Carbohydrate Polymers 95 (2013) 25–31

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

# Improvement of enzyme transport in wood chips for thermomechanical pulp refining

Andre Pelletier[a], Kecheng Li[a,*], Yu Zhao[a], George Court[b], James Luo[c], Mark Frith[d]

[a] University of New Brunswick, Department of Chemical Engineering, Fredericton, New Brunswick, Canada
[b] Irving Paper Ltd., Saint John, New Brunswick, Canada
[c] Novozymes Biotech Inc., Franklinton, NC, United States
[d] NewPage Port Hawkesbury Ltd., Port Hawkesbury, Nova Scotia, Canada

ARTICLE INFO

*Article history:*
Received 31 August 2012
Received in revised form 25 January 2013
Accepted 9 February 2013
Available online 26 February 2013

*Keywords:*
Molecular probe
Enzymatic pretreatment
Mechanical refining
Energy savings
Penetration

ABSTRACT

Wood chips, macerated wood chips and coarse wood fibers were compressed and allowed to decompress in enzyme solutions prior to mechanical refining. Electrical consumption in refining was monitored in order to investigate potential energy savings during refining. Wood treated with Celluclast® 1.5 L showed a reduction in refining energy up to a 36%. Canadian Standard Freeness was used to track degree of refining. Decompressing in the enzyme solution gave a 15% reduction in energy consumption compared to having the already decompressed substrate treated with enzymes. This indicated that improving the transportation of enzymes into wood chips would further enhance the enzyme effect on refining. In this study, confocal laser scanning microscopy (CLSM) was used to track a dyed dextran of 70k molecular weight (the size of some of the larger enzymes) which was used as molecular probe to investigate the potential added penetration of enzymes induced by compression/decompression in the selected chip sizes.



## 1. Introduction

Most mechanical grade pulps being manufactured today are thermomechanical pulps (TMPs). The resulting high yield pulps (up to 95%) are bulky, having good opacity and excellent printability. Unfortunately it is a very energy intensive process and produces fibers which are stiff and contain most, if not all, of the original lignin. This gives a paper with lower strength, higher pitch content (giving runability problems) and higher color reversion rate when comparing to chemical pulps. These qualities and drawbacks come from the fact that TMPs undergo a purely mechanical separation, leaving its chemical composition more or less unaffected. Fibers obtained remain stiff and contain lignin reducing interfibre bonding potential. This leads to a weak sheet prone to breaks.

The past few decades have seen a great deal of research into biopulping, a process where wood chips are inoculated with naturally occurring fungi which possess the ability to degrade woody materials. The main focus has been on lignin-degrading basidomycetes 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, Attridge, Myers, & Blanchette, 1993; Akhtar, Blanchette, Meyers, & Kirk, 1998; Ferraz et al., 2008). However, this kind of application does have disadvantages. In order to achieve the highest energy savings, certain steps must be taken. Chips should be sterilized prior to inoculation by the desired fungus. This will limit the growth of potential competitive species. Piles must be kept within a very specific range of humidity and temperature to maximize growth. And this can take several weeks (Pere, Siika-Aho, & Viikari, 2000). This growth period creates a problem. For this type of treatment to be widely applicable, there is need for a large amount of on-site storage capacity that could be used as a production "buffer" where a residence time of four weeks is not an issue.

These fungi actually produce enzymes (biological catalysts) which degrade and fragment wood fibers to forms more readily digested. Enzyme discovery, preparation and purification have come a long way since initial fungal trials were conducted. Now, direct enzymatic applications offer a more appealing solution. Although the complete mechanisms behind the abilities of the white-rot fungi to degrade wood are not fully understood, enzymes are most definitely an integral part of said mechanism (Leatham, Myers, & Wegner, 1990). The physical action of fungal hyphae certainly plays a role in enzyme delivery but it is the enzymes themselves that eventually play the major role in decomposition (Wilson, 1959). By direct application of the required enzymes,

* Corresponding author. Tel.: +1 506 452 6084; fax: +1 506 453 4767.
*E-mail addresses:* apelleti@unb.ca (A. Pelletier), kecheng@unb.ca (K. Li).

0144-8617/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2013.02.025

growth time is not a factor. Since growth no longer matters, proper sterilization is not an issue. As more active enzymes are discovered, treatment times will continue to drop. Some studies report significant energy savings with relatively short treatment times (as low as 30 min) (Meyer, Lecourt, & Peng, 2008).

Various parameters can affect the overall efficiency of an enzyme treatment. Two key parameters have to do with substrate size, more precisely specific surface area (Chandra et al., 2007), and available pore size in wood. While fungi have a natural enzyme delivery system with their hyphae, enzyme solutions, on the other hand, do not and thus their penetration to hydrolysis sites is limited by their large size relative to the pore size in fibers (Flournoy, Kirk, & Highley, 1991; Maiti & Whitmire, 1997; Mooney, Mansfield, Touhy, & Saddler, 1998). Impregnators have been used in chemical pulping to improve treatments and in mechanical pulping to improve air displacement in chip steaming. It was shown in a technical report that applying pressure to dextran solutions of increasing molecular size had limited penetration into wood chips even when pressure was applied to these solutions. Impregnation combining an Andritz Impressafiner™ with specific enzymes gave energy savings during subsequent refining of alkaline peroxide mechanical pulp (Hart et al., 2009).

Our preliminary, unpublished results show that treating coarse pulp with Celluclast® 1.5 L solution could increase its beatability in a PFI mill, lowering final freeness at a given number of revolutions. These results were confirmed on a larger scale using a KRK refiner with coarse chips. However, when attempting to treat larger wood substrates such as chips and macerated chips, no significant changes in energy could be obtained. These results indicated that enzyme efficiency was inhibited by the larger substrate size, most likely due to the penetration issues.

In this current study, three different sizes of Black Spruce (chips, macerated chips and very coarse pulp) were treated with Celluclast® 1.5 L prior to refining in a laboratory scale single disc KRK refiner. A final Canadian Standard Freness (CSF) target of between 90 and 120 mL of freeness was chosen for the final pulp. A compression/decompression device, designed and built in-house, was used to see if this could improve enzyme penetration. A dyed dextran of a given molecular size (70,000 Mw) was used as molecular probe in combination with confocal laser scanning microscopy (CLSM) to visualize if compression/decompression can increase penetration into the different substrate sizes or if accessible surface is the only important factor.

## 2. Materials and methods

### 2.1. Materials

Wood chips: 100% black spruce wood chips were collected after washing and screening from Irving Pulp & Paper, Saint John, NB. Chips were also compressed with an Andritz Impressafiner™ leading to an increase in surface area. This process consumed, on average, 75 kWh/oven dried ton (ODT). Some wood chips were also refined in the KRK refiner to produce a coarse pulp with even greater surface area. These three different substrates were all tested for moisture (Tappi method T210 cm-03) before further treatments were applied. The three different substrate sizes considered are shown in Fig. 1. These particular sizes were chosen based on their uses and availability within industry and could be easily obtained. It is clear from Fig. 1 that the accessible surface area increases from whole chips, to impressafined wood, down to coarse pulp.

Celluclast® 1.5 L, referred to as enzyme solution in the following sections, was provided by Novozyme Inc. (USA). Its optimal temperature and pH are from 40 to 60 °C and 4.0 to 5.0 respectively. 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 with the exception of the dyed Dextrans which were purchased from Invitrogen Life Technologies and were used as purchased.

### 2.2. Coarse pulp preparation

Chips were preheated in an atmospheric steamer for 15 min. 55 °C water was added to the chips to bring the consistency to approximately 20% (liquor to wood ratio of 5:1). This mixture was hand fed into a hopper which in turn was screw fed into the refiner. Care was taken to maintain the correct L/W throughout the process. The plate gap was set to 1.0 mm and the average energy consumption was 850 kWh/ODT. CSF of the pulp obtained was not measurable and contained mostly matchsticks and fiber bundles.

### 2.3. Enzyme activity determination

A standard enzyme activity determination method was applied in this study (Adney & Baker, 2008). In this method, we applied enzymes to hydrolyze standard substrates, then used a spectrophotometer (Multon Roy, Spectronic 1001 Plus) to test the absorbance of the colored solution obtained after applying the DNS method as described by Miller (1959). The enzyme activity used for dosage calculations was filter paper units (FPU) and has been found to vary between 80 and 160 FPU/ml depending on the batch. This highlights the importance of proper assaying before using the enzyme solution.

### 2.4. Hydrolysis conditions

To simplify the hydrolysis procedures, the average value was adopted for its applicable pH and temperature range. According to enzyme activity, appropriate enzyme dosages were applied in each hydrolysis. 200 g of oven dried wood (ODW) was made into a solution of 10% consistency with tap water at the appropriate temperature. This 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 bleaching bag. After adding the appropriate amount of the 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 1 h. During this time bags were massaged every 15 min to ensure proper heat and enzyme distribution throughout the hydrolysis. These massages also allow for enzyme-pulp mixing.

Control pulps were prepared alongside every different enzyme condition 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. Pulps were then filtered (a 100 mL sample of filtrate was kept for reducing sugar analysis) and rewatered to the refining consistency.

### 2.5. Impressafined pulp recompression and decompression in hydrolysis liquor

Wood chips from the same source were macerated in an Impressafiner™. This provided macerated wood chips to test. This substrate was either used as was 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.


**Fig. 1.** (a) Original wood chip sample, (b) macerated wood chips, and (c) Very coarse pulp prepared using a KRK refiner.

### 2.6. Reducing sugar determination

The filtrates collected from the hydrolysis liquors were tested for reducing sugar concentration following the same colorimetric method as described above (Miller, 1959).

### 2.7. Refining conditions

The refiner was first run for an appropriate amount of time (1 h) to ensure proper shaft thermal balance thus maintaining correct gap measurements throughout the entire process. Once this was achieved, 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 ODW 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 ODW. This loss was accounted for when adjusting consistency and feed time was also modified accordingly. Consistencies were chosen to be 15, 20 and 20% for the first, second and third stage 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.

### 2.8. CSF testing

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

### 2.9. CLSM imaging

CLSM images were taken on a Leica TCS-SP2 Confocal LM. Sample preparation was done using the small lab-scale compression device with dyed dextran solutions. These impregnated samples were then directly imaged. Whole chips, impressafined chips and coarse pulp were all treated with a dyed dextran solution (0.02% in distilled water), in a wood to liquid ratio of 1:10, to observe the penetration in the various substrates. A control without compression was also done with the substrates being soaked in a solution of the same concentration over night. Chips and macerated chips sizes were carefully selected and were all roughly 25 mm × 35 mm while thickness was between 3 and 5 mm. After treatment and selection, transverse sections of wood were obtained from the middle of the sample with razor blades. These were fixed to glass slides and directly observed in the CLSM. The excitation wavelength was 595 nm and emissions between 610 and 620 nm were collected.

## 3. Results and discussion

### 3.1. Impact of substrate size and enzyme selection on reducing sugar production

Fig. 2 shows the reducing sugars produced using varying dosages of the enzyme solution on all three different substrates sizes. As seen in this figure it is obvious that the increased surface area of the refined coarse pulp had a noticeable impact on the amount of reducing sugars produced. Although the macerated chips appeared to have a increase in accessible surface area when compared to the whole chips, as seen in Fig. 1, the amount of reducing sugars released after a 1 h hydrolysis were comparable to the amounts released by the whole chip treatment at the same dosages, except for the highest dosage where there was a slight improvement in sugar production. The increase in surface area for the macerated wood chips is obviously less significant at the microscopic level than what the macroscopic examination would suggest. The sugars produced are still relatively small, even for the highest amount of reducing sugars produced (18 mg/g ODW) which was obtained with the coarse pulp substrate and the highest dosage of enzyme used (5 FPU/g ODW). This amount of reducing sugar produced corresponds to a 1.8% loss in oven dry mass.

Using a compression/decompression cycle on the macerated wood chips also had an effect on the amount of reducing sugar produced during hydrolysis. When compression times of 10 min and 20 min were used, hydrolysis was able to liberate 2.8 and 3.0 mg/g ODW, respectively. The difference between these two trials is

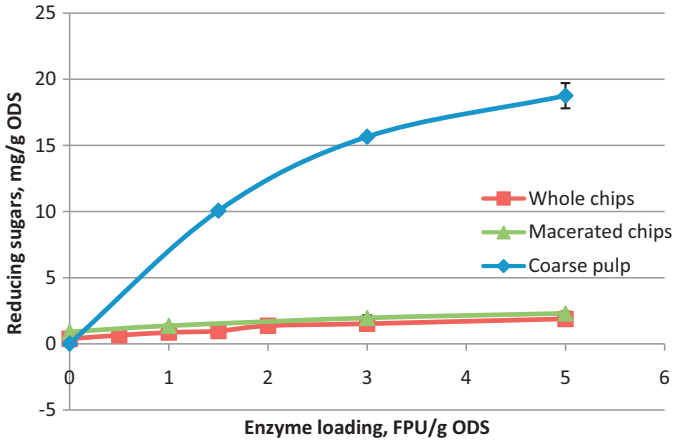


**Fig. 2.** Reducing sugars released from Black Spruce substrates of differing size after a 1 h hydrolysis using the enzyme solution.

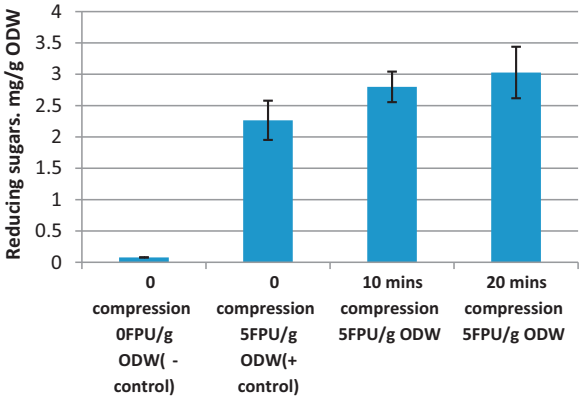


**Fig. 3.** Reducing sugars released from macerated wood chips using various mechanical pretreatments and after a 1 h hydrolysis using the enzyme solution.

negligible and falls within the standard deviations found for these two measurements. This indicates that compression does little to affect actual sugar production; however this does not indicate if the depth of penetration is changed which could impact SEC. Both the compression trials showed a slight increase over the positive control of macerated chips that were simply placed in the hydrolysis liquor with no compression which produced 2.3 mg of reducing sugars per gram of ODW. This slight increase is likely due to the increased penetration of the enzyme into the wood chip which gives the enzyme solution access to more reaction sites. Fig. 3 gives a graphical representation of this.

### 3.2. Enzyme treatment and effects of substrate size on energy consumption

The substrates obtained during the reducing sugar tests were immediately refined after being hydrolysed. This avoided the need of boiling the pulps to deactivate the enzyme since the heat generated during refining is sufficiently high to denature these enzymes (Illikainen, Harkonen, Ullmar, & Niinimaki, 2007). Fig. 4 shows the comparison between different substrate sizes after hydrolysis with the enzyme solution. In this comparison, the whole chips and the macerated chips show variable decreases and some increases in the amount of energy consumed during refining with no clear trend. It is possible that there is some optimization that could be done to determine a suitable dosage which could be used. The variability was likely caused by the fact that the size difference of whole and macerated chips can be quite significant. There was a decrease in SEC at the 1 FPU/g ODW dosage of 8.6% for the chips and a smaller 2.7% decrease at 3 FPU/g ODW for the macerated wood chips. However the deviations in the measurements, some as high as 10%, can make this difficult to say with certainty. The coarse substrate trial however shows a clear and significant impact of enzyme dosage on the refining energy consumption. The largest drop was a 36% decline in refining energy compared to the control and this was achieved at the highest dosage tested of 5 FPU/g ODW. In this figure it is apparent that the control for the whole chips is lower than for those of the compressed and coarse substrates but the CSF obtained after the 3 stages of refining was much higher, ~225 mL as opposed to 125 and 90 mL for the compressed and coarse substrates respectively. The average difference of about 500 kWh/ODMT (low of 250 and a high of 750 kWh/ODMT) between refining the chip control and coarse substrate control along with the added 800 kWh/ODMT, shown in the figures as the bar extension over the standard deviation bars which is to prepare the coarse substrate, can seem like a big difference but when considering the final stage of pulp making, fiber development, is often the most energy intensive and that going from ~230 mL to ~80 mL CSF can consume as much as 1200 kWh/ODMT (Walter, Paulsson, & Wackerberg, 2009).

### 3.3. Effects of compression/decompression on specific energy consumption

Since the changes in surface area alone did not help in improving energy consumption for the macerated wood chips but as evidenced by the work done by Hart et al. (2009) and the increase in the amount of reducing sugar produced using impregnation, it was decided to attempt to have the macerated wood chips recompressed and allowing them to decompress in hydrolysis liquor. Since the first trial did not show any significant savings up to

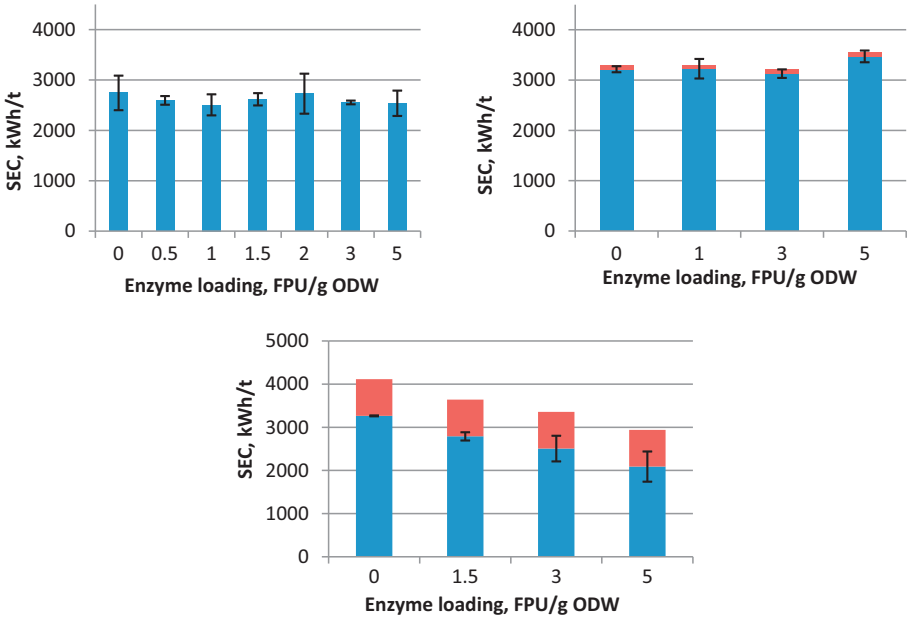


**Fig. 4.** Refining energy consumption of the three different substrate sizes being studied using increasing dosages of the enzyme solutions. The bars that appear in the macerated chip and coarse substrate graphs on top of the main bars represent the additional energy required to produce those specific substrate sizes. (Clockwise from top left: whole chips, macerated chips and coarse pulp.)

5 FPU/g ODW, a dosage of 10 FPU/g ODW was used in this experiment. When no compression was done with an enzyme hydrolysis the amount of energy reduction was only slightly better at 3.4% than the reduction obtained at the 5 FPU/g ODW dosage. However, when compression was applied, the reductions in SEC were much better getting as high as 15.3% when the macerated wood chips were compressed for 10 min. At 20 min of compression, an 8.9% reduction in SEC was obtained. The difference could be due to difficulties in having a uniform distribution of hydrolysis liquor during the expansion period. Another possibility is that holding the compression for longer led to slower expansion of the wood which could be thought of as a transition between elastic and plastic deformation. Fig. 5 shows the SEC results for the different pulp treatments used.

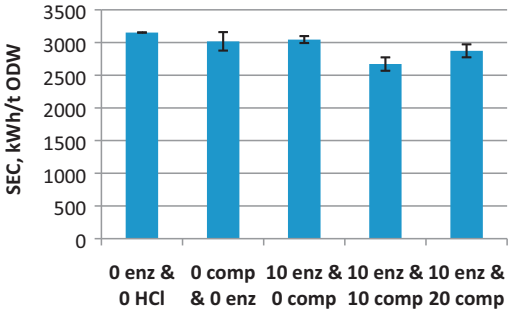


**Fig. 5.** Comparison between various pulp treatments on refining energy consumption. The macerated wood chips were used as is without recompression, treated with enzymes without compression and treated with enzymes following different compression times.

### 3.4. CLSM imaging

The chips and macerated chips were soaked overnight in a solution of 0.02% dyed 70k Mw dextrans. Coarse pulp was not tested as this substrate would be completely stained and no useful information would be obtained. The size of dextran was chosen based on some known enzyme sizes and that many are under 70 kDa (Ariffin, Abdullah, Umi Kalsom, Shirai, & Hassan, 2006; Beldman, Searle-van Leeuwen, Rombouts, & Voragen, 1988). The assumption was then made that any enzyme smaller than this should be able to penetrate at least as far as the molecular probe. The top two images in Fig. 6 shows one end and the outer edge of a middle section of a transverse slice from the middle of a chip. The outer edges of the chip are stained as well as some penetration from the end. This endwise penetration is to be expected as it follows the grain of the wood and is in accordance with well accepted chemical pulping knowledge (Gullichsen & Sundqvist, 1995; Gustafsson, Jimenez, McKean, & Chian, 1988; Stone & Förderreuther, 1956). There is very limited penetration (>150 μm) in the thickness of the

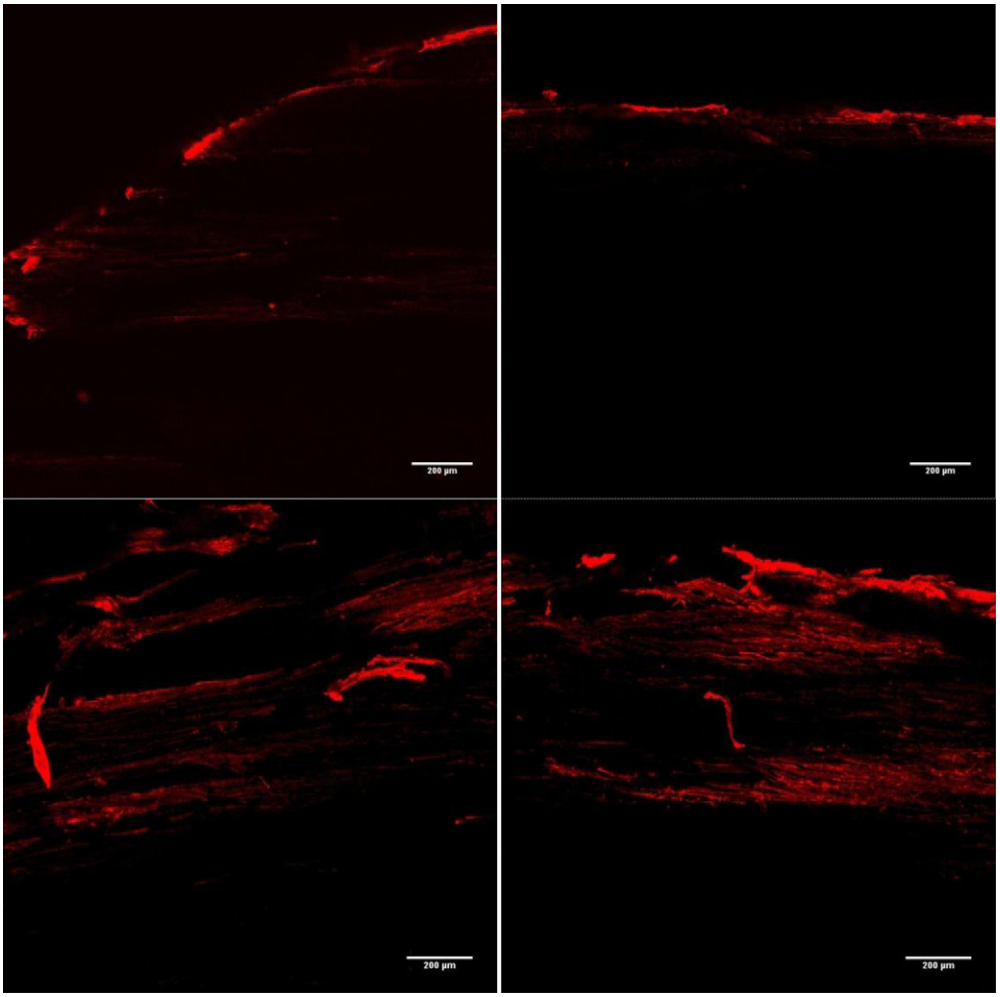


**Fig. 6.** Transverse section from the middle of a whole chip stained with the molecular probe. These images are stacks of single CLSM images in which fluorescence was observed (top two images) Transverse section from the middle of a macerated chip stained with the molecular probe. These images are stacks of single CLSM images in which fluorescence was observed (bottom two images).

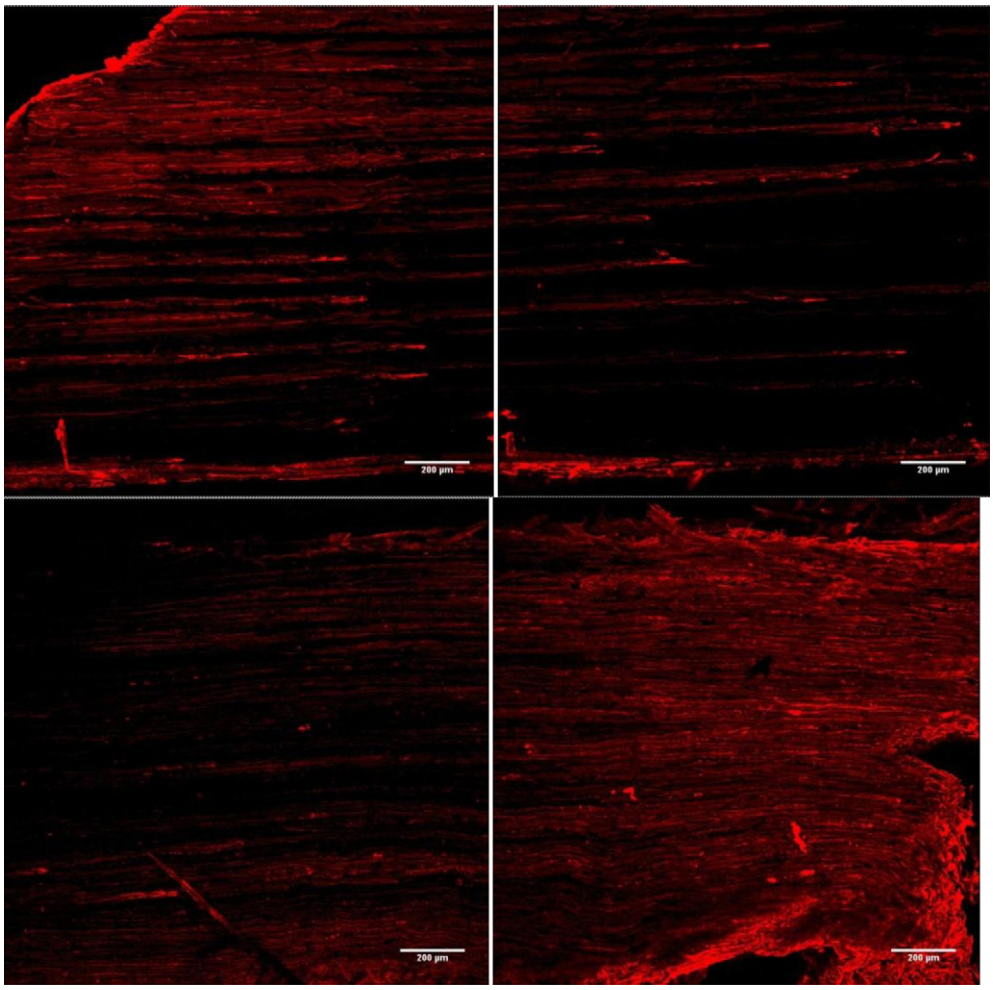


**Fig. 7.** Transverse section from the middle of a whole chip compressed and decompressed in the staining solution. These images are stacks of single CLSM images in which fluorescence was observed (top two images). Transverse section from the middle of a macerated chip compressed and decompressed in the staining solution. These images are stacks of single CLSM images in which fluorescence was observed (bottom two images).

chip which is also well established in chemical pulping literature. Consideration was also given to the possibility that the dye leached from the dextran backbone migrating deeper into the wood without its conjugate. However, the stability of this system has been investigated and leaching was found to be negligible (Plaschke, Czolk, Reichert, & Ache, 1996; Senarath-Yapa & Saavedra, 2001). The bottom two images in Fig. 6 shows a macerated wood chip of similar dimensions than those of the whole chip picture above. The macerated wood chips contain numerous cracks and fractures which create many more avenues for the molecular probe to penetrate deeper into the wood. Due to the broken nature of this wood, imaging was much more difficult and the images represent limited focal planes. However it is evident that there is more penetration into the thickness of the chips as well as from the end.

When a compression/decompression cycle was applied to the chips, penetration into the ends was greatly improved. Penetration into the thickness of the chip was not much affected, however, endwise penetration was greatly enhanced into the chip through natural channels; the lack of penetration from that direction would not be an issue in an industrial setting. The top two images in Fig. 7 shows a section from a compressed treated chip. The section was taken from a similar region as the section for the corresponding images in Fig. 6. In this image it is clear that penetration into the wood chip reaches much further than when no compression/decompression was used, with some dyed pathways reaching several millimeters into the chips (~3 mm).

Similar observations can be made for the macerated wood chips that were submerged in the staining solution during a compression/decompression cycle. Penetration was not greatly improved through the thickness of the chip but penetration from the ends was enhanced. Final depth of the penetration was similar to that of the wood chip in the top two images in Fig. 7; however more channels were used and this is most likely due to cracks and fractures formed during the maceration process which opened more avenues for the molecular probe to penetrate. The bottom two images in Fig. 7 shows the penetration from one end of a macerated wood chip. Again penetration was almost able to reach the middle of the chip (~3.0 mm). The end of the chip in this case is almost completely saturated with the stain and many more channels reach the depth mentioned.

## 4. Conclusion

It is clear from the results that adding one or more compression/decompression cycles to an enzyme treatment with Celluclast® 1.5 L can improve its ability to increase sugar production as well as lower SEC. A 15.3% drop in SEC was observed when comparing between macerated wood chips that were compressed/decompressed in the hydrolysis liquor and when they were simply soaked in the solution. This was accompanied by a slight increase in sugar production going from 2.3 mg/ODW to

2.8 mg/g ODW for the positive control and the 10 min compression/decompression cycle respectively.

Using 70 kDa dyed dextran as a molecular probe was an effective method to model the improved penetration of the enzyme solution. It was evident from the obtained images that compression/decompression was able to increase penetration of large molecules into chips. It was also able to show that the added cracks and fractures that were created during the destructuring process was able to further enhance the ability of the molecular probe to penetrate into the ends of the chips.

## Acknowledgements

The National Science and Engineering Council of Canada (NSERC), the Atlantic Innovation Fund (AIF), Irving Pulp & Paper Ltd. and Andritz are acknowledged for their financial support. The authors would also like to thank Novozymes Inc. for providing the enzymes.

## References

Adney, B., & Baker, J. (2008). *Meaurement of cellulase activities, Laboratory analytical procedure (LAP)*. National Renewable Energy Laboratory, Technical Report, NREL/TP-510-42628.

Akhtar, M., Attridge, M. C., Myers, G. C., & Blanchette, R. A. (1993). Biomechanical pulping of Loblolly Pine chips with selected white-rot fungi. *Holzforschung*, *47*(1), 36–40.

Akhtar, M., Blanchette, R. A., Meyers, G., & Kirk, T. K. (1998). An overview of biomechanical pulping research. In R. A. Young, & M. Akhtar (Eds.), *Environmentally friendly technologies for the pulp and paper industry* (pp. 309–383). NY: John Wiley and Sons.

Ariffin, H., Abdullah, N., Umi Kalsom, M. S., Shirai, Y., & Hassan, M. A. (2006). Production and characterization of cellulase by *Bacillus pumilus* EB3. *International Journal of Engineering and Technology*, *3*(1), 47–53.

Beldman, G., Searle-van Leeuwen, M. F., Rombouts, F. M., & Voragen, A. G. J. (1988). The cellulase of *Trichorderma viride*: Purification, characterization and comparison of all detectable endoglucanases exoglucanases and $\beta$-glucosidases. *European Journal of Biochemistry*, *146*, 301–308.

Chandra, R. P., Bura, R., Mabee, W. E., Berlin, A., Pan, X., & Saddler, J. N. (2007). Substrate pretreatment: The key to effective enzymatic hydrolysis of lignocellulosics? *Advances in Biochemical Engineering/Biotechnology*, *108*, 67–93.

Ferraz, A., Guerra, A., Mendonca, R., Masarin, F., Vicentim, M. P., Aguiar, A., et al. (2008). Technological advances and mechanistic basis for fungal biopulping. *Enzyme and Microbial Technology*, *43*(2), 178–185.

Flournoy, D. S., Kirk, T. K., & Highley, T. L. (1991). Wood decay by brown-rot fungi: Changes in pore structure and cell wall volume. *Holzforschung*, *45*(5), 383–388.

Gullichsen, J., & Sundqvist, H. (1995). On the importance of impregnation and chip dimensions on the homogeneity of kraft pulping. In *Proceedings of the 1995 Pulping Conference* Chicago, (pp. 227–234).

Gustafsson, R. R., Jimenez, G., McKean, W., & Chian, D. S. (1998). The role of penetration and diffusion in pulping non-uniformity of softwood chips. In *Proceedings of the 1988 Pulping Conference* New Orleans, (pp. 685–691).

Hart, P. W., Waite, D. M., Thibault, L., Tomashek, J., Rousseau, M.-E., Hill, C., et al. (2009). Selective enzyme impregnation of chips to reduce the specific refining energy in alkaline peroxide mechanical pulping. *Holzforschung*, *63*, 418–423.

Illikainen, M., Harkonen, E., Ullmar, M., & Niinimaki, J. (2007). Power consumption distribution in a TMP refiner: Comparison of the first and second stages. *Tappi Journal*, *6*(9), 18–23.

Leatham, G., Myers, G., & Wegner, T. (1990). Biomechanical pulping of aspen chips: Energy savings resulting from different fungal treatments. *Tappi Journal*, *73*(5), 197–200.

Maiti, B., & Whitmire, D. (1997). Xylanase molecular size, pulp pore-structure and pulp delignification. *Chemical Engineering Communications*, *162*(1), 169–178.

Meyer, V., Lecourt, M., & Peng, F. (2008). Ecotarget: Bio-mechanical refining. In *Ecotarget Final Conference* Stockholm.

Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*, *31*(3), 426–428.

Mooney, C. A., Mansfield, S. D., Touhy, M. G., & Saddler, J. N. (1998). The effect of initial pore volume and lignin content on the enzymatic hydrolysis of softwoods. *Bioresource Technology*, *64*, 113–119.

Pere, J., Siika-Aho, M., & Viikari, L. (2000). Biomechanical pulping with enzymes: Response of coarse mechanical pulp to enzymatic modification and secondary refining. *Tappi Journal*, *83*(5), 1–8.

Plaschke, M., Czolk, R., Reichert, J., & Ache, H. J. (1996). Stability improvement of optochemical sol-gel film sensors by immobilisation of dye-labelled dextrans. *Thin Solid Films*, *279*(1–2), 233–235.

Senarath-Yapa, M. D., & Saavedra, S. S. (2001). Dye leaching from a doped sol-gel is eliminated by conjugation to a dendrimer. *Analytica Chimica Acta*, *432*(1), 89–94.

Stone, J. E., & Förderreuther, C. (1956). Studies of penetration and diffusion into wood. *Tappi Journal*, *39*(10), 679–683.

Walter, K., Paulsson, M., & Wackerberg, E. (2009). Energy efficient black spruce TMP by using acid hydrogen peroxide: Part 1. A pilot plant study. *Nordic Pulp and Paper Research Journal*, *24*(3), 255–265.

Wilson, C. L. (1959). Penetration and invasion of *Ceratocystis Piceae* in White Oak Wood. *Mycologia*, *51*(3), 311–317.