chips (on the left) and enzyme treated chips (on the right). In these images some differences can be observed. The top left image of a fiber bundles shows slightly less separation and slightly more overlap than the treated sample to the right. The right side sample shows the beginnings of some fibrillation along with more distinct separation between the individual fibers. The middle and bottom left fiber surfaces show ML and P wall remainders covering fiber bundles, while in the images to the right, the deeper P wall, transitioning to the secondary (S1, S2, or S3) wall, is exposed, as evidenced by the directionality of the microfibrils (Barnett *et al.* 2004; Donaldson 2008).

Similar images were taken after refining the untreated and enzyme-treated macerated chips. Since some minor changes were observed for the whole chips while little to no change in energy consumption was observed (Pelletier *et al.* 2013), it was expected that the macerated chips with better energy savings observed would show greater changes to fiber separation.

Figure 7 shows SEM images of untreated (on the left) and enzyme-treated (on the right) macerated chips after one stage of mechanical refining. Maceration alone seems to have improved fiber separation which resulted in some energy savings on its own. This has been observed in prior works (Sabourin 1998; Kure *et al.* 1999).

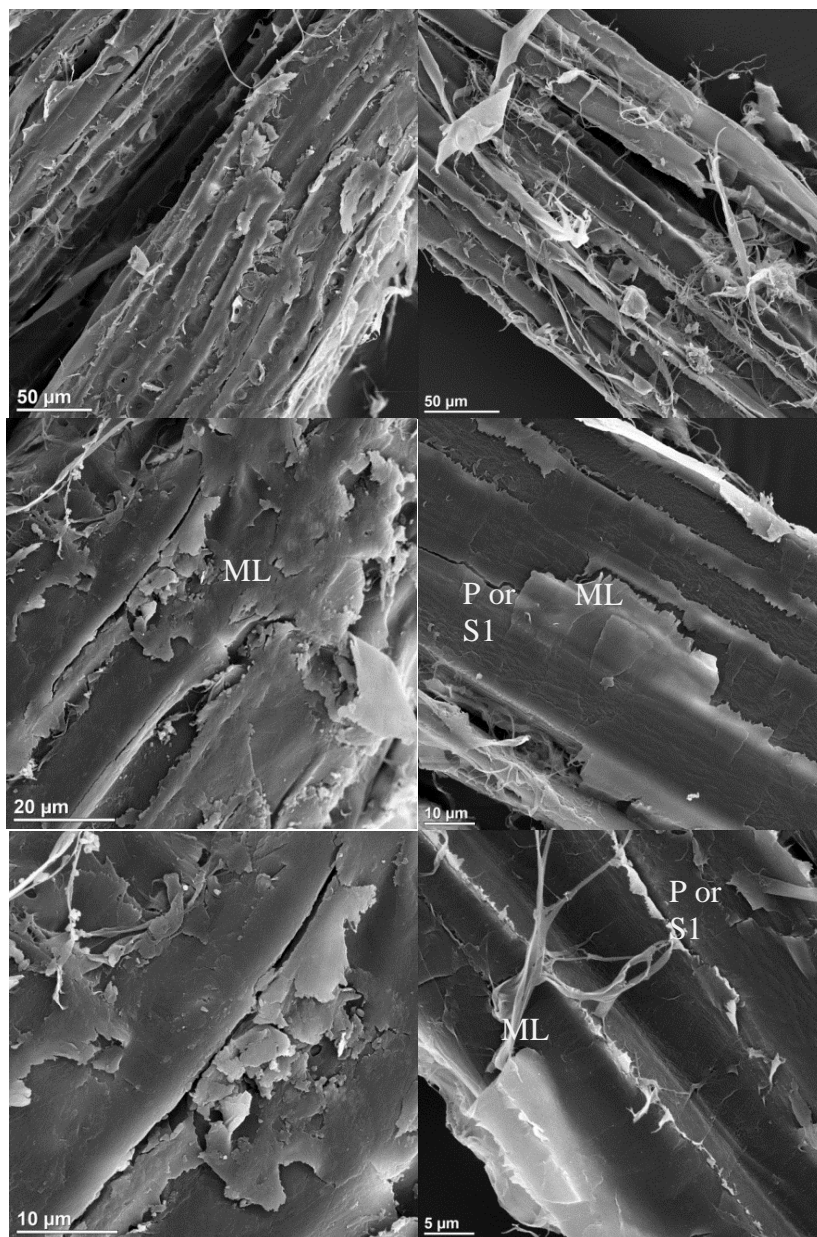


Fig. 6. SEM images, at various magnifications, of control (left side) where all fibers are covered in ML and P remainders. Enzyme-treated (right side) whole chips after one stage of refining, showing some increased fibrillation and some exposed P or S1.

The top two images in Fig. 7 are very similar to the top two images in Fig. 5. Fiber bundles appear smaller, containing fewer fibers resulting in better separation. Also, when using higher magnification, it becomes evident that the enzyme-treated macerated chips had more exposed microfibrils than in any other of the treatment conditions. It is clear when comparing the left and right side images for the bottom two rows that the peeling observed in the enzyme treated samples reached deeper into fiber cell wall and exposed more cellulose microfibrils.

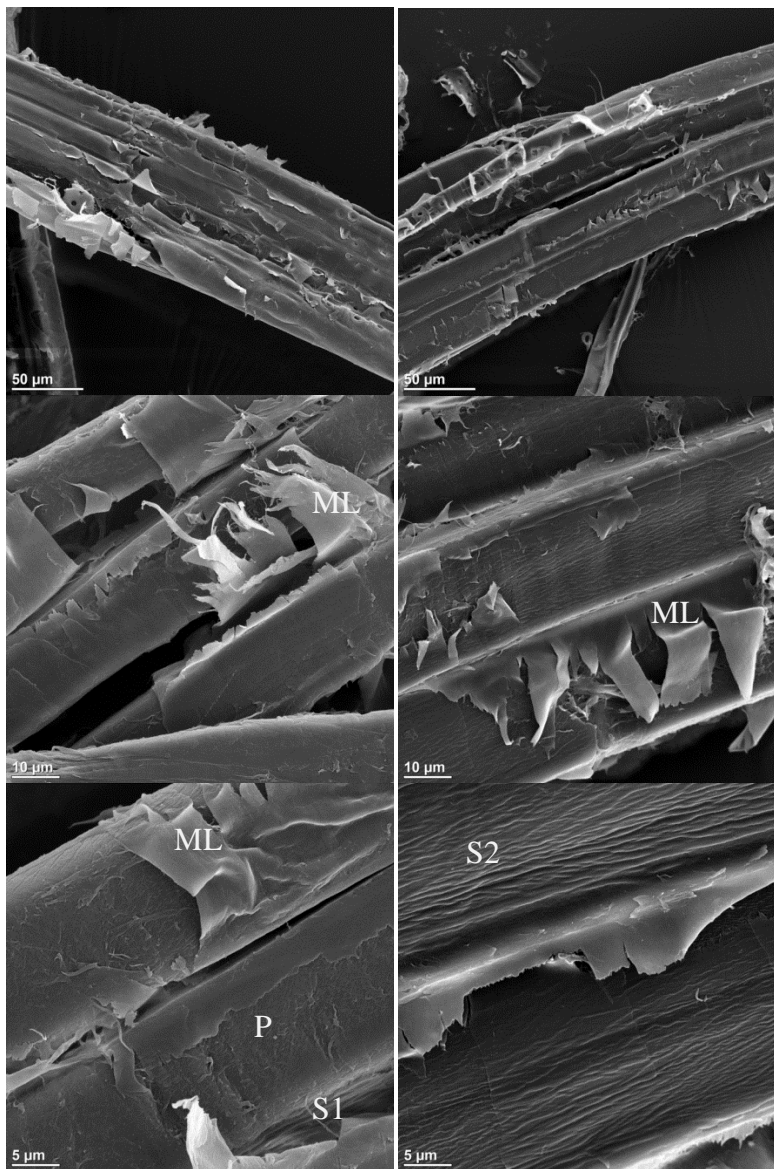


Fig. 7. SEM images, at various magnifications, of control (left side). Enzyme-treated (right side) macerated chips after one stage of refining

TEM imaging of the same samples revealed further differences between the various treatments that help explain the differences in SEC. Figure 8 again compares untreated whole chips (on the left) and enzyme-treated chips (on the right). The images to the left show some severe mechanical tearing and cutting with some disruptions making it completely through the fiber wall.

Any delaminations that do occur usually happen in the middle lamella or at the interface between that layer and the primary cell wall. While enzyme penetration is limited in whole chips, there is evidence that some enzyme action did take place, as is evidenced by some changes in mechanical action in the images on the right. In these images, some separation occurs into the secondary cell wall.

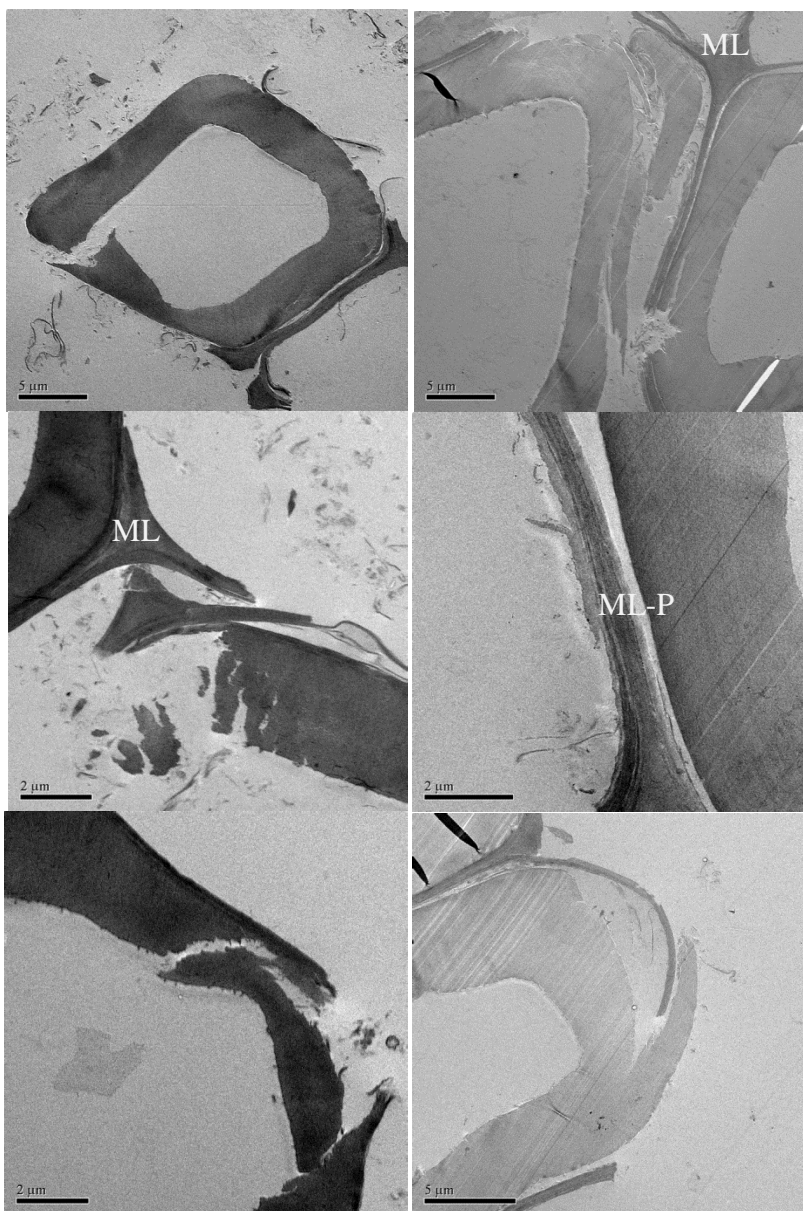


Fig. 8. TEM images, at various magnifications, of control (left side) and enzyme-treated (right side) whole chips after one stage of refining

The macerated chips showed some significant differences from the whole chips without needing any enzyme treatment. Figure 9 shows the untreated (on the left) and (on the right) enzyme-treated macerated chips. In the images on the left, it is evident that the fibers produced from the macerated chips had thinner walls and seemed more collapsible. They seemed more like what one would expect from a secondary or third stage of refining. It seems that maceration alone could greatly improve refinability of wood. However, fiber separation continued to occur mainly in the middle lamella and primary cell wall. On the right side, there is more evidence that macerated chips were more susceptible to enzyme treatments. Images here show several areas of cell wall erosion caused by enzyme attack. These weakened points also allow for easier mechanical separation which causes more of these separations to occur into the secondary wall.

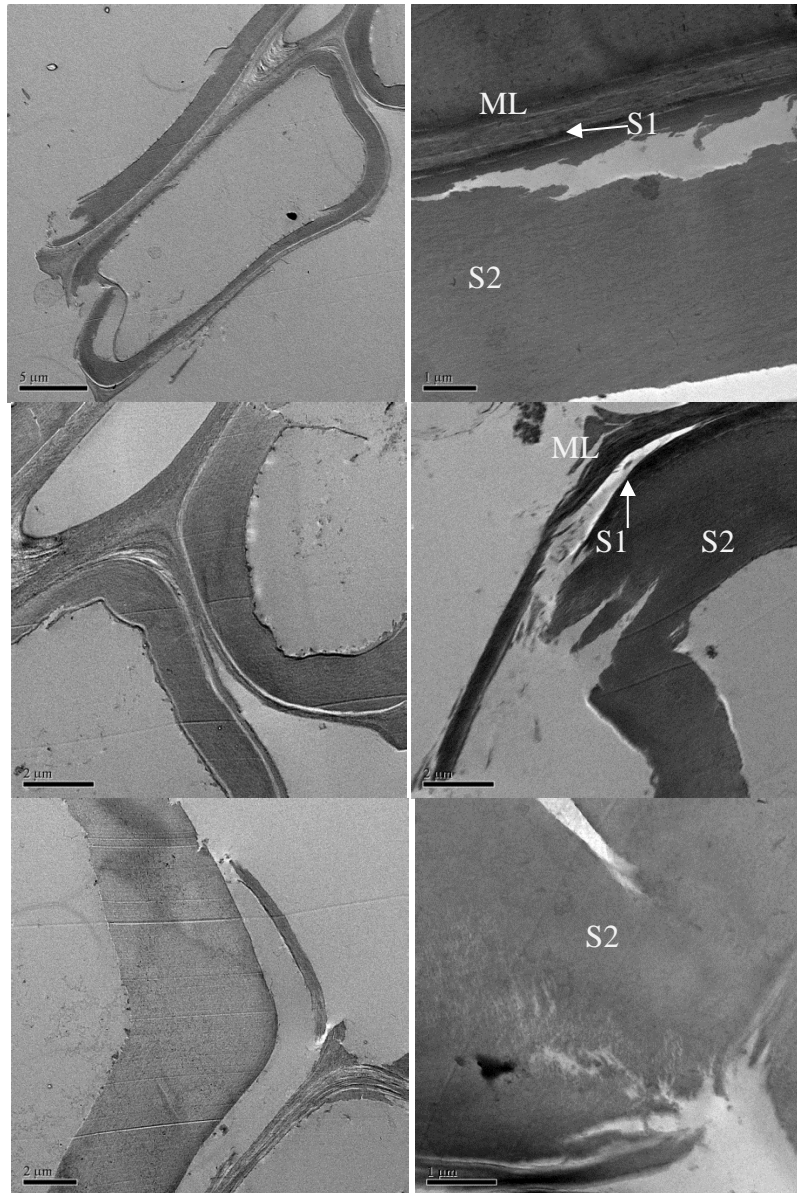


Fig. 9. TEM images, at various magnifications, of control (left side) and enzyme-treated (right side) macerated chips after one stage of refining

CONCLUSIONS

1. SEM images showed that there was some improvement in fiber separation when using enzymes. These included improved separation from bundles as well as the beginnings of fibrillation during the first stage of refining.
2. Higher magnification images showed that the primary and secondary walls were becoming exposed after one stage of refining for the enzyme-treated samples with the enzyme-treated macerated samples showing much more exposed microfibrils than the enzyme-treated whole chips.

3. Non-treated whole and macerated chips showed very little change. But maceration alone seems to improve initial fiber separation even without enzyme treatment.
4. TEM images supported these results by showing fiber separation clearly occurring in the secondary wall for enzyme treated samples, more so for macerated chips than whole chips.
5. TEM images also provided evidence for enzymatic dissolution of fiber wall materials. It is this action that provides the weakened bonds cut during refining.
6. From the distribution of energy savings, it can be said that most of the weakened areas created by enzyme attack are taken advantage of during the first stage of refining. This is especially true when no efforts are made to improve penetration where ~60% of the energy savings occur in the first stage.
7. When compression/decompression is used to help improve enzyme penetration, the energy savings distribution is shifted to a 40/30/30 split, indicating that the enzyme weakened bonds deeper in the wood and/or fiber structure also leading to the best energy savings.

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